Maigreur constitutionnelle : de son diagnostic et ses caractéristiques physiologiques à l'exploration cellulaire du muscle squelettique

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MAIGREUR CONSTITUTIONNELLE : DE SON DIAGNOSTIC ET SES CARACTÉRISTIQUES PHYSIOLOGIQUES À L'EXPLORATION CELLULAIRE DU MUSCLE SQUELETTIQUE

Présentée et soutenue publiquement par

Mélina BAILLY

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« Ce qui est incompréhensible, c'est que le monde soit compréhensible. »

Albert EINSTEIN

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5-HTTLPR : serotonin-transporter-linked polymorphic region
26RFa : neuropeptide pyroglutamilated RFamide peptide
ACTH : adrenocorticotropic hormone
ALK : anaplastic lymphoma kinase
AM : anorexie mentale
ANOVA : analysis of variance
ATP : adenosine triphosphate
BSQ : body shape questionnaire
CI : complexe I de la chaîne respiratoire
CII : complexe II de la chaîne respiratoire
CIV : complexe IV de la chaîne respiratoire
CAFA : indices des capillaires par surface de section transversale de fibre
CapTor : indice de tortuosité des capillaires
CC : indice des capillaires au contact
CFPE : indice d'échange capillaires–périmètre de fibre
CoA : coenzyme A
CTP1 : carnitine O-palmitoyltransferase 1
CTP2 : carnitine O-palmitoyltransferase 2
CTX : C-terminal telopeptide
DEBQ : Dutch eating behavior questionnaire
DER : dépense énergétique de repos
DET : dépense énergétique totale
DHEAS : dehydroepiandrosterone sulfate
DHPR : récepteur à la dihydropyridine
DMO : densité minérale osseuse
DSM-V : diagnostic and statistical manual of mental disorders
DXA : absorptiométrie biphotonique à rayons X
EDE : eating disorder examination
EDI : eating disorder inventory
EGCUT : Estonian genome center of the university of Tortu
FAT/CD36 : transporteur des acides gras
FITM1 : fat storage-inducing transmembrane 1
FITM2 : fat storage-inducing transmembrane 2
FSH : follicle-stimulating hormone
FT3 : free triiodothyronine
GH : growth hormone
GLP-1 : glucagon-like peptide-1
GLUT4 : glucose transporter type 4
HOMA-IR : homeostasis model assessment of insuline resistance
IGF-1 : insulin-like growth factor-1
IMC : indice de masse corporelle
IMTG : triglycérides intramusculaires
LC : longueur cumulée des capillaires en contact avec la fibre musculaire
LH : luteinizing hormone
MC : maigreur constitutionnelle / maigre constitutionnel
MM : masse maigre
MOSPA : Monica optional study of physical activity questionnaire
α-MSH : α-melanocyte-stimulating hormone
NAP : niveau d'activité physique
NPY : neuropeptide Y
OMS : Organisation Mondiale de la Santé
OPG : osteoprotegerin
PECAM-1 : platelet endothelial cell adhesion molecule
PF : périmètre de la fibre musculaire
pQCT : tomodensitométrie quantitative périphérique
PTH : parathormone
PYY : peptide YY
QR : quotient respiratoire
RANKL : receptor activator of nuclear factor kappa-B ligand
RyR : récepteur à la ryanodine
SERCA : pompe calcium-ATPase du réticulum sarcoplasmique
SLN : sarcolipin
SHBG : sex hormone-binding globulin
STILTS : study into lean and thin subjects
T : témoin (sujets normo-pondérés)
T3 : triiodothyronine
TFEQ : three-factor eating questionnaire
UCP3 : uncoupling protein 3
UEA 1 : ulex europaeus agglutinin

PUBLICATIONS ET COMMUNICATIONS

RELATIVES À LA THÈSE

ARTICLE | **Bailly, M.**, Germain, N., Galusca, B., Courteix, D., Thivel, D., & Verney, J. (2020). Definition and diagnosis of constitutional thinness: A systematic review. *British Journal of Nutrition*, 124(6), 531-547. https://doi.org/10.1017/S0007114520001440 **(Publié)**

ARTICLE | **Bailly, M.**, Germain, N., Galusca, B., Courteix, D., Thivel, D., & Verney, J. (2020). Invited Letter to Editor in response to: Constitutional thinness: body fat metabolism and skeletal muscle are important factors. *British Journal of Nutrition*, 124(9), 999-1000. https://doi.org/10.1017/S0007114520002159 **(Publié)**

ARTICLE | **Bailly, M.**, Boscaro, A., Pereira, B., Courteix, D., Germain, N., Galusca, B., Boirie, Y., Thivel, D., & Verney, J. (under review). Underweight but not underfat: Is Fat-Free mass a key factor in constitutional thinness? *European Journal of Clinical Nutrition*. **(En révisions)**

ARTICLE | **Bailly, M.**, Boscaro, A., Pereira, B., Féasson, L., Boirie, Y., Germain, N., Galusca, B., Courteix, D., Thivel, D., & Verney, J. (submitted). Is constitutional thinness really different from anorexia nervosa? A systematic review and meta-analysis. *Reviews in Endocrine & Metabolic Disorders.* **(Soumis)**

ARTICLE | **Bailly, M.**, Feasson, L., Pereira, B., Boileau, A., Hourdé, C., Germain, N., Galusca, B., Courteix, D., Thivel, D., & Verney, J. (2020). Two new reliable immunohistochemical methods for simultaneous identification of capillaries, the three types of fibers and basal lamina in human skeletal muscle. *Histochemistry and Cell Biology*, 154(3), 327-337. https://doi.org/10.1007/s00418-020-01895-5 **(Publié)**

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Applied Physiology, Nutrition, and Metabolism, 45(11), 1287-1298. https://doi.org/10.1139/apnm-2020-0068 **(Publié)**

POSTER | **Bailly, M.**, Germain, N., Galusca, B., Féasson, L., Courteix, D., & Verney, J. (2019). Caractérisation du phénotype musculaire chez des sujets présentant une maigreur constitutionnelle. *22ème JED (Journées de l'École Doctorale SVSAE)*, Clermont-Ferrand, France. **(Présenté) – ANNEXE**

NON RELATIVES À LA THÈSE

ARTICLE | Fillon, A., Beaulieu, K., Miguet, M., **Bailly, M.**, Finlayson, G., Julian, V., Masurier, J., Pereira, B., Duclos, M., Boirie, Y., & Thivel, D. (2020). Delayed meal timing after exercise is associated with reduced appetite and energy intake in adolescents with obesity. *Pediatric Obesity*, 15(9), e12651. https://doi.org/10.1111/ijpo.12651 **(Publié) – ANNEXE**

ARTICLE | Fillon, A., Beaulieu, K., Miguet, M., **Bailly, M.**, Finlayson, G., Julian, V., Masurier, J., Mathieu, M.-E., Pereira, B., Duclos, D., Boirie, Y., & Thivel, D. (2020). Does exercising before or after a meal affect energy balance in adolescents with obesity? *Nutrition, Metabolism and Cardiovascular Diseases*, 30(7), 1196-1200. https://doi.org/10.1016/j.numecd.2020.04.015 **(Publié) – ANNEXE**

COMMUNICATION ORALE | Fillon, A., Miguet, M., **Bailly, M.**, Julian, V., Pereira, B., Masurier, J., Beaulieu, K., Finlayson, G., Duclos, M., Boirie, Y., & Thivel, D. (2019). Effect of the exercise timing on energy intake and appetite sensations in adolescents with obesity. ECOG Workshop, Katowice, Pologne. **(Présentée)**

POSTER | Fillon, A., Miguet, M., **Bailly, M.**, Julian, V., Pereira, B., Masurier, J., Beaulieu, K., Finlayson, G., Duclos, M., Boirie, Y., & Thivel, D. (2019). Does exercising before or after a meal optimize overall energy balance in adolescents with obesity? ECOG Workshop, Katowice, Pologne. **(Présenté)**

PARTIE I – REVUE DE LA LITTÉRATURE ET OBJECTIFS

1. CONTEXTE

1.1. Introduction du travail de doctorat

Dès le milieu du XXème siècle, le concept d'une maigreur dite « constitutionnelle » est apparu dans les premiers écrits scientifiques. Cette idée d'une maigreur d'origine physiologique, semblant défier les notions de base de l'énergétique, a rapidement divisé la communauté scientifique. Comment expliquer qu'une personne jeune, en bonne santé, et s'alimentant normalement, puisse rester si maigre ? Si dans nos sociétés obésogènes modernes cette difficulté à la prise de poids pourrait parfois sembler enviable, il n'en reste pas moins que les personnes présentant une maigreur constitutionnelle (MC) témoignent en consultation de leurs inquiétudes et expriment clairement leur souhait de prendre du poids. Scepticisme ou désintérêt : seule une quarantaine d'études cliniques s'est intéressée à la MC en près de 90 ans. Qui plus est, le nombre extrêmement faible de publications ainsi que leur forte hétérogénéité mènent à une littérature équivoque en ce qui concerne la définition de la MC, son diagnostic, sa physiologie, et même son existence. Au regard de ces éléments, ce travail de doctorat a eu pour premier objectif de proposer une approche holistique du diagnostic et de la caractérisation physiologique de la MC. Dans un second temps, nous avons exploré le tissu musculaire du sujet MC dans le but d'apporter de nouvelles pistes à la compréhension de ce profil particulier.

1.2. Approche historique

1.2.1. Naissance du concept

Dès 1933, le Professeur et Docteur allemand Erich Grafe fait état d'un type de maigreur qu'il qualifie de « constitutionnelle » dans son ouvrage *Metabolic Diseases and their Treatment* (Grafe 1933) (**Figure 1**). Il rend compte de l'existence d'individus présentant une maigreur importante malgré un état apparent de bonne santé, dont l'origine n'est pas connue (Grafe 1933). Il décrit de « bons mangeurs » qui ne prennent pourtant pas de poids et souligne ce paradoxe en mentionnant une disproportion entre l'apport alimentaire important et le faible poids corporel de ces individus. De plus, le Professeur Grafe rapporte avoir été témoin de certains cas de MC « familiale ».

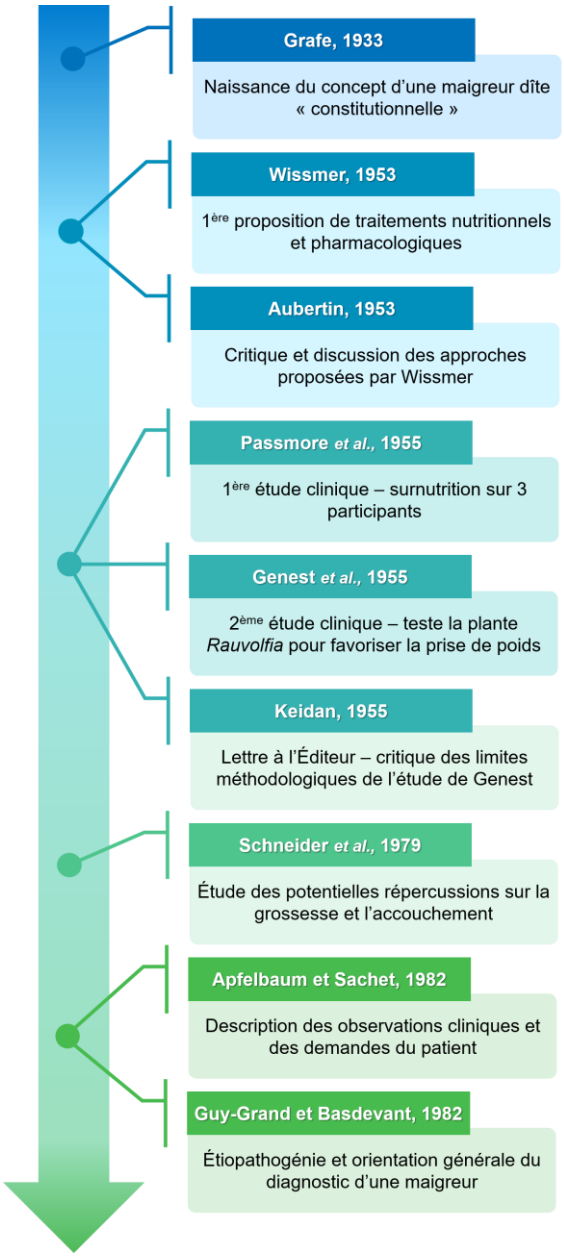


FIGURE 1 – APPROCHE HISTORIQUE DE LA MAIGREUR CONSTITUTIONNELLE

Il explique également que ces personnes maigres constitutionnelles ainsi que leurs proches craignent une origine pathologique sous-jacente à cet état de forte maigreur et recherchent alors une aide médicale. Pourtant, le Pr. Grafe rapporte ne pas déceler de quelconque pathologie pouvant expliquer la maigreur à l'examen de ces personnes, si ce n'est l'état de forte maigreur en lui-même (Grafe 1933).

1.2.2. Premières propositions de prise en charge

Une vingtaine d'année plus tard, Wissmer s'interroge sur le contraste entre la prolificité des travaux concernant l'obésité et son traitement face à la « discrétion » concernant les états de maigreur (Wissmer 1953). Si l'auteur débute son propos par une définition assez claire de la MC, il semble finalement englober sous le terme de MC les cas d'amaigrissement par insuffisance alimentaire. Cette confusion sera d'ailleurs vivement critiquée par Aubertin qui fait part de sa déception face à ce manque de clarté et déclare à propos de l'article de Wissmer : « on est quelque peu surpris de voir englober sous le terme de maigres constitutionnels les amaigris par insuffisance alimentaire, volontairement instituée pour des raisons d'esthétique, les anorexiques mentaux, les sous-alimentés par impécuniosité, les éthyliques, les édentés, les dyspeptiques de toutes sortes, les hépatiques, etc., sans parler de ceux qui se dépensent trop et ne mangent pas en proportion » (sic) (Aubertin 1953). Wissmer semble cependant indiquer qu'en ce qui concerne la MC, une thérapeutique similaire aux autres causes de maigreur peut être proposée suite à la réparation du dérèglement causal. Il préconise en pratique un apport calorique quotidien de 3 500 à 4 000 kcal en joignant un exemple détaillé de régime devant conduire à une prise de poids (Wissmer 1953). Là encore, la proposition de régime de Wissmer est à nouveau largement critiquée par Aubertin qui s'exprime ainsi à propos des rations proposées par Wissmer : « de deux choses l'une : ou bien le patient est un maigre sthénique qui mange correctement ; il serait un peu ridicule de lui faire peser ses rations. Ou bien il est plus ou moins anorexique ; et il n'absorbera certainement pas tout ce que Wissmer conseille de lui donner » (sic) (Aubertin 1953). Pour Aubertin, « l'essentiel consiste, en réalité, à orienter l'alimentation du malade vers les aliments les plus nutritifs et à chercher éventuellement à en concentrer la valeur » (sic) (Aubertin 1953). Durant cette année 1953, les échanges par articles interposés entre Aubertin et Wissmer semblent particulièrement tournés vers la recherche de traitements nutritionnels ou pharmacologiques pouvant aider les personnes présentant une MC à prendre du poids. Les deux auteurs y abordent par exemple l'intérêt du Fenugrec, une plante médicinale aujourd'hui reconnue pour ses nombreuses propriétés bénéfiques sur la santé (effets antioxydants, anti-

inflammatoire, antibactériens, anti cancers…) (Venkata et al. 2017)). Aubertin disait à propos du Fenugrec : « il s'agit d'un mélange de principes actifs qui trouvent tout particulièrement leurs indications dans le traitement de la maigreur. […] L'usage du foin grec (Fenugrec) […] dont les graines étaient déjà utilisées dans la plus haute antiquité […] fut introduit en Italie […] pour engraisser le bétail. En Orient, le goût des femmes grasses […] incitait les jeunes filles désireuses de se marier […] à faire un large usage de fenugrec […] » (sic) (Aubertin 1953). La suite de leurs échanges apparaît assez surréaliste. Aubertin préconise par exemple d'injecter « sous la peau, vingt minutes avant chacun des deux principaux repas, 10 unités d'insuline des cures d'insuline […] vingt jours par mois pendant plusieurs mois » (sic). Ces deux auteurs discutent par la suite de l'utilisation de testostérone agissant sur l'anabolisme des protéines ; ainsi que l'utilisation de cortisone ou d'adrenocorticotropic hormone (ACTH) à petites doses. Aubertin écrit : « il n'est pas douteux que ces hormones provoquent une stimulation de l'état général, et un certain degré d'embonpoint qu'il n'est pas toujours facile de distinguer de la bouffissure liée à la rétention d'eau et de sel » et rappelle le caractère « peu durable » de ce traitement pouvant « s'accompagner d'effets associés regrettables » (Aubertin 1953). L'ensemble des propos tenus dans l'article de Aubertin n'a pas été relevé, étant donné le caractère non scientifique et inapproprié de certains éléments (Aubertin 1953). Bien que la plupart des traitements proposés en 1953 apparaîtraient aujourd'hui surréalistes, en particulier chez des sujets « bien portants » (Aubertin 1953), il nous a semblé intéressant de retracer les premières propositions, parfois très surprenantes, de traitements de la MC.

Deux ans plus tard, en 1955, Passmore réalise la première étude clinique, à notre connaissance, portant sur la MC, bien que l'étude ne porte que sur trois participants (Passmore et al. 1955a). L'auteur s'interroge sur les mécanismes pouvant expliquer que des personnes en bonne santé, avec un bon appétit, puissent rester excessivement maigres. Il soulève en effet cette croyance commune selon laquelle certaines personnes maigres auraient un mystérieux moyen de dépenser l'excès d'énergie, et s'intéresse à la balance énergétique de ces trois participants MC face à un apport énergétique accru : 4000 kcal/jour sur

une période de 10 à 14 jours (Passmore et al. 1955a). Dans cette étude menée en milieu hospitalier à l'infirmerie royale d'Édimbourg, les patients sont monitorés toute la journée : standardisation de l'activité physique (temps passé en position allongée, assise, ou temps de marche), mesures de la dépense énergétique de repos (DER) (calorimétrie indirecte) toutes les quatre heures, prises de sang, apports alimentaires (pesée), échantillons d'urine, accumulation des fèces (pesée) et estimation des flux azotés. Bien qu'il soit difficile d'apporter des conclusions sur un tel échantillon (trois participants sans groupe témoin), l'auteur n'a pas observé de preuve d'un excès d'oxydation énergétique. D'après l'analyse des fèces, plus de 90 % de l'excès d'énergie a été absorbé (Passmore et al. 1955a). Dans une seconde étude, le même auteur approfondit l'analyse du gain de poids des trois participants et souligne que les sujets n'ont pris que 70 % de la prise de poids attendue (Passmore et al. 1955b). Le premier sujet a réalisé une surnutrition de 10 jours et a pris 1.5 kg au lieu 2.2 kg ; et les deux autres sujets ont réalisé une surnutrition de 14 jours ont respectivement pris 2.5 kg et 2.6 kg au lieu de 3.4 kg et 3.6 kg. Cette étude pionnière suggère ainsi une difficulté à la prise de poids.

1.2.3. Premières observations cliniques

En 1979, une étude s'intéresse aux potentielles conséquences du faible capital adipeux préalable à la grossesse dans un contexte de MC (Schneider et al. 1979). Sur 16 261 dossiers d'accouchements, 53 dossiers concernant des femmes MC ont été retenus ainsi que 100 dossiers témoins. Cette étude rétrospective montre que les femmes MC auraient tendance ($p=0.08$) à davantage accoucher avant le terme de 37 semaines que les femmes du groupe témoin (Schneider et al. 1979). Le poids de naissance du nouveau-né à partir de la 39ème semaine de grossesse serait également légèrement diminué, le poids de naissance restant stable aux environs de 2.9 kg à partir de la 39ème semaine. Cependant, avant la 39ème semaine, le poids de naissance est comparable entre les deux groupes et aucune conséquence pathologique périnatale ne semble être observée. L'étude ne montre pas de différence entre les mères présentant une MC et les mères du groupe témoin en ce qui concerne la prise de poids totale de la

mère, la proportion d'accouchements par voie basse ou la proportion d'accouchements nécessitant une extraction instrumentale (Schneider et al. 1979).

En 1982, deux articles interrogent la quasi absence de travaux scientifiques portant sur la MC en dépit du nombre important de consultations médicales des individus atteints de maigreur (Apfelbaum and Sachet 1982). Les auteurs soulèvent effectivement le peu d'intérêt porté à cette condition par l'ensemble de la communauté scientifique et médicale qui ne semble pas considérer cet état de maigreur comme pathologique. Les auteurs soulignent que les courbes de mortalité/poids provenant de compagnies d'assurance montrent un risque de mortalité lié à la maigreur, même si ces chiffres recouvrent tous types de maigreur, et non exclusivement les cas de MC. Face à la quasi absence de données dans la littérature, Apfelbaum et Sachet font part de leurs observations cliniques en milieu hospitalier et des demandes que peuvent faire les patients MC durant les consultations (Apfelbaum and Sachet 1982). Les auteurs notent qu'à l'interrogatoire, les patients MC expliquent que leur état de maigreur persiste depuis le plus jeune âge, avec une courbe de poids en dessous de celle des enfants du même âge dès les 18 premiers mois de vie, suivie d'un poids stable à l'âge adulte (Apfelbaum and Sachet 1982). L'interrogatoire rigoureux des apports alimentaires témoigne d'une alimentation riche et variée, similaire à celle des sujets normo-pondérés voire plus calorique, du fait de la volonté de prendre du poids. Les auteurs racontent qu'en consultation, le patient MC est inquiet des potentielles conséquences pathologiques de sa forte maigreur et insatisfait de sa morphologie avec un mal-être dû au manque de formes perçu comme un manque de féminité chez les femmes, et au manque de masse perçu comme « castrateur » et comme signe de non-virilité chez le sujet masculin (Apfelbaum and Sachet 1982). En outre, l'individu MC semble décrire une forme de stigmatisation sociale envers sa maigreur et rapporte fréquemment une place socio-professionnelle non satisfaisante. Les auteurs rapportent qu'à l'examen clinique, la musculature semble correcte avec une maigreur plutôt généralisée et une adiposité cependant fréquemment réduite (pli cutané mince). Ils décrivent également des fonctions génitales *a priori*

normales, sans aménorrhée chez la femme. Apfelbaum et Sachet témoignent également d'analyses biologiques sans anomalies notables du bilan endocrinien (fonctions hypophysaires et thyroïdiennes normales), du profil lipidique, du taux de protides sanguin, du test de tolérance au glucose, et de la fréquence des hypertriglycéridémies et hypercholestérolémies. Face à ces observations cliniques *a priori* normales, les auteurs n'encouragent pas forcément la prise d'un traitement spécifique à visée de prise de poids, sauf en cas de requête appuyée et persistante de la part du sujet, et proposeraient davantage une psychothérapie légère visant à une meilleure acceptation par le patient de son état de maigreur (Apfelbaum and Sachet 1982). Néanmoins, les auteurs proposent différentes mesures thérapeutiques diététiques ou médicamenteuses visant à la prise de poids en cas de demandes répétées du patient. Ils préconisent dans ce cas une surnutrition massive à base de lipides et de sucres rapides afin d'atteindre des rations caloriques quotidiennes de l'ordre de 3 500 kcal, tout en soulignant que la réussite d'un tel régime serait modérée et de courte durée chez le patient MC. Concernant les thérapeutiques médicamenteuses, les auteurs semblent recommander qu'elles restent exceptionnelles et ne constituent qu'un dernier recours. Ils déconseillent les médicaments orexigènes (puisque le sujet MC ne semble pas manquer d'appétit) ainsi que les anabolisants, mais proposent éventuellement un traitement à l'insuline (article de 1982 – (Apfelbaum and Sachet 1982)). Les auteurs semblent conclure que la meilleure stratégie à adopter face à la MC est surtout l'acceptation de cet état de maigreur qui ne semblerait pas, *a priori*, induire de désordres de santé majeurs et qui paraîtrait difficilement contrôlable et possible à réguler (Apfelbaum and Sachet 1982). La même année et dans la même revue, Guy-Grand et Basdevant publient l'*Étiopathogénie et orientation générale du diagnostic d'une maigreur* (Guy-Grand and Basdevant 1982). Les auteurs s'intéressent à la définition même de la maigreur et à son diagnostic et soulignent l'importance sémantique de différencier les notions de maigreur, d'amaigrissement et de dénutrition. Ils décrivent ensuite les différents points importants à considérer à l'examen clinique d'une maigreur afin d'en déterminer son étiologie. Guy-Grand et Basdevant font ainsi la distinction entre les différentes causes possibles de maigreur en les séparant en deux catégories : la maigreur due à une

réduction des apports énergétiques et la maigreur avec conservation ou augmentation des apports, à laquelle appartient ainsi la MC (Guy-Grand and Basdevant 1982). Ainsi, les auteurs expriment également l'importance de bien distinguer la MC de l'anorexie mentale (AM). Dans le cas d'une MC, ils décrivent un état d'équilibre nutritionnel avec un apport qualitatif non déséquilibré ainsi qu'un apport quantitatif global « suffisant » avec une ration calorique quotidienne de l'ordre de 1 900 à 2 500 kcal. De plus, Guy-Grand et Basdevant apportent également la notion d'une hérédité de la MC en mentionnant de potentiels antécédents familiaux (Guy-Grand and Basdevant 1982).

1.3. Constats actuels

1.3.1. Un trouble peu connu et peu reconnu

Il semble exister une différence de paradigme majeure entre le monde des « non-experts » et des « experts » en ce qui concerne la conceptualisation de la MC. Si parmi les non-experts, nombreux sont les témoignages de connaissance de cas de MC : « elle/il » mange tout ce qu'elle/il veut/peut et pourtant elle/il reste très maigre alors qu'elle/il aimerait grossir », une partie importante de la communauté médicale et scientifique, en revanche, semble faire preuve de scepticisme face à cette MC qui remet en question la vision dogmatique selon laquelle une augmentation des apports alimentaires se traduit obligatoirement par une prise de poids chez une personne jeune et en bonne santé. Ce scepticisme est peut-être renforcé par la difficulté d'expliquer aisément cette résistance à la prise de poids par les paramètres usuels de la balance énergétique, bien que la preuve du concept semble avoir été confirmée par une étude contrôlée (Germain et al. 2014, 2016a). En effet, la MC semble constituer le premier modèle humain de résistance à la prise de poids malgré une surnutrition et révélant un « gap » énergétique non expliqué à ce jour (Germain et al. 2014, 2016a). Si dans nos sociétés modernes obésogènes les « non-experts » semblent parfois considérer ce trouble comme une « chance », la communauté scientifique et médicale ne montrerait pas d'inquiétude épidémiologique particulière pour ce trouble qui présenterait, *a priori*, peu d'enjeux en termes de santé publique comparativement à la

situation opposée qu'est l'obésité. Ce désintérêt se traduit dans la littérature scientifique par un nombre d'investigations cliniques extrêmement faible : sur le dernier siècle, seule une quarantaine d'investigations cliniques très hétéroclites a été menée sur la MC. En conséquence, la connaissance de la MC reste très limitée et encore peu robuste, tant sur sa caractérisation que sur d'éventuels conseils ou stratégies pouvant aider cette population. Si la MC est peu connue, elle est en conséquence peu reconnue, et souvent mal ou non diagnostiquée.

1.3.2. Distinction entre maigreur constitutionnelle et anorexie mentale

L'une des principales erreurs de diagnostic dans le cas d'une MC consiste en la confusion avec l'AM (Estour et al. 2014). Pourtant, le diagnostic différentiel entre une MC et une AM représente un enjeu majeur en termes de prise en charge et de suivi. Bien que ces deux populations partagent le critère commun d'un indice de masse corporelle (IMC) très faible, inférieur à 17.5 kg/m², le tableau clinique semble très différent (Estour et al. 2014). Le patient souffrant d'AM présente généralement des troubles alimentaires et psychologiques, une balance énergétique négative, une dynamique de perte de poids importante, ainsi qu'un bilan biochimique anormal caractéristique d'une sous-nutrition. Au contraire, la personne MC ne montre pas de signe de désordre alimentaire ou de trouble psychologique, présente un poids faible mais stable sans dynamique de perte de poids, des règles physiologiques normales, ainsi qu'un bilan biochimique sans anomalie majeure (Estour et al. 2014).

1.3.3. Maigreur et risques de santé

L'intérêt d'explorer les causes et conséquences de la MC est parfois questionné et discuté en argumentant que pas ou peu d'évidences ont démontré que cet état de maigreur était préjudiciable en termes de santé. Plusieurs éléments semblent néanmoins s'opposer à cette vision. Tout d'abord, si les premiers bilans biochimiques ne semblent effectivement pas inquiétants, le nombre très faible d'études ne permet pas d'apporter de conclusions robustes. Il ne s'agit que de premiers éléments, et l'absence

de préjudices sur l'ensemble des composantes physiologiques de la santé reste à prouver. Nous rappellerons également qu'il ne s'agit pas d'une légère minceur mais d'un sous-poids important, comparable à celui de sujets présentant une AM. Outre le manque de puissance quantitative du nombre d'études (moins d'une quarantaine), le manque de puissance « qualitative » est également à souligner. En effet, les domaines d'études sur la MC sont extrêmement variés (composition corporelle, balance énergétique, bilans biochimiques, mais également génétique, variabilité de la fréquence cardiaque, neurologie, dyspepsie fonctionnelle, ophtalmologie…). La majorité des études portent cependant sur une population MC assez spécifique : les femmes jeunes (20-25 ans) et très peu de données existent dans la population plus âgée ou dans la population masculine. À notre connaissance, aucune étude longitudinale n'a suivi l'évolution de la MC dans le temps. Alors que peu de données permettent de déterminer l'impact de la MC sur la santé du jeune adulte, il semble que nous n'ayons aucun recul à l'heure actuelle sur les conséquences de la MC avec l'évolution de l'âge. Or, selon le concept d'« obesity paradox » chez la population âgée, l'excès pondéral pourrait être paradoxalement lié à un risque de mortalité plus faible (Oreopoulos et al. 2009). Un IMC inférieur à 25 kg/m² chez les femmes et hommes âgés serait ainsi associé à une mortalité augmentée (Kvamme et al. 2012). Nous connaissons également la courbe en J de l'association entre IMC et mortalité toutes causes confondues suite à l'étude de 1.46 million d'adultes âgés de 19 à 84 ans, montrant qu'un faible IMC est lié à un risque relatif de mortalité augmenté (Berrington de Gonzalez et al. 2010). Bien que de nombreuses limitations puissent être avancées, ces observations interrogent sur les conséquences de la MC à long terme. D'autre part, une faible densité minérale osseuse (DMO) (Galusca et al. 2008; Fernández-García et al. 2009; Hasegawa et al. 2011), une faible masse osseuse et un risque fracturaire accru ont été observés chez les sujets MC, malgré un remodelage osseux apparemment normal (Galusca et al. 2008, 2016). Considérant l'histoire naturelle de perte de masse osseuse avec le vieillissement, le risque d'ostéoporose pourrait être plus important dans cette population, même si cela reste à prouver. Nous retiendrons donc la difficulté de conclure quant aux potentielles conséquences sur la santé à court et long terme de la MC au regard du

faible nombre d'études et de leurs disparités. Néanmoins, plusieurs éléments encouragent la nécessité de soulever cette question.

1.3.4. Maigreur : bien-être et société

Au-delà du motif habituel de consultation de l'individu MC concernant son inquiétude en termes de santé, la personne fait généralement part d'un mal-être vis-à-vis de sa maigreur excessive, tant d'un point de vue personnel que sociétal. Le souhait de grossir fait presque systématiquement l'objet de la consultation médicale (Bossu et al. 2007; Germain et al. 2007; Santonicola et al. 2012; Estour et al. 2013; Galusca et al. 2015; Gunes et al. 2016). La difficile image de soi impliquée par la forte maigreur entraîne parfois une souffrance psychologique réelle chez le sujet MC, qui rapporte la difficulté d'assumer cette maigreur extrême envers soi-même mais également envers les autres. Au-delà de cette forte maigreur souvent vécue comme un complexe par le patient, s'ajoute une forme de handicap sociétal due à une forte stigmatisation décrite par cette population (Estour et al. 2013). Si dans l'enfance ou l'adolescence, filles comme garçons, peuvent subir des moqueries de leurs camarades ou l'inquiétude de l'infirmière scolaire vis-à-vis de leur maigreur, la situation ne s'améliore pas forcément à l'âge adulte. Les femmes doivent particulièrement lutter contre une stigmatisation de la société et parfois même du corps médical, les classant dans l'AM ; ce qui est d'autant plus complexe que le déni est l'un des traits caractéristiques de l'AM (American Psychiatric Association 2013; Estour et al. 2013).. Il s'avère difficile pour le patient MC de se faire entendre tant le vieil adage « quand on mange on a un poids normal » reste présent (Estour et al. 2018). Cette méconnaissance de la MC peut engendrer de lourdes conséquences psychosociales mais également professionnelles avec un frein à l'embauche ou à la titularisation : nous citerons l'exemple d'une femme MC s'étant vue refuser un emploi en raison de sa supposée anorexie (expérience clinique) (Estour et al. 2012, 2018). Face à ce constat, il semble primordial de communiquer et de justifier l'existence même de la MC tant dans la sphère sociétale que médicale (Estour et al. 2013).

D'autre part, il semble important de souligner que la MC ne toucherait pas davantage les femmes que les hommes, bien que la plupart des investigations cliniques actuelles aient été effectuées chez des femmes. Il est possible que cette prédominance d'études menées chez les femmes soit liée aux études utilisant la MC en tant que groupe « contrôle de sous poids », comparativement au groupe de patientes souffrant d'AM ; la prévalence de l'AM étant nettement supérieure chez les femmes que chez les hommes (Hoek 2006). Cette prédominance d'études menées chez les femmes pourrait également illustrer un taux de consultation plus faible chez les hommes que chez les femmes MC.

Ainsi que nous l'avons observé à travers cette première approche contextuelle de la MC et comme le montre la **Figure 2**, peu d'investigations ont été menées à l'heure actuelle sur l'existence, les causes et les conséquences de la MC. Pourtant, cette méconnaissance de la MC entraîne non seulement une incapacité à accompagner et à conseiller au mieux cette population, mais aboutit également à des difficultés quant au diagnostic d'une MC.

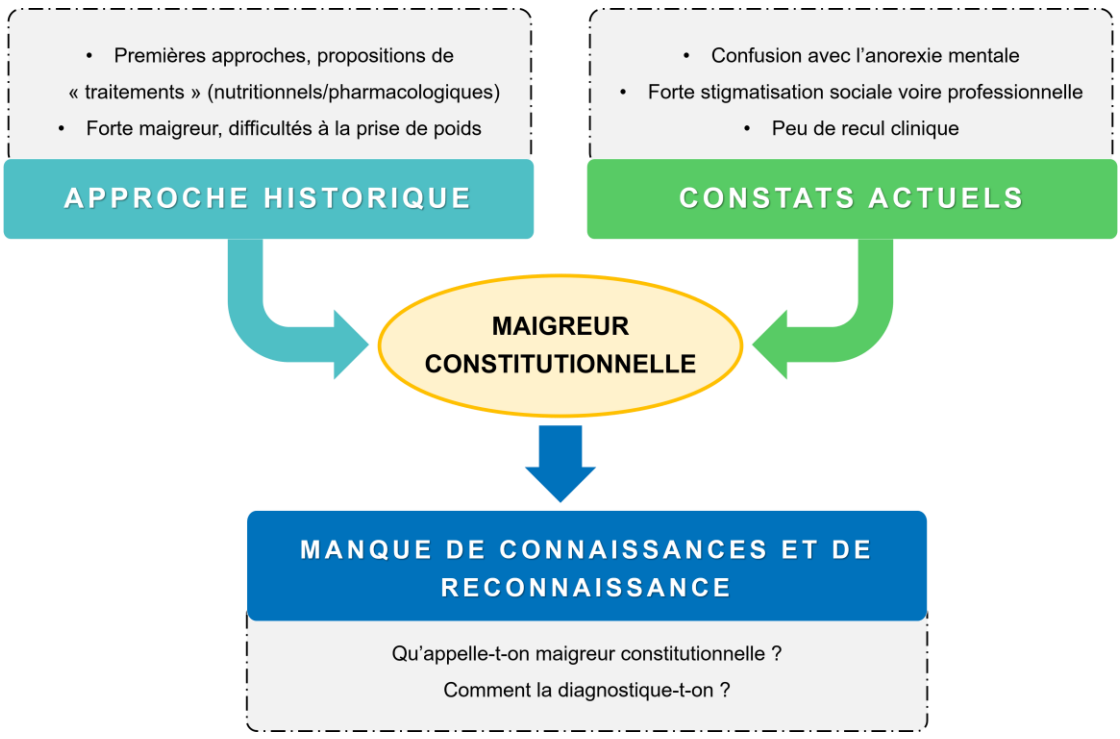


FIGURE 2 – DES CONSTATS HISTORIQUES ET ACTUELS VERS UNE APPROCHE DIAGNOSTIC

2. DIAGNOSTIC DE LA MAIGREUR CONSTITUTIONNELLE

À notre connaissance, la MC est si peu connue et par conséquent, diagnostiquée, que sa prévalence reste difficile à établir à ce jour (Estour et al. 2014). Néanmoins, Orthofer rapporte que 881 personnes sur les 47 102 personnes (femmes et des hommes confondus) de l'« Estonian Genome Center of the University of Tartu (EGCUT) cohort » présenteraient une MC (exclusion des troubles psychologiques de prise alimentaire, des pathologies ou traitements affectant le système endocrinien, des cas de grossesse, et des athlètes professionnels) (Orthofer et al. 2020). Pour Orthofer et collaborateurs, la cohorte EGCUT (Leitsalu et al. 2015) serait l'une des plus grandes et mieux phénotypées au monde (Orthofer et al. 2020). À partir de ces données, nous avons ainsi calculé une prévalence de la MC de 1.9 % (femmes et hommes confondus). D'après l'Organisation Mondiale de la Santé (OMS), la prévalence de la maigreur, mais cette fois-ci « toutes causes confondues », pour la France serait de 2.7 % pour les femmes et de 0.4 % pour les hommes (World Health Organization 2020).

Si la prévalence globale de la MC est difficile à établir, la proportion de femmes et hommes MC apparaît encore plus complexe à définir. Néanmoins, parmi les 1 622 participants MC de la cohorte « Study Into Lean and Thin Subjects » (STILTS) (Riveros-McKay et al. 2019), 1 325 étaient des femmes et 297 des hommes, ce qui correspond à 81.7 % de femmes et 18.3 % d'hommes parmi ces sujets MC. Ces chiffres peuvent donner une estimation très approximative de la proportion de femmes et d'hommes MC, mais restent à relativiser vis-à-vis de potentiels facteurs pouvant influencer le genre à l'inclusion (Riveros-McKay et al. 2019). Parmi les 881 individus MC de la cohorte EGCUT, un tiers est représenté par les hommes et deux tiers par les femmes (Orthofer et al. 2020). Si une proportion importante d'hommes MC semble exister, les études cliniques réalisées jusqu'ici sont très majoritairement conduites chez les femmes. Quelques études ont inclus des sujets MC des deux genres ; mais seules trois études, à notre connaissance, ont été menées chez une population d'hommes MC exclusivement (Diaz et al. 1992; Mazzeo et al. 2004; Marra et al. 2019).

2.1. Définition et sémantique de la maigreur

2.1.1. Ambiguïté concernant la composition corporelle

Dans l'encyclopédie *Larousse*, la « maigreur » est définie comme l'« état de quelqu'un, d'un animal, qui est maigre, sans graisse, ni chair » (Larousse 2020a) et l'adjectif « maigre » réfère à quelqu'un « qui a peu de chair et de graisse sur les os » (Larousse 2020b). La « chair » est définie comme un « tissu musculaire et conjonctif du corps humain et animal que recouvre la peau » (Larousse 2020c). L'encyclopédie Larousse complète sa définition de la maigreur ainsi : « on appelle maigre un sujet chez lequel le rapport masse grasse/poids corporel est inférieur à 10 % chez l'homme et à 14 % chez la femme » (Larousse 2020a). Ces éléments témoignent d'une dualité : la maigreur réfère-t-elle à la notion de masse corporelle « totale », ou bien plus spécifiquement à l'absence de « masse grasse » et donc implicitement à une forte proportion de masse maigre (MM) ? Tel que l'avaient déjà soulevé Guy-Grand et Basdevant, la définition même de la maigreur est équivoque (Guy-Grand and Basdevant 1982). Dès 1982, ces auteurs constatent qu'il y aurait deux définitions de la maigreur : l'insuffisance pondérale « globale » ou bien l'insuffisance de masse grasse uniquement. Pour Guy-Grand et Basdevant, l'insuffisance pondérale est définie par un poids inférieur à 10-20 % du poids idéal théorique pour la taille, et l'insuffisance de masse grasse correspond, comme pour l'encyclopédie *Larousse* ou la définition proposée par Apfelbaum et Sachet (Apfelbaum and Sachet 1982), à une masse grasse inférieure à 14 % chez la femme et 10 % chez l'homme (Guy-Grand and Basdevant 1982). Les auteurs soulignent que l'équivalence entre ces deux définitions nécessite d'être débattue (Guy-Grand and Basdevant 1982). Selon eux, la définition correspondant à l'insuffisance « pondérale » semble être plus fréquemment utilisée. Ils mettent également en avant que cette définition basée sur l'insuffisance pondérale ne donne alors aucune indication sur la composition corporelle, et soulèvent avec pertinence que même en présence d'un pourcentage de masse grasse faible, un individu peut également présenter une diminution de MM absolue. Cette dualité de sens se retrouve également dans une notion de dynamique : la perte de poids n'est pas synonyme de l'amaigrissement qui, *stricto sensu*, réfère au fait de devenir

plus maigre, et donc finalement à une perte en masse grasse. On différenciera également la « dénutrition » se rapportant à des carences alimentaires et des besoins énergétiques non couverts de la « malnutrition » qui renvoie à trouble qualitatif plus que quantitatif.

2.1.2. Ambiguïté concernant le degré de gravité

En société, la notion de MC est parfois interprétée comme une légère minceur plus que comme une maigreur réelle avec des IMC similaires à ceux de patients AM. Cette ambiguïté se retrouve également dans la littérature scientifique anglophone. L'utilisation des mots clés tels que « thinness » ou « leanness » dans les moteurs de recherche bibliographique mènent à de nombreuses publications dans lesquelles les « lean groups » désignent en réalité des groupes de participants normo-pondérés servant de groupe contrôle « mince » comparativement à des sujets présentant une obésité. Dans la littérature scientifique, le terme « lean » est très fréquemment utilisé pour qualifier un groupe témoin de participants « minces » par opposition à un groupe de participants présentant une obésité. Ces « lean groups » présentent alors un IMC dans les normes (18.5 – 25 kg/m²) et ne constituent donc pas un groupe de sujets MC. On note une absence de terminologie majeure dans la littérature scientifique : la MC est généralement désignée en tant que « constitutional thinness » (minceur constitutionnelle) (Tolle et al. 2003; Ling et al. 2016; Galusca et al. 2018), avec l'expression « persistent/healthy thinness » (minceur persistante/saine) (Slof et al. 2003; Mazzeo et al. 2004; Riveros-McKay et al. 2019) ou bien de façon plus ambiguë par l'appellation « constitutional leanness » (maigreur constitutionnelle) (Marra et al. 2007, 2009, 2009; Pasanisi et al. 2013; Florent et al. 2019), mais il existe également un grand nombre d'études qui n'utilisent aucune terminologie référant au moins à la notion d'une maigreur constitutionnelle/persistante (van Binsbergen et al. 1990; Scalfi et al. 1992; Hinney et al. 1997; Bosy-Westphal et al. 2004; Fernández-García et al. 2009) alors que la description de leur groupe en sous poids correspond à celle de sujets MC. D'autres études au contraire mentionnent une « constitutional thinness » alors que le diagnostic de leur groupe de MC apparaît discutable (Slof et al. 2003; Mazzeo et

al. 2004). Cette terminologie relative à la MC est ambiguë et plusieurs articles n'utilisent pas de désignation particulière pour la MC. Cela soulève un problème majeur quant au référencement, et donc au partage, des études menées sur la MC, ce qui renforce et favorise encore davantage la méconnaissance de la MC.

2.2. Diagnostic étiologique de la maigreur

2.2.1. Apports des premiers travaux

Dès les premières publications discutant la MC, celle-ci est caractérisée comme un état de maigreur important malgré des apports caloriques suffisants et un état général apparent de bonne santé (Grafe 1933; Passmore et al. 1955a; Genest et al. 1955; Keidan 1955; Apfelbaum and Sachet 1982). En 1982, Guy-Grand et Basdevant consacrent un article à l'étiopathogénie de la maigreur et au diagnostic d'une MC. Ils y développent l'intérêt de dissocier la maigreur dite « constitutionnelle » non pathologique de la maigreur dite « acquise » (Guy-Grand and Basdevant 1982). La MC y est décrite comme un état de conservation voire d'augmentation des apports alimentaires par opposition aux cas de maigreur causée par une réduction des apports. Parmi ces cas de réduction d'apports alimentaires, ils listent différentes origines possibles telles que : socio-économiques, psychologiques, gastro-intestinales, pathologiques ou d'intoxication telles que décrites sur le **Tableau 1**.

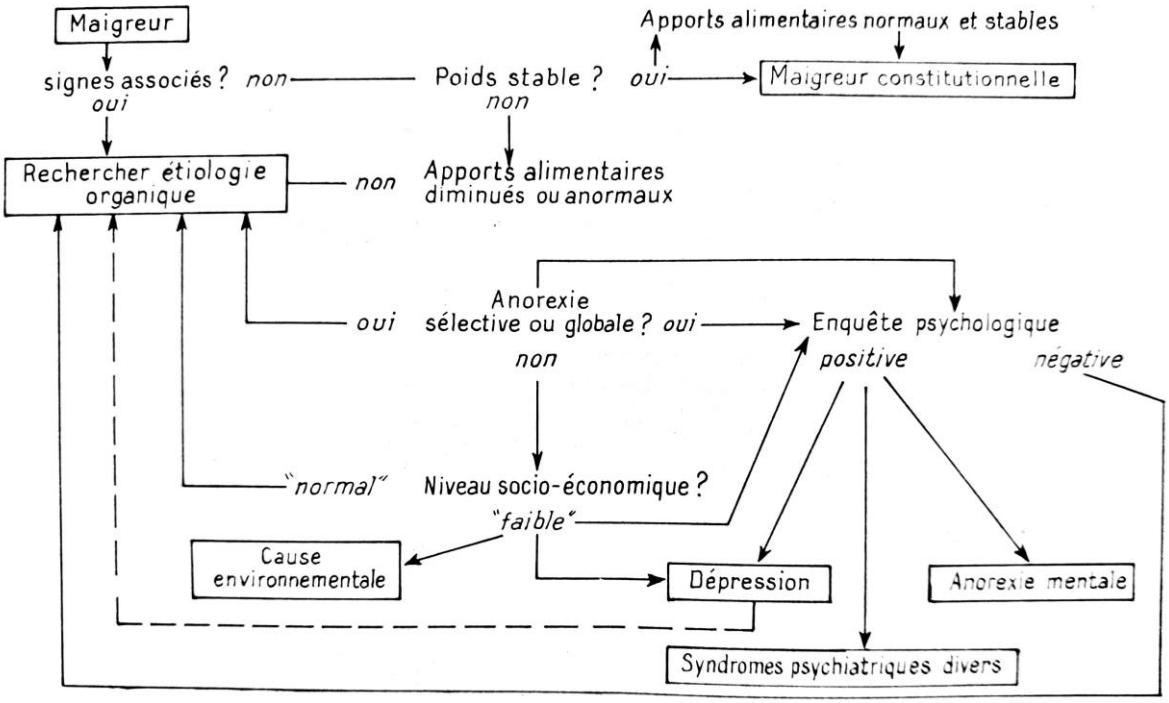
TABLEAU 1 – PRINCIPALES CAUSES DES MAIGREURS

Conservation ou augmentation des apports	Réduction des apports
Maigreur constitutionnelle	Origine socio-économique : - Famine - Marginaux - Vieillards
Augmentation du - Catabolisme - Hyperthyroïdie - Hyperactivité anxieuse ou maniaque	Origine psychologique : - Dépression - Anorexie mentale - Régimes déséquilibrés - Syndromes psychiatriques
Médicaments : - Extraits thyroïdiens	Origine gastro-intestinale : - Ulcère, gastrectomie - Malabsorption - Obstruction (brides, cancers) - Maladies hépato-biliaires
Pertes digestives : - Entéropathies exsudatives - Stéatorrhées (sprue) - Fistules digestives - Parasitoses	Maladies générales : - Cancers et leucémies - Maladies infectieuses - Viroses tuberculose - Insuffisance rénale chronique - Endocrinopathies (insuffisance hypophysaire, surrénalienne) - Maladies métaboliques avec hypercalcémie ou hypokaliémie
	Intoxications : - Saturnisme - Alcoolisme - Drogues

Issu de publication (Guy-Grand and Basdevant 1982)

Les auteurs insistent également sur l'importance de procéder à l'anamnèse pondérale lors de l'examen du sujet maigre. Ils précisent qu'un sujet MC présente une stabilité de son statut pondéral (équilibre nutritionnel) qui s'oppose à d'autres types de maigreur dus à une perte de poids ou à un amaigrissement, récent ou non. Cette notion d'histoire pondérale faisant état d'une maigreur stable et présente depuis l'enfance/adolescence est largement appuyée par Apfelbaum et Sachet qui décrivent effectivement une courbe pondérale ayant toujours été basse (Apfelbaum and Sachet 1982). Guy-Grand et Basdevant relèvent également la nécessité de procéder à une enquête alimentaire sur les aspects quantitatifs mais également qualitatifs dans l'examen d'une maigreur. Finalement, les auteurs proposent le premier

schéma de diagnostic étiologique de la maigreur, illustré sur la **Figure 3** ci-dessous (Guy-Grand and Basdevant 1982).



Issu de publication (Guy-Grand and Basdevant 1982)

FIGURE 3 – SCHEMA DE DIAGNOSTIC ETIOLOGIQUE D'UNE MAIGREUR

Bien que ce schéma puisse présenter certaines limites, il reste, à notre connaissance, le seul à ce jour existant dans la littérature scientifique de la MC et prend en compte différents éléments de diagnostic en évaluant la présence ou l'absence de : symptômes associés à la maigreur, stabilité pondérale, apports alimentaires normaux, difficultés au niveau socio-économique, présence ou non de désordres psychologiques liés à la prise alimentaire (Guy-Grand and Basdevant 1982). En effet, si certaines causes de maigreur sont facilement identifiables, le diagnostic différentiel entre un cas d'AM et de MC peut s'avérer complexe et délicat.

2.2.2. Diagnostic différentiel : anorexie mentale et maigreur constitutionnelle

Les enjeux du diagnostic différentiel entre AM et MC sont grands et pourtant l'existence même de la MC reste parfois contestée par certains experts qui affirment qu'elle n'existe pas et correspondrait à des patients AM étant parvenus à tromper le corps médical. De nombreuses études cliniques ont pourtant rapporté des différences physiologiques significatives entre AM et MC (Bossu et al. 2007; Marra et al. 2007, 2009, 2019; Galusca et al. 2008, 2012, 2015; Estour et al. 2017). Ce type d'affirmation montre néanmoins la nécessité d'étayer la littérature scientifique faisant preuve de l'existence de la MC et de sa distinction avec l'AM. Deux publications se sont spécifiquement intéressées au diagnostic différentiel entre MC et AM (Estour et al. 2014, 2017). La revue publiée par Estour et ses collaborateurs en 2014 montre un profil hormonal très différent entre AM et MC, ainsi qu'en atteste le **Tableau 2**. Tandis que le bilan biochimique des individus MC ne présente pas d'anomalies majeures, les patientes AM montrent généralement les signes biochimiques d'une carence alimentaire avec des taux diminués de free triiodothyronine (FT3), leptine, insulin-like growth factor-1 (IGF-1), estradiol, luteinizing hormone (LH), follicle-stimulating hormone (FSH) et augmentés de growth hormone (GH), cortisol et ACTH (**Tableau 2)**. Si l'on peut imaginer un biais potentiel de « tromperie » de la part d'un patient AM sur des tests psychologiques ou d'enquête alimentaire, un bilan sanguin n'est cependant pas manipulable, ce qui réfuterait ainsi la thèse selon laquelle la MC n'existerait pas et correspondrait à une erreur de diagnostic d'AM.

TABLEAU 2 – DIFFERENCES PSYCHIATRIQUES, HORMONALES ET DE BALANCE ENERGETIQUE ENTRE ANOREXIE MENTALE ET MAIGREUR CONSTITUTIONNELLE

Anorexia nervosa	Constitutional thinness
BMI ≤ 17,5 kg/m²	
Presence of eating disorders	No eating disorders; de-restriction
Psychological disorders	No psychological disorders
Amenorrhea	Physiological menses
Hormonal abnormalities= undernutrition T3 ↓, leptin ↓, IGF-1 ↓, GH ↑ Cortisol↑, ACTH ↑, 17 β E2 ↓, LH ↓, FSH ↓	No hormonal abnormalities = no sign of undernutrition
Blunted fat mass	Diminished fat mass
Negative energy balance	Equilibrated / positive energy metabolism
Weight loss / broken weight growth curve	Stable weight within lower percentile of growth curve

Issu de publication (Estour et al. 2014)

Le **Tableau 2** résumerait ainsi les principales différences entre AM et MC. Contrairement au patient AM, la personne MC ne présenterait pas de désordre psychologique de prise alimentaire (Bossu et al. 2007; Germain et al. 2014; Estour et al. 2017), un statut pondéral stable sans amaigrissement (Bossu et al. 2007; Estour et al. 2017) et des règles physiologiques normales (Marra et al. 2007; Pasanisi et al. 2013; Germain et al. 2016b). Soulignons également que la masse grasse serait extrêmement diminuée chez le patient AM, mais le serait moins chez le sujet MC (Germain et al. 2007; Galusca et al. 2012; Estour et al. 2017). La régulation hormonale de l'appétit semblerait également bien différente entre les sujets AM et MC, ainsi que rapporté par différentes études (Tolle et al. 2003; Miljic et al. 2006; Germain et al. 2007, 2009, 2016b). Ces éléments tendent ainsi à démontrer la distinction entre l'AM et la MC. Néanmoins, les résultats sont nombreux et parfois complexes à interpréter. Cela soulève le besoin de mutualiser et de clarifier les différentes données pouvant être utilisées pour le diagnostic différentiel, d'autant que ce diagnostic s'est complexifié en 2013 avec la parution de la cinquième version du « *diagnostic and statistical manual of mental disorders* » (DSM-V). En effet, ce dernier comprend de nombreux changements par rapport à sa version précédente, dont le retrait du critère d'aménorrhée dans le diagnostic de l'AM (American Psychiatric Association 2013). Cela renforce d'autant plus l'importance d'utiliser des critères particulièrement fiables et discriminants entre AM et MC. Si en 2014 Estour publie

un article sur les risques d'erreurs de diagnostic différentiel entre AM et MC, l'auteur publie peu après une étude s'intéressant spécifiquement aux marqueurs biologiques, anthropométriques et psychologiques différenciant la MC de l'AM et des sujets normo-pondérés témoins (T) (Estour et al. 2017). L'analyse révèle notamment que les niveaux de FT3 et de leptine apparaissent fortement spécifiques et sensibles dans la différenciation de la MC par rapport à l'AM (Estour et al. 2017). Dans la mesure où le dosage sanguin en FT3 semble assez accessible et peu coûteux, l'auteur préconiserait, *a minima*, l'évaluation de la FT3 dans le diagnostic d'une MC (Estour et al. 2017). Concernant les paramètres psychologiques, le seul paramètre ayant démontré une réelle capacité de différenciation entre MC et AM serait l'échelle de la restriction alimentaire du Dutch Eating Behavior Questionnaire (DEBQ) (Estour et al. 2017).

2.2.3. Critères de diagnostic actuellement utilisés

Au-delà des éléments de diagnostic permettant de distinguer la MC de l'AM spécifiquement abordés par certaines publications (Estour et al. 2014, 2017), la question du diagnostic complet de la MC demeure importante et complexe. Afin de mieux appréhender les critères de diagnostic considérés à ce jour, nous nous proposons ici de nous intéresser aux critères d'inclusion des participants MC actuellement utilisés dans les études cliniques.

2.2.3.1. Notion de maigreur et fluctuation

Face à une personne présentant une silhouette apparente de maigreur corporelle, deux questions semblent naturellement émerger : comment *évaluer* cette maigreur, et à partir de quel *seuil* peut-on évoquer une « maigreur » ? Si une grande partie des études semblent utiliser un seuil d'IMC pour définir l'état corporel d'une maigreur (Scalfi et al. 1992; Tagami et al. 2004; Galusca et al. 2008), les percentiles d'IMC peuvent également être utilisés (Hinney et al. 1997), ainsi que le pourcentage du poids « idéal » (Schneider et al. 1979; van Binsbergen et al. 1990), ou encore la ressemblance par rapport à des profils

de silhouettes (Slof et al. 2003; Mazzeo et al. 2004). Ces définitions semblent ainsi plutôt référer à la définition de la maigreur en tant qu'insuffisance pondérale. D'autres études considèreraient plutôt la notion de faible masse grasse (Diaz et al. 1992; Margaritelis et al. 2019). Enfin, un certain nombre d'études semblent décrire une situation de MC sans pour autant avoir clairement reporté de « seuil » de maigreur pour l'inclusion des participants MC (Marra et al. 2007; Santonicola et al. 2012; Pasanisi et al. 2013). Outre les divergences de critères utilisés, les seuils utilisés apparaissent également très hétérogènes. Par exemple, le seuil d'IMC utilisé semble beaucoup varier en fonction des études ; allant de 16.5 kg/m² (Bossu et al. 2007; Galusca et al. 2008) à 20.0 kg/m² (Petretta et al. 1997). Si l'histoire pondérale des individus apparaît comme un critère important pour certaines études (Santonicola et al. 2012; Germain et al. 2014), d'autres ne mentionnent pas ce critère (Tagami et al. 2004; Florent et al. 2019). Lorsque la stabilité pondérale des sujets est vérifiée lors de l'inclusion des participants MC, la notion de « stabilité » diffère cependant en fonction des études. Par exemple, Scalfi et collaborateurs vérifient la stabilité du poids dans les 2 ans précédant l'expérimentation (Scalfi et al. 1992), Galusca et collaborateurs requièrent un poids stable tout au long de la période post-pubertaire (Galusca et al. 2018) tandis que Bossu et collaborateurs reconstruisent également l'histoire pondérale du sujet, de sa naissance à ses 18 ans (Bossu et al. 2007). Finalement, une forte hétérogénéité est observée concernant les critères relatifs à la maigreur à l'instant t des sujets, mais également vis-à-vis des potentielles fluctuations passées de cet état de maigreur.

2.2.3.2. Absence de causes évidentes

Un état apparent de bonne santé, sans connaissance d'un quelconque trouble pathologique est souvent mentionné lors de l'inclusion de participants MC (Germain et al. 2009; Riveros-McKay et al. 2019; Marra et al. 2019), bien que d'autres études, cependant, ne requièrent pas clairement l'absence de maladies associées parmi les critères d'inclusion de sujets MC (Tolle et al. 2003; Slof et al. 2003; Fernández-García et al. 2009). L'absence de désordres psychologiques liés à la prise alimentaire est généralement un critère

d'inclusion des participants MC (Tagami et al. 2004; Santonicola et al. 2012; Germain et al. 2014), même si de rares études ne le mentionnent pas (Paschalis et al. 2013; Gunes et al. 2016). Néanmoins, ce critère semble être exprimé de façon plus ou moins explicite dans la littérature avec des outils et des méthodes variés. Certaines études mentionnent simplement une « absence » de troubles alimentaires chez leur participants MC (Schneider et al. 1979; Diaz et al. 1992), d'autres lèvent également l'ambiguïté par la présence d'un groupe AM (Tolle et al. 2003; Germain et al. 2007; Miljic et al. 2007), d'autres expliquent avoir procédé à une interview spécifique de leurs participants MC (Hinney et al. 1997; Slof et al. 2003; Santonicola et al. 2012), d'autres rapportent avoir eu recours à l'utilisation de questionnaires tels que le Three-Factor Eating Questionnaire (TFEQ), le DEBQ, Eating Disorder Inventory (EDI), Eating Disorder Examination (EDE), Body Shape Questionnaire (BSQ) et SCOFF[1] (Germain et al. 2014; Ling et al. 2016; Riveros-McKay et al. 2019; Florent et al. 2019), et certaines études réalisent des bilans sanguins pour vérifier l'absence de carences nutritionnelles (Germain et al. 2014; Ling et al. 2016; Galusca et al. 2018). Pour une proportion importante d'études, les participants MC seraient recrutés parmi des personnes ayant des difficultés à prendre du poids (Germain et al. 2007; Galusca et al. 2008; Estour et al. 2017). La notion de résistance à la prise de poids ne semble cependant pas abordée dans les critères d'inclusion de la majorité des études (Petretta et al. 1997; Hasegawa et al. 2011; Florent et al. 2019). La présence de règles physiologiques serait un critère fréquemment utilisé pour l'inclusion de sujets MC (Bossu et al. 2007; Hasegawa et al. 2011; Margaritelis et al. 2019), même si quelques études ne semblent pas l'avoir considéré (Hinney et al. 1997; Slof et al. 2003; Riveros-McKay et al. 2019). Un volume important d'activité physique, notamment présent chez certains sportifs ou personnes souffrant d'AM, peut également être une cause de déséquilibre de la balance énergétique. Néanmoins, beaucoup d'études ne semblent pas

[1] SCOFF questions: Do you make yourself **S**ick because you feel uncomfortably full? Do you worry you have lost **C**ontrol over how much you eat? Have you recently lost more than **O**ne stone in a 3-month period? Do you believe yourself to be **F**at when others say you are too thin? Would you say that **F**ood dominates your life?

prendre en compte l'activité physique des sujets lors de leur inclusion (Tolle et al. 2003; Germain et al. 2009; Hasegawa et al. 2011). Parmi les études considérant l'activité physique des sujets, la plupart mentionne simplement l'absence d'activités physiques « excessive », sans détailler l'utilisation d'un questionnaire spécifique ou d'une quantification de cette activité (Scalfi et al. 1992; Galusca et al. 2012; Paschalis et al. 2013). Certaines études utilisent le questionnaire « Monica optional study of physical activity » MOSPA (Iqbal et al. 2006) pour vérifier l'absence d'un volume d'activité physique trop important (Germain et al. 2014; Galusca et al. 2015). On constate également des différences entre les études en ce qui concerne le niveau d'activité (fréquence, durée, intensité) étant considéré comme suffisant pour risquer de biaiser le diagnostic de MC. La **Figure 4** illustre les principaux éléments rapportés par la littérature dans cette première approche du diagnostic de la MC.

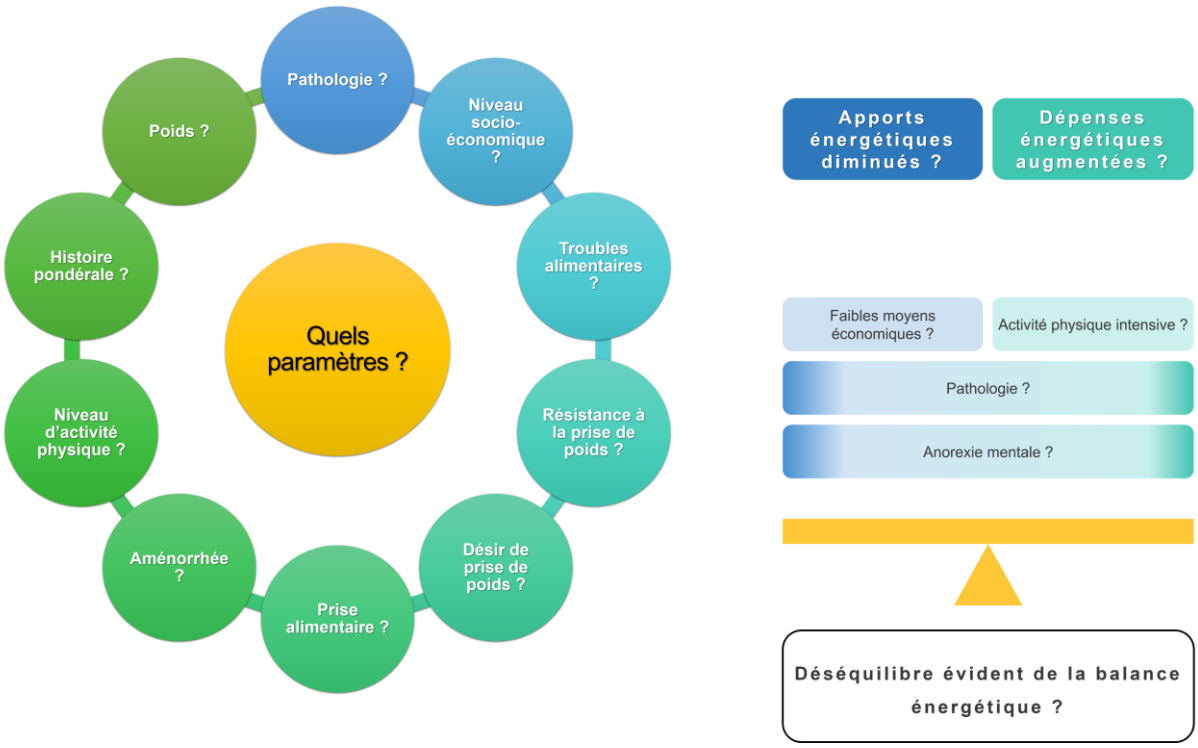


FIGURE 4 – QUESTIONNEMENTS CLASSIQUES LORS D'UNE PREMIERE APPROCHE DU DIAGNOSTIC DE MAIGREUR CONSTITUTIONNELLE

2.2.3.3. Absence de consensus

Au regard de la multiplicité des critères utilisés dans le diagnostic d'une MC, il semble qu'actuellement, la définition et le diagnostic de ce trouble manquent d'harmonie. On constate de nombreuses divergences concernant les critères considérés, les méthodes adoptées pour évaluer ces critères, mais aussi les seuils utilisés. Ce constat soulève la nécessité d'investiguer plus en profondeur la diversité des critères utilisés actuellement pour le diagnostic d'une MC, afin de mieux les identifier, et à terme, d'harmoniser les critères du diagnostic ; ce qui fera l'objet de l'**étude 1** de ce travail de thèse. Un diagnostic reposant sur des indicateurs cliniques fiables et pertinents semble effectivement constituer la première étape primordiale avant toute démarche d'accompagnement médical ou de recherche clinique.

3. PHYSIOLOGIE DE LA MAIGREUR CONSTITUTIONNELLE

Au-delà de multiplicité des critères de diagnostic utilisés lors de l'inclusion de participants MC dans les études, les domaines d'études ainsi que les résultats semblent également refléter une hétérogénéité.

3.1. Balance énergétique

Face à un individu présentant une forte maigreur, il semble que la première question soulevée soit celle de l'équilibre de la balance énergétique.

3.1.1. Apports alimentaires

L'étude de relevés alimentaires semble effectivement confirmer que les apports alimentaires des personnes MC sont similaires à ceux de personnes normo-pondérées témoins (T) (Bossu et al. 2007; Germain et al. 2014; Galusca et al. 2018; Ling et al. 2019) et supérieurs à ceux des patients AM (Bossu et al. 2007) (**Tableau 3**). Plusieurs études rapportent même des valeurs moyennes d'apports énergétiques supérieures chez les personnes MC comparativement à des sujets normo-pondérés T, bien que cela reste non significatif (Germain et al. 2014; Galusca et al. 2018; Ling et al. 2019). Qualitativement, l'apport énergétique apparaît similairement proportionné en pourcentage de lipides, glucides et protéines entre les sujets MC et T (Bossu et al. 2007; Germain et al. 2014; Galusca et al. 2018; Margaritelis et al. 2019). En revanche, les patients AM auraient davantage d'apports protéiques que les sujets MC et T, et moins d'apports lipidiques que les sujets T (Bossu et al. 2007). En terme de répartition de l'apport calorique au cours de la journée, les sujets MC fractionneraient davantage leurs apports et grignoteraient plus que les sujets T (Germain et al. 2014; Ling et al. 2019) en raison d'une satiété rapidement atteinte durant les repas (Estour et al. 2018). Il est cependant à noter que la fiabilité des relevés alimentaires n'est pas connue à ce jour dans cette population, et reste soumise à de possibles biais de reports de la consommation alimentaire (Poslusna et al. 2009; Naska et al. 2017).

TABLEAU 3 – APPORTS ET DEPENSES ENERGETIQUES CHEZ DES FEMMES PRESENTANT UNE MAIGREUR CONSTITUTIONNELLE

	CT (n = 7)	C (n = 7)	AN (n = 6)	P Value
Self-reported food intake, kJ/day	7,565±908	7,961±1,452	4,894±703	a, c
Protein intake, %	13.01±2.2	14.5±19	18.8±5.4	a, c
Carbohydrate intake, %	50.7±7.5	46.4±5.1	50.6±6.6	NS
Lipids intake, %	36.2±6.7	39.0±5.6	30.5±8.1	a
TEE, kJ/day	8,382±988	8,793±845	8,001±2152	NS
RMR, kJ/day	4,839±473	5,576±209	3,810±937	a, b, c
RQ	0.82±0.01	0.83±0.01	0.89±0.02	a, c
FQ	0.85±0.01	0.84±0.01	0.86±0.01	NS
AEE, kJ/day	3,542±464	3,207±410	4,191±967	NS
PAL	1.75±0.12	1.57±0.07	2.14±0.30	NS
TEE/FFM ratio	259.5±40.6	208.0±29.5	234.4±69.5	b
RMR/FFM ratio	148.6±5.4	131.8±10.4	111.3±25.0	b, c

Valeurs présentées en tant que moyennes ± SD. CT (sujets maigres constitutionnels), C (sujets témoins), AN (patients présentant une anorexie mentale), TEE : dépense énergétique totale, RMR : dépense énergétique de repos, RQ : quotient respiratoire, FQ : quotient alimentaire, AEE (TEE – RMR) : dépense énergétique liée à l'activité, PAL (TEE/RMR) : niveau d'activité physique, FFM : masse maigre
[a] $p<0.05$ C vs. AN, [b] $p<0.05$ C vs. CT, [c] $p<0.05$ AN vs. CT, NS : non significatif

Issu de publication (Bossu et al. 2007)

Par ailleurs, la littérature a soulevé l'hypothèse d'une mauvaise absorption lipidique qui pourrait expliquer l'état de maigreur de la population MC (Germain et al. 2014; Ling et al. 2016). Néanmoins, une étude semble avoir réfuté cette hypothèse en ne montrant pas de signe de stéatorrhée chez les personnes MC (Ling et al. 2019).

L'utilisation de questionnaires évaluant le contrôle cognitif relatif à la prise alimentaire révèle également des résultats intéressants. Par exemple, l'utilisation du DEBQ et de l'EDE (Germain et al. 2014; Estour et al. 2017) indique que les sujets MC semblent présenter des scores de restriction cognitive plus faibles que les sujets T (Germain et al. 2014; Estour et al. 2017) ; la restriction cognitive étant définie par Herman et Polivy comme un effort cognitif soutenu afin de lutter contre l'envie de manger dans une optique de contrôle du poids corporel (Herman and Polivy 1980). Cette observation semble cohérente puisque les sujets MC ne chercheraient pas à restreindre leur prise alimentaire, dans la mesure où ils désireraient au contraire plutôt prendre du poids. Le questionnaire EDE montre également une préoccupation pour le

poids et l'alimentation plus faible chez les sujets MC que chez les patients AM, et similaire entre les participants MC et T (Estour et al. 2017) ; ce qui tend à montrer l'absence de régulation psychologique et cognitive de la prise alimentaire chez l'individu MC. Au contraire, les patients AM présentent effectivement un profil psychologique témoignant d'une forte préoccupation pour le poids et l'alimentation (Estour et al. 2017). Le questionnaire EDI montre également une faible préoccupation envers la quête de minceur chez les sujets MC, contrairement aux patients AM (Estour et al. 2017). Ainsi, malgré un nombre d'études relativement faible, la littérature semble relativement consensuelle concernant les apports alimentaires et le profil psychologique chez les sujets MC comparativement aux sujets T et AM. Les sujets MC semblent présenter des apports alimentaires quantitativement et qualitativement normaux malgré un grignotage important mais ne présenteraient pas de distorsion quelconque concernant l'attitude envers l'alimentation, si ce n'est une faible restriction cognitive alimentaire pouvant s'interpréter aisément vis-à-vis de leur résistance à la prise de poids.

De façon intéressante, une étude s'est également intéressée à l'évaluation de la dyspepsie fonctionnelle (sensations de digestion difficile) chez des sujets MC comparativement à des sujets T et des patients présentant des troubles psychologiques liés à la prise alimentaire (Santonicola et al. 2012). Des symptômes de détresse postprandiale (symptômes liés au repas tels que sensation de plénitude, de satiété rapide) serait rapporté chez 56 % des sujets MC contre 18 % des sujets T, 83 % des sujets boulimiques, 90 % des patients AM et 90 % des sujets présentant un autre désordre du comportement alimentaire. Cette étude semble donc montrer qu'un sujet MC sur deux ressentirait un inconfort à la fin du repas, ce qui semble dû à une satiété plus rapidement atteinte chez les sujets MC *vs.* T (Santonicola et al. 2012). Cette satiété vite atteinte pourrait laisser envisager que l'important grignotage présent chez les sujets MC constituerait un mécanisme de compensation (Germain et al. 2014; Ling et al. 2019). Les sujets MC ne présenteraient cependant pas ou peu de sensations de plénitude gastrique post prandiale gênante, de même que pas ou peu de douleurs épigastriques, contrairement aux patients AM

(Santonicola et al. 2012). L'étude rapporte également une absence de nausées ou de brûlures d'estomac chez les sujets MC. Finalement, bien que les résultats de cette étude soient complexes à interpréter en raison de l'absence de certains tests statistiques, il semblerait que les sujets MC ne présentent pas ou peu de dyspepsie fonctionnelle, excepté une satiété rapidement atteinte durant les repas (Santonicola et al. 2012).

3.1.2. Dépense énergétique

Contrairement à l'étude des apports alimentaires, les observations concernant la dépense énergétique des sujets MC divergent, y compris en termes de dépense énergétique totale (DET). L'étude de la DET en utilisant la méthode de l'eau doublement marquée semble montrer des valeurs similaires entre les sujets MC (2 003 ± 236 (SD) kcal/jour) et T (2 102 ± 202 (SD) kcal/jour) (Bossu et al. 2007) (**Tableau 3**). L'estimation de la DET, calculée en tant que produit entre la DER mesurée par calorimétrie indirecte et le niveau d'activité physique (NAP) mesuré par accélérométrie, montrerait également une DET similaire entre sujets MC et T (Germain et al. 2014). En revanche, une autre étude ayant mesuré la DET en chambre calorimétrique a rapporté une DET inférieure chez les sujets MC (1 820 ± 244 (SD) kcal/jour) *vs.* T (2 180 ± 284 (SD) kcal/jour) (Ling et al. 2019).

Le métabolisme de repos a également été investigué dans plusieurs études dont les résultats divergent. Si certaines études observent une DER (absolue) similaire entre les sujets MC et T (Scalfi et al. 1992; Petretta et al. 1997; Marra et al. 2007), d'autres rapportent une DER inférieure chez les sujets MC (Bosy-Westphal et al. 2004; Bossu et al. 2007; Hasegawa et al. 2011; Germain et al. 2014; Estour et al. 2017; Ling et al. 2019) (**Tableau 3**). La comparaison de la DER (absolue) entre les sujets MC et AM semble légèrement moins discordante. La plupart des études montrent une DER supérieure chez les sujets MC par rapport aux patients AM (Petretta et al. 1997; Marra et al. 2007, 2019; Pasanisi et al. 2013), bien qu'une étude n'observe pas de différence (Scalfi et al. 1992). Considérant que la masse musculaire

explique en grande partie la DER (Bogardus et al. 1986), la faible DER des sujets MC pourrait s'expliquer par la faible MM de ces sujets (Bossu et al. 2007; Marra et al. 2009), et justifiant dès lors l'intérêt d'explorer le ratio entre le DER et la MM. Les résultats de DER/MM diffèrent entre les études, et le genre pourrait également influencer les résultats. Chez les femmes, le ratio DER/MM apparaît similaire entre les sujets MC et T pour la plupart des études (Marra et al. 2007; Hasegawa et al. 2011; Germain et al. 2014; Galusca et al. 2018; Ling et al. 2020), excepté pour une étude où ce ratio DER/MM a été trouvé plus élevé chez les sujets MC (35.5 ± 1.3 (SD) kcal/jour/kg) (Bossu et al. 2007) (**Tableau 3**). Les deux études ayant investigué ce ratio chez des hommes MC ont montré une DER/MM plus élevée chez les sujets MC que T (Marra et al. 2019; Ling et al. 2020). Les résultats des études paraissent plus homogènes en ce qui concerne la comparaison des groupes MC *vs.* AM. Les sujets MC semblent montrer un ratio DER/MM plus élevé que les patients AM (Bossu et al. 2007; Marra et al. 2007, 2019; Pasanisi et al. 2013). Concernant la nature des substrats oxydés au repos, l'étude du quotient respiratoire (QR) ne semble pas montrer de différence entre les sujets MC, T et AM (Bossu et al. 2007; Marra et al. 2007, 2019; Germain et al. 2014; Galusca et al. 2018).

La dépense énergétique liée à l'activité physique apparaît très peu investiguée. Elle semblerait similaire pour les sujets MC et T (Bossu et al. 2007; Germain et al. 2014; Galusca et al. 2018) et aurait tendance à être inférieure chez les MC (847 ± 111 (SD) kcal/jour) comparativement aux sujets AM (1 002 ± 231 (SD) kcal/jour) (Bossu et al. 2007) (**Tableau 3**). Le NAP est également très peu exploré. Une étude montre qu'il serait inférieur chez les sujets MC comparativement aux sujets T (Ling et al. 2019), tandis que d'autres études semblent montrer un NAP similaire entre les sujets MC et T (Bossu et al. 2007; Galusca et al. 2018). Les sujets MC pourraient avoir tendance à présenter un plus faible NAP que les patients AM (Bossu et al. 2007).

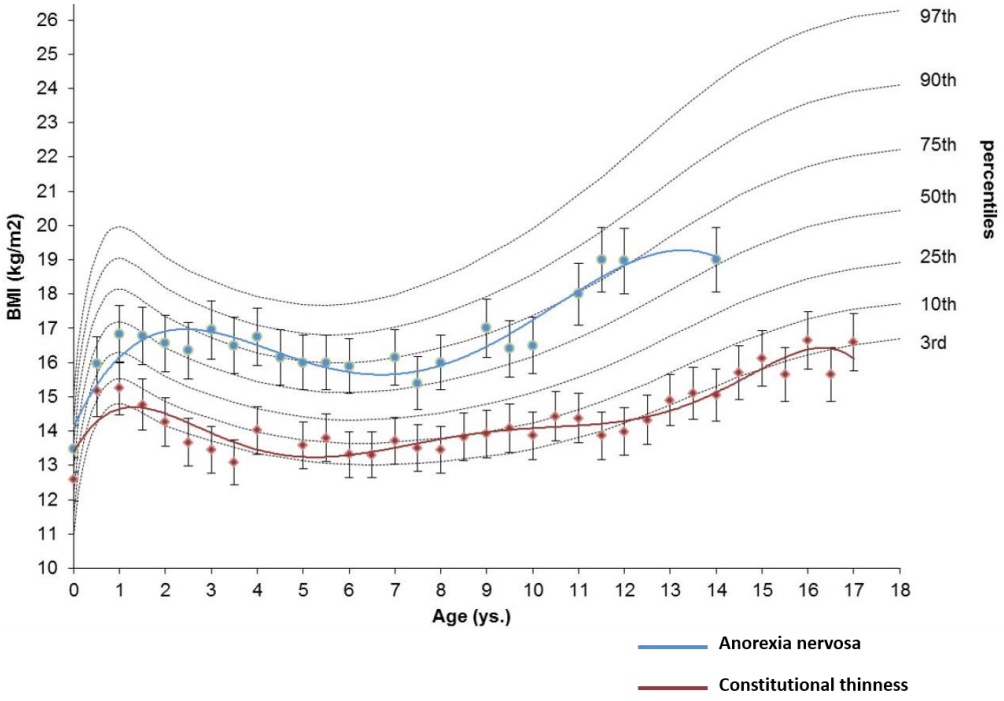
Finalement, l'évaluation du profil énergétique de la MC ne semble pas, pour l'instant, expliquer l'état de maigreur la caractérisant. Face à l'absence d'une explication évidente, plusieurs investigations scientifiques se sont tournées vers des paramètres plus spécifiques. Une étude s'est notamment intéressée à la réponse thermique suivant une prise alimentaire et semble montrer une thermogenèse post-prandiale diminuée chez les sujets MC par rapport au groupe T, mais également par rapport au groupe AM (Scalfi et al. 1992). Une autre étude s'est focalisée sur l'exploration de l'activité du tissu adipeux brun chez des personnes MC (Pasanisi et al. 2013). Cette étude révèle la présence d'une activité de ce tissu parmi les 7 sujets MC participant à l'étude, tandis que seuls 3 sujets parmi les 24 sujets T de l'étude présentent une activité du tissu adipeux brun. De plus, cette activité serait plus élevée chez les 7 sujets MC que chez les 3 sujets T chez lesquels une activité avait été détectée (Pasanisi et al. 2013). La notion d'une énergie dépensée sous l'effet de l'agitation non liée à des déplacements dans l'espace, nommée « fidgeting » dans la littérature scientifique, peut également constituer une dépense d'énergie particulière. Les sujets MC sembleraient effectivement présenter davantage de « fidgeting » que des sujets T et AM (Marra et al. 2007).

D'autre part, une étude s'est plus spécifiquement intéressée aux aspects structurels et fonctionnels hypothalamiques chez des patients AM avec un groupe contrôle T, mais également avec un groupe contrôle MC (Florent et al. 2019). L'étude identifie des dysfonctionnements neurochimiques hypothalamiques ainsi que des différences structurelles chez les patients AM. Ces observations ne sont pas retrouvées chez le groupe contrôle T, mais ne sont pas non plus retrouvées chez le groupe MC. Dans la mesure où l'hypothalamus contient des systèmes intégratifs impliqués dans la balance énergétique notamment, ces résultats renforcent la distinction entre l'AM et la MC ainsi que l'absence de désordres majeurs apparents de la balance énergétique des sujets MC (Florent et al. 2019).

3.2. Anthropométrie et composition corporelle

3.2.1. Anthropométrie

Les participants MC présentent bien sûr un poids corporel largement inférieur aux personnes normo-pondérées (Bossu et al. 2007; Germain et al. 2014; Galusca et al. 2018). La plupart des études observent par ailleurs un sous-poids similaire entre les participants MC et AM (Germain et al. 2007, 2016b; Marra et al. 2007, 2019; Pasanisi et al. 2013). En termes de valeurs, les patients AM de ces études présentent des valeurs moyennes de poids légèrement en-dessous de celles observées pour les participants MC, mais sans différence significative ; ce qui est probablement dû à un biais de sélection lié à un appariement pondéral des sujets MC et AM à l'inclusion. *A priori*, le plus faible poids des sujets MC ne provient pas d'une taille inférieure puisqu'elle semble similaire entre les individus MC, T et AM (Scalfi et al. 1992; Galusca et al. 2008; Germain et al. 2009). Ainsi, les observations concernant l'IMC sont similaires à celles que nous venons de mentionner concernant le poids des sujets. L'ensemble des études rapportent ainsi un IMC largement inférieur chez les sujets MC comparativement aux sujets T (Tolle et al. 2003; Marra et al. 2007; Estour et al. 2017). Les résultats montrent généralement un IMC similaire entre les sujets MC et AM (Galusca et al. 2008; Germain et al. 2009; Pasanisi et al. 2013) (possiblement dû à un biais d'appariement sur le poids) ou parfois supérieur chez les sujets MC vs. AM (Scalfi et al. 1992; Miljic et al. 2006; Galusca et al. 2015). En ce qui concerne l'évolution dans le temps du statut pondéral, les études semblent montrer que l'IMC moyen des individus MC est toujours resté autour du 3ème percentile pendant la croissance et jusqu'à l'âge de 18 ans, contrairement aux patients AM chez qui l'IMC évolue autour du 50ème percentile avant l'apparition de la maladie (Bossu et al. 2007; Estour et al. 2017), ainsi que présenté sur la **Figure 5** ci-dessous. Le rapport entre tour de taille et tour de hanche serait similaire entre les sujets MC et T (Galusca et al. 2018; Ling et al. 2019). Ainsi, la littérature semble être plutôt consensuelle en ce qui concerne la comparaison entre les sujets MC et T, mais apparaît cependant plus contrastée vis-à-vis de la comparaison avec l'AM.



BMI : indice de masse corporelle

Issu de publication (Estour et al. 2017)

FIGURE 5 – COURBES DE CROISSANCE DES SUJETS MAIGRES CONSTITUTIONNELS ET DE PATIENTS SOUFFRANT D'ANOREXIE MENTALE

3.2.2. Masse grasse et masse maigre

La majorité des études semblent montrer que les sujets MC ont des pourcentages de masse grasse inférieurs à ceux de sujets normo-pondérés T (Tagami et al. 2004; Bossu et al. 2007; Galusca et al. 2008; Marra et al. 2009, 2019; Hasegawa et al. 2011; Paschalis et al. 2013; Germain et al. 2014; Estour et al. 2017; Margaritelis et al. 2019; Ling et al. 2019) (**Tableau 4**). Néanmoins, quelques études rapportent un pourcentage de masse grasse similaire entre les sujets MC et T (Tolle et al. 2003; Bosy-Westphal et al. 2004; Germain et al. 2016b). Comparativement aux patients AM, les sujets MC présenteraient des pourcentages de masse grasse supérieurs (Tolle et al. 2003; Bossu et al. 2007; Germain et al. 2007, 2009; Marra et al. 2007; Galusca et al. 2008, 2012, 2015; Fernández-García et al. 2009; Estour et al. 2017) (**Tableau 4**) bien que quelques études ne rapportent pas cette différence (Marra et al. 2009, 2019;

Pasanisi et al. 2013). Au-delà de l'hétérogénéité de significativité de ces résultats, des valeurs très hétérogènes sont rapportées par les études. Il est surprenant de constater que le pourcentage de masse grasse varie de 11.9 % (Pasanisi et al. 2013) à 24.6 % (Galusca et al. 2018) chez les individus MC, de 20.1 % (Paschalis et al. 2013) à 32.9 % (Galusca et al. 2015) chez les normo-pondérés T, et de 7.1 % (Tagami et al. 2004) à 19.2 % (Marra et al. 2009) chez les patients atteints d'AM. Ces écarts peuvent être expliqués par l'utilisation de méthodes d'évaluation de la masse grasse différentes entre les études. Certaines l'estiment par absorptiométrie biphotonique à rayons X (DXA) (Bosy-Westphal et al. 2004; Bossu et al. 2007; Germain et al. 2009; Hasegawa et al. 2011; Galusca et al. 2012; Estour et al. 2017), d'autres par impédancemétrie (Tolle et al. 2003; Tagami et al. 2004; Marra et al. 2007; Pasanisi et al. 2013), et d'autres par la mesure des plis cutanés (Marra et al. 2009; Paschalis et al. 2013; Margaritelis et al. 2019). Cela montre donc l'intérêt de considérer le type de méthode utilisée, d'autant que si la fiabilité de ces trois méthodes de mesures semble plutôt correcte chez le sujet T (Erselcan et al. 2000), elle reste à démontrer chez le sujet MC. Par exemple, une mesure par DXA semble apporter un réel gain de précision chez le sujet obèse (Erselcan et al. 2000), et le même type d'observation pourrait éventuellement exister pour le désordre pondéral opposé qu'est la MC.

Concernant la MM, la littérature rapporte des moyennes inférieures chez les sujets MC par rapport aux sujets T (Bosy-Westphal et al. 2004; Bossu et al. 2007; Marra et al. 2009, 2019; Hasegawa et al. 2011) (**Tableau 4**), à l'exception d'une étude (Marra et al. 2007). Aucune différence significative ne semble être rapportée par la littérature en ce qui concerne la comparaison entre les sujets MC et AM de la MM (Bossu et al. 2007; Pasanisi et al. 2013; Estour et al. 2017; Marra et al. 2019) (**Tableau 4**).

TABLEAU 4 – COMPOSITION CORPORELLE CHEZ DES FEMMES PRESENTANT UNE MAIGREUR CONSTITUTIONNELLE

	CT (n = 7)	C (n = 7)	AN (n = 6)	P Value
Weight, kg	42.7±3.0	54.1±4.5	40.8±4.0	a, b
BMI, kg/m^2	16.1±0.6	21.2±0.8	15.8±0.8	a, b
FFM, %	81.7±2.1	73.8±4.1	90.6±5.4	a, b, c
FM, %	18.3±2.1	26.9±4.1	9.4±5.4	a, b, c
FFM, kg	32.5±2.9	37.8±1.6	34.1±1.9	a, b
FM, kg	7.7±1.2	14.9±2.1	3.8±2.4	a, b
Leptin, ng/ml	8.3±3.4	9.0±3.1	2.8±2.2	a, c
Free T3, pmol/l	3.7±0.5	3.8±0.5	2.4±0.4	a, c
IGF-1, ng/ml	225±93	274±60	168±62	a

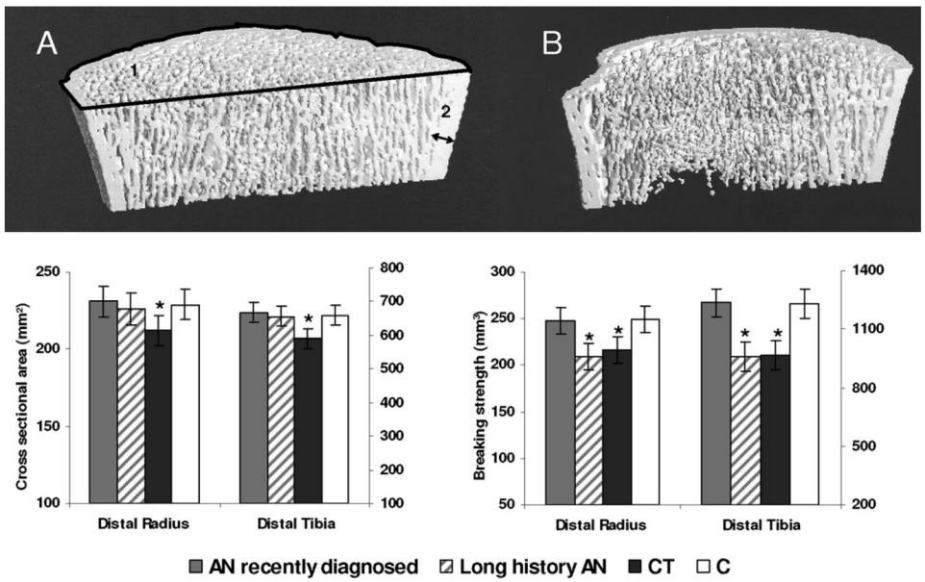
Valeurs présentées en tant que moyennes ± SD. CT (sujets maigres constitutionnels), C (sujets témoins), AN (patients présentant une anorexie mentale), (BMI : indice de masse corporelle, FFM : masse maigre, FM : masse grasse
[a] p<0.05 C *vs.* AN, [b] p<0.05 C *vs.* CT, [c] p<0.05 AN *vs.* CT, NS : non significatif)

Issu de publication (Bossu et al. 2007)

3.2.3. Tissu osseux

Peu d'études ont investigué le tissu osseux chez l'individu MC. Les quelques études réalisées semblent montrer une masse osseuse inférieure chez les sujets MC comparativement aux sujets T (Bosy-Westphal et al. 2004; Hasegawa et al. 2011), et une masse osseuse similaire entre les sujets MC et AM (Fernández-García et al. 2009). La DMO serait inférieure chez les sujets MC par rapport aux sujets T (Fernández-García et al. 2009; Galusca et al. 2018), et serait similaire entre les sujets MC et AM (Fernández-García et al. 2009). La DMO du col du fémur serait également inférieure chez les sujets MC par rapport aux sujets T, mais similaire entre les sujets MC et AM (Galusca et al. 2008; Estour et al. 2017). Au niveau des vertèbres lombaires, les études montrent des résultats divergents, avec une DMO trouvée inférieure (Galusca et al. 2008; Fernández-García et al. 2009) ou similaire (Estour et al. 2017) chez les sujets MC comparativement aux sujets T. Les sujets MC présenteraient une DMO au niveau des vertèbres lombaires similaire (Galusca et al. 2008; Fernández-García et al. 2009) ou supérieure aux sujets AM (Estour et al. 2017). De mêmes niveaux de vitamine D sont observés entre les sujets MC, T et AM (Galusca et al. 2008, 2018; Estour et al. 2017). Ainsi, seuls peu de résultats concernant le tissu osseux des individus MC existent dans la littérature, et ces résultats peuvent différer entre les études. Néanmoins, une publication a

spécifiquement porté sur l'analyse du tissu osseux chez l'individu MC et a observé des surfaces de section osseuses diminuées par rapport aux sujets T tandis que les patients présentant une AM de courte et de longue durée ne présentent pas de diminution de surface de section osseuse (Galusca et al. 2008) (**Figure 6**). Similairement aux patientes présentant une AM de longue durée, les sujets MC auraient une force de rupture plus faible que celle des sujets T, suggérant un risque de fracture potentiellement accru (Galusca et al. 2008). La microarchitecture osseuse apparaît également modifiée chez l'individu MC qui présenterait, au niveau du tibia, une épaisseur corticale amincie, des travées osseuses moins nombreuses ainsi que des espaces inter trabéculaires plus importants (Galusca et al. 2008) (**Figure 6**).

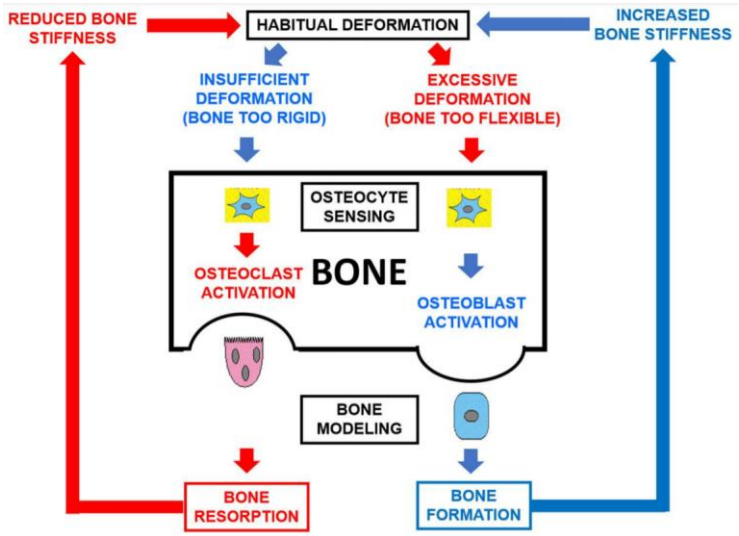


Reconstruction d'images (tomodensitométrie quantitative périphérique tridimensionnelle (3D-pQCT)) du tibia distal chez des sujets C (témoins) (**A**) et des sujets CT (maigres constitutionnels) (**B**). Section transversale mesurée par 3D-pQCT et force de rupture calculée du radius distal et du tibia distal chez des sujets AN (patients présentant une anorexie mentale) récemment diagnostiqués, des sujets AN ayant une longue histoire de la maladie, des sujets CT, et des sujets C. La surface de section transversale (1) et l'épaisseur corticale (2) sont indiquées en A. Les barres indiquées par « * » sont significativement différentes des sujets témoins à p=0.05, sur la base de l'analyse of variance (ANOVA) et du test de Tukey.

Issu de publication (Galusca et al. 2008)

FIGURE 6 – ÉVALUATION DU TISSU OSSEUX PAR 3D-PQCT CHEZ DES FEMMES PRESENTANT UNE MAIGREUR CONSTITUTIONNELLE

Les marqueurs du tissu osseux tels que l'ostéocalcine, la parathormone (PTH), le C-terminal telopeptide (CTX) ou l'osteoprotegerin (OPG) seraient similaires entre les participants MC et T, tandis que les patients AM présenteraient des valeurs diminuées d'ostéocalcine, mais augmentées de CTX et d'OPG (Galusca et al. 2008; Estour et al. 2017). En revanche, les individus MC auraient des taux de Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL) diminués associé à un ratio OPG/RANKL par conséquent augmenté par rapport aux sujets T et AM (Galusca et al. 2008). Une corrélation positive est observée entre l'ostéocalcine et le CTX chez les sujets MC et T suggérant un équilibre entre formation et résorption osseuse, contrairement aux patients AM (Galusca et al. 2008). Ainsi, malgré une faible masse osseuse, les personnes MC semblent pourtant présenter un remodelage osseux normal. La faible DMO et la faible masse osseuse observées chez les sujets MC pourrait uniquement résulter de la faible charge mécanique appliquée au tissu osseux en raison du faible poids corporel de cette population. Ainsi qu'illustré par la théorie du mécanostat de Harold Frost (Frost 1987) (**Figure 7**), si les ostéocytes du tissu mécanique osseux des sujets MC reçoivent peu d'information mécanique (peu de déformation), les ostéocytes vont plutôt activer les ostéoclastes que les ostéoblastes, de façon à ce que le tissu osseux devienne moins rigide.



Issu de publication (Lüscher et al. 2019)

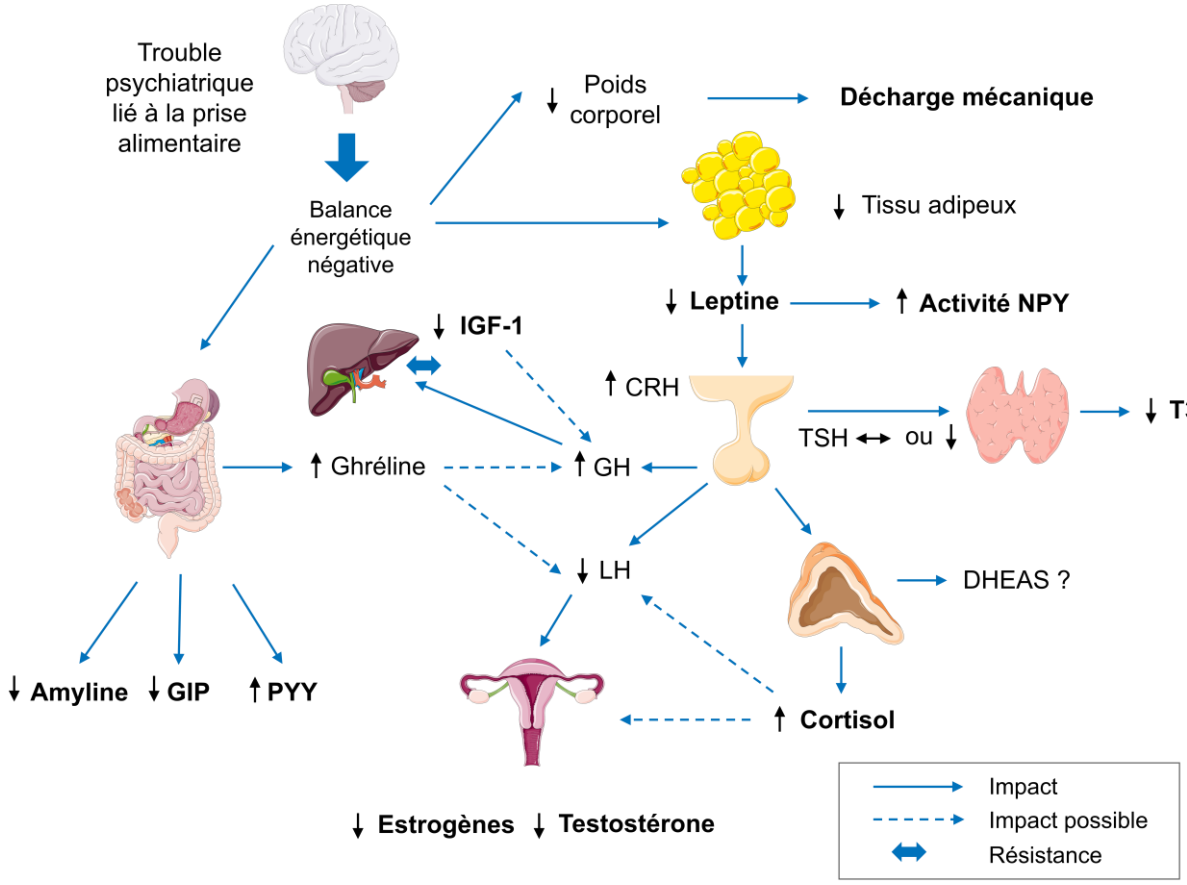
FIGURE 7 – THEORIE DU MECANOSTAT DE FROST

Bien que la DMO apparaisse faible chez le sujet MC (Fernández-García et al. 2009; Hasegawa et al. 2011; Galusca et al. 2018), le risque fracturaire en condition de vie réelle n'a cependant jamais été investigué à ce jour chez cette population. Il est possible d'argumenter que l'impact lors d'une chute sera probablement plus faible chez les sujets MC du fait de leur faible poids corporel, ce qui pourrait permettre à leur tissu osseux de résister à la contrainte mécanique sans nécessairement occasionner de fracture. Considérant l'absence de découplage entre la formation et la résorption osseuse et l'absence de preuve d'un risque fracturaire réellement accru chez ces sujets, aucun traitement n'est actuellement proposé (Estour et al. 2018). De nouvelles études sont nécessaires pour explorer les conséquences de ces premières observations cliniques sur le risque fracturaire, à court et long terme (Galusca et al. 2016).

L'analyse de la composition corporelle chez l'individu MC semble montrer une diminution de la masse grasse, de la MM et également de la masse osseuse. Ces premiers résultats tendraient ainsi à suggérer une maigreur homogène qui toucherait tous les secteurs de la composition corporelle, sans pour autant observer de déséquilibre de la balance énergétique. Néanmoins, dans la mesure où le nombre de participants inclus dans les études cliniques s'intéressant à la MC reste faible, une approche systématique de revue de littérature semble nécessaire afin d'obtenir des conclusions scientifiques plus solides.

3.3. Marqueurs hormonaux et nutritionnels

Chez le patient AM, la littérature a révélé que le déficit de prise énergétique dû aux troubles psychiatriques de ces patients engendrait de nombreux désordres endocriniens (Dede et al. 2014), ainsi qu'illustré sur la (**Figure 8**).



Adapté à partir de publication (revue) (Dede et al. 2014)

FIGURE 8 – CONSEQUENCES ENDOCRINIENNES DE LA REDUCTION DE POIDS CORPOREL ET DE MASSE GRASSE CHEZ LE PATIENT SOUFFRANT D'ANOREXIE MENTALE

La **Figure 8** montre ainsi que les sujets AM présentent un faible taux de leptine qui s'explique probablement par la faible masse grasse de ces sujets (Ostlund et al. 1996; Dede et al. 2014), ainsi qu'un niveau élevé de ghréline (Dede et al. 2014). Ils présentent également un taux de triiodothyronine (T3) plus faible qui serait à interpréter comme un signe évident de dénutrition (Moshang et al. 1975). Enfin, les concentrations en hormones sexuelles (Krassas 2003) et en IGF-1 (Misra and Klibanski 2014) sont abaissées alors que les taux de GH et de cortisol sont élevés (Misra and Klibanski 2014). Le faible niveau d'IGF-1 du patient AM aurait pour cause une désensibilisation des récepteurs du foie à la GH. Le niveau de GH resterait ainsi élevé en raison de l'absence de rétrocontrôle négatif du fait de la diminution du niveau d'IGF-1 (Misra and Klibanski 2014). Au regard des nombreuses anomalies endocriniennes

observées chez le sujet AM, une question émerge naturellement : le sujet MC présente-t-il des anomalies endocriniennes ?

3.3.1. Bilan biochimique

Une importante proportion d'études s'étant intéressées à la MC a procédé à des séries de tests sanguins dans le but d'évaluer le fonctionnement global des différents organes et systèmes biologiques. L'objectif de ces analyses biochimiques sanguines avait pour but de détecter des anomalies potentielles chez le sujet MC afin d'appréhender son fonctionnement physiologique, mais aussi de comparer les profils biochimiques entre le sujet MC et AM. Bien qu'il apparaisse complexe de retracer tous les résultats liés aux nombreux paramètres biochimiques dosés parmi les différentes études, il émergerait globalement une absence d'anomalie majeure dans le bilan biochimique du sujet MC, contrairement au sujet AM. Les sujets MC présenteraient des taux de FT3, d'IGF-1 et de cortisol normaux (Tolle et al. 2003; Galusca et al. 2012; Germain et al. 2016b) ; contrairement aux patients AM qui auraient de faibles niveaux de FT3 et d'IGF-1 (Petretta et al. 1997; Germain et al. 2007; Galusca et al. 2012) et des niveaux élevés de cortisol (Miljic et al. 2006; Galusca et al. 2015; Germain et al. 2016b), bien qu'une étude ne semble pourtant pas montrer de différence d'IGF-1 significative entre les sujets MC et AM (Bossu et al. 2007). Plusieurs études observent des taux de GH similaires entre les sujets MC et T et inférieurs chez les sujets MC *vs.* AM (Germain et al. 2007; Galusca et al. 2008, 2015). D'autres études, en revanche, ne rapportent pas de différence significative de GH entre les sujets MC et AM (Fernández-García et al. 2009; Estour et al. 2017). *A priori*, les hormones relatives à la fonction sexuelle telles que 17β-estradiol, LH, FSH, sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS) et testostérone seraient estimées à des taux normaux chez les sujets MC, similaires à ceux des participants T (van Binsbergen et al. 1990; Germain et al. 2007; Estour et al. 2017). Concernant le 17β-estradiol, les sujets AM présenteraient des taux plus faibles que les individus MC (Tolle et al. 2003; Estour et al. 2017). En revanche, pour les autres hormones sexuelles, les études semblent montrer des résultats assez divergents en ce qui concerne la

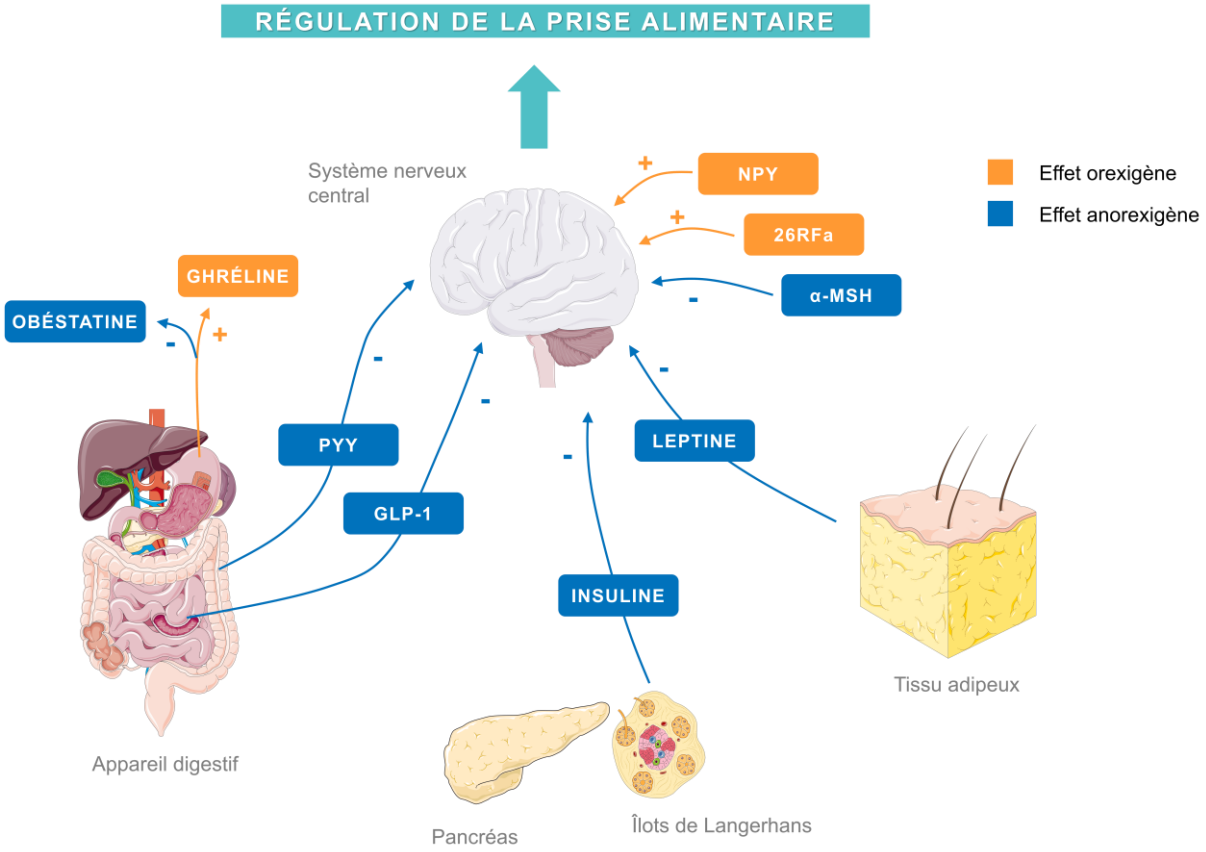
comparaison des sujets MC et à celle des patients AM (Germain et al. 2007, 2009; Estour et al. 2017). La glycémie des sujets MC serait similaire aux participants T (Petretta et al. 1997; Tagami et al. 2004; Hasegawa et al. 2011; Galusca et al. 2018), et similaire (Miljic et al. 2006) ou supérieure (Petretta et al. 1997) à celle des patients AM. Les dosages sanguins d'insuline chez les sujets MC montrent des taux similaires (Tagami et al. 2004; Galusca et al. 2018) ou inférieurs (Ling et al. 2019) à ceux des sujets normo-pondérés T, et similaires (Miljic et al. 2006) à ceux des patients AM. Le score d'insulino-résistance calculé avec l'indice de l' homeostasis model assessment of insuline resistance (HOMA-IR) serait similaire entre les sujets MC et T (Tagami et al. 2004; Galusca et al. 2018). Les taux sanguins de triglycérides seraient également semblables entre les participants MC et T (Hasegawa et al. 2011; Germain et al. 2014; Ling et al. 2019).

Si la présente revue de littérature permet d'aborder très succinctement le profil biochimique du sujet MC, nous retiendrons que (1) le sujet MC ne semblerait pas présenter d'anomalies biochimiques majeures (2) les résultats biochimiques sont cependant nombreux et parfois divergents. Ce constat suscite un intérêt particulier pour la mise en place d'une approche systématique voire méta-analytique afin d'apporter des conclusions exhaustives et robustes face à la quantité et à la diversité des résultats.

3.3.2. Hormones régulatrices de l'appétit

Dans le cadre d'une MC, il apparaît non seulement important de s'interroger sur la prise alimentaire effective, mais également sur les aspects de la régulation hormonale de l'appétit chez ces sujets. Concernant la ghréline, seule hormone orexigène produite par l'estomac (**Figure 9**), plusieurs études rapportent des niveaux similaires entre les sujets MC et T (Tolle et al. 2003; Germain et al. 2009, 2014). Cependant, une autre étude observe des niveaux inférieurs chez les sujets MC (Germain et al. 2007). Comparativement aux patients AM, les participants MC auraient des taux de ghréline inférieurs (Tolle et al. 2003; Miljic et al. 2006; Germain et al. 2007). De façon plus isolée, quelques des études se sont

intéressées à des neuropeptides orexigènes tels que le neuropeptide pyroglutamilated RFamide peptide (26Rfa) (Galusca et al. 2012) ou le neuropeptide Y (NPY) (Galusca et al. 2015) mais également sur d'autres régulateurs à effet anorexigène tels que le peptide YY (PYY) (Germain et al. 2007, 2014), le glucagon-like peptide-1 (GLP-1) sécrété par l'intestin (Germain et al. 2007, 2014), l'obéstatine (issue du même précurseur que la ghréline) (Germain et al. 2009, 2014) ou l' α-melanocyte-stimulating hormone (α-MSH) (Galusca et al. 2015) (**Figure 9**). Par comparaison aux sujets T, les sujets MC présenteraient des niveaux similaires de NPY (Galusca et al. 2015), supérieurs (Germain et al. 2007) ou similaires (Germain et al. 2014) de PYY, inférieurs (Germain et al. 2007) ou similaires (Germain et al. 2014) de GLP-1, similaires d'obéstatine (Germain et al. 2009, 2014) et similaires d'α-MSH (Galusca et al. 2015). Le taux de 26RFa des sujets MC tendrait à être plus important que chez les participants T (Galusca et al. 2012). Comparativement à l'AM, la MC serait caractérisée par des niveaux inférieurs de 26RFa (Galusca et al. 2012), similaires de NPY (Galusca et al. 2015), supérieurs de PYY (Germain et al. 2007), inférieurs de GLP-1 (Germain et al. 2007), similaires d'obéstatine (Germain et al. 2009) et supérieurs d'α-MSH (Galusca et al. 2015). Ainsi, l'interprétation de ces résultats ne révèle pas clairement d'effet global orexigène ou anorexigène chez les sujets MC, étant donné que plusieurs hormones ayant le même effet sur la régulation de la prise alimentaire varient de façon opposée chez le sujet MC. Néanmoins, ces résultats montrent une fois encore que le sujet MC diffère du patient AM, y compris sur la régulation de sa prise alimentaire.

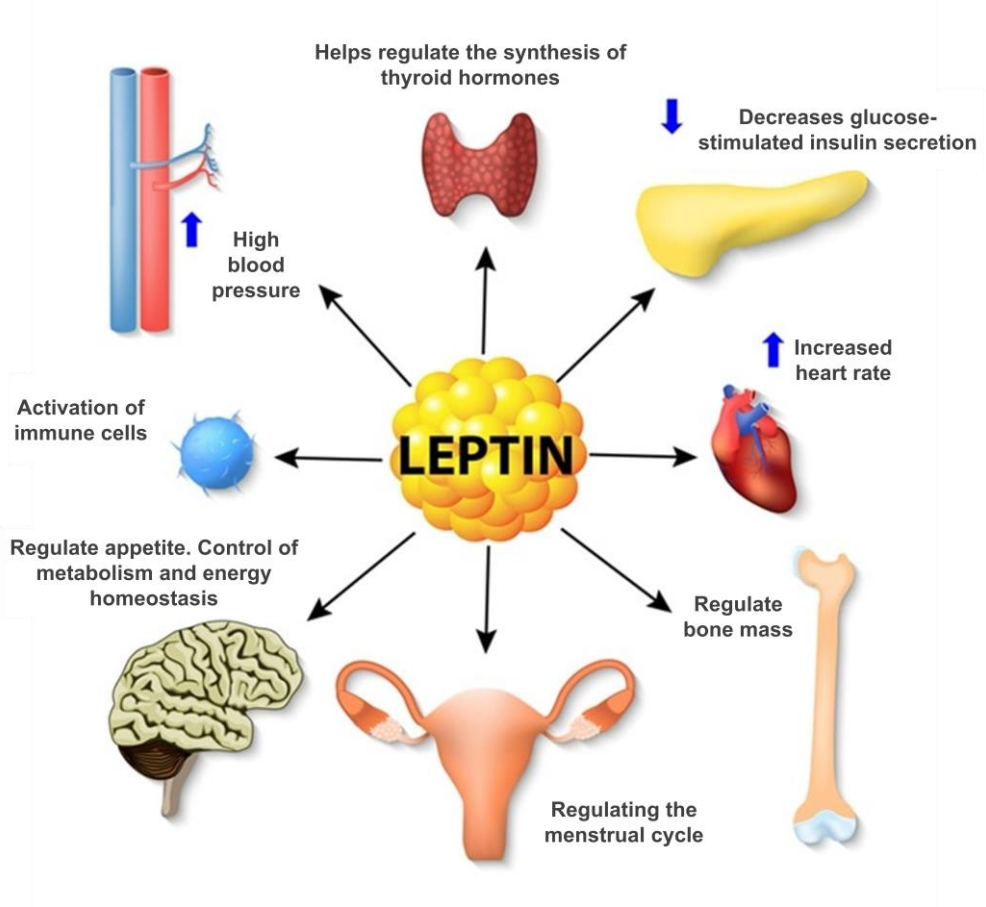


Adaptée à partir du serveur médical d'images libres de droit ("Smart Servier Medical Art" n.d.)

FIGURE 9 – REGULATION HORMONALE DE L'APPETIT

La leptine, sécrétée par le tissu adipeux aussi bien sous-cutané que viscéral, compte parmi les hormones de régulation de l'appétit à effet anorexigène les plus étudiées dans le cadre de la MC (**Figure 9**). Plus d'une dizaine d'études s'y sont intéressées, relevant généralement des niveaux intermédiaires chez l'individu MC comparativement aux patients AM présentant des valeurs très inférieures, et aux sujets T présentant des valeurs généralement supérieures (Miljic et al. 2006; Galusca et al. 2008, 2012, 2015; Germain et al. 2009, 2014, 2016b; Fernández-García et al. 2009; Estour et al. 2017). Quelques études rapportent même des niveaux de leptine n'étant pas significativement inférieurs chez les sujets MC par rapport aux sujets T (Bossu et al. 2007; Germain et al. 2007; Ling et al. 2019). Ainsi que le montre la (**Figure 10**), les implications physiologiques de la leptine, au-delà de la régulation de l'appétit,

apparaissent multiples. Face à la divergence des résultats obtenus dans les différentes études, il apparaît particulièrement intéressant de mettre en place une approche systématique, voire méta-analytique, dans le but d'apporter des conclusions robustes.



Issu de publication (Liji 2017)

FIGURE 10 – IMPLICATIONS PHYSIOLOGIQUES DE LA LEPTINE

Face à ces observations, il apparaît difficile de distinguer un profil de régulation de l'appétit type chez la MC tant cette régulation est fine et complexe. De plus, les méthodologies varient entre les articles avec des niveaux hormonaux pouvant être mesurés à jeun, à différents instants au long de la journée (et des repas), moyennés ou estimés par l'aire incrémentale sous la courbe. De même, les formes hormonales dosées peuvent différer entre forme totale ou forme active, ce qui peut conduire à une hétérogénéité

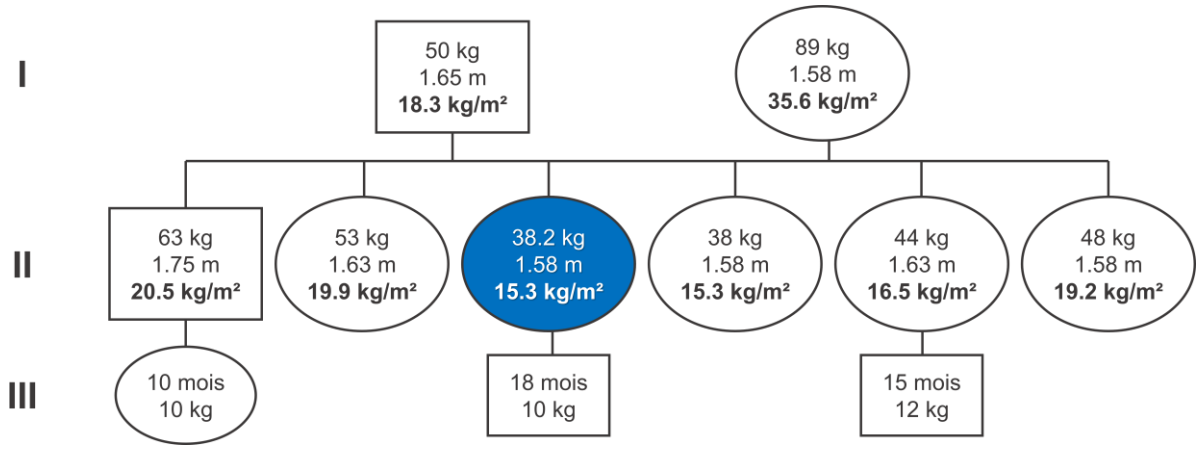
des résultats. Par exemple, dans une même étude ayant dosé la ghréline totale ainsi que sa forme active acylée (Germain et al. 2007), les résultats montrent des niveaux différents avec un niveau de ghréline total inférieur mais de ghréline acylée similaire chez les sujets MC comparativement aux patients AM. Même si l'analyse de la régulation de l'appétit reste multiple et complexe, il est important de constater que de nombreuses différences sont décrites dans la littérature concernant la MC. Le profil hormonal de la MC semble ainsi se dégager de celui de l'AM mais également de celui du sujet T, suggérant un profil de régulation de l'appétit spécifique chez le sujet MC. Néanmoins, la diversité des méthodes utilisées et des résultats rend la conclusion et l'interprétation complexes, ce qui suggère le besoin de davantage de travaux centrés sur les questions de régulation de l'appétit chez le sujet MC.

3.4. Hérédité et approches génétiques

Malgré l'intérêt de nombreuses publications, la question de la génétique de la MC est souvent abordée de façon complexe dans la littérature, au sens où l'analyse génétique est souvent réalisée sur d'importantes cohortes de sujets dans lesquelles le réel diagnostic d'une MC est généralement discutable. De nombreuses études très intéressantes ne rapportent des résultats qu'en fonction de l'IMC des individus, ce qui est cependant insuffisant pour discuter la notion de MC. Une attention particulière doit ainsi être apportée au prérequis indispensable qu'est le diagnostic de la MC.

Dès 1997, Hinney et ses collaborateurs se sont intéressés au polymorphisme du gène du transporteur de la sérotonine (serotonin-transporter-linked polymorphic region (5-HTTLPR)) pouvant avoir des implications dans la régulation du poids et l'apparition des troubles alimentaires, mais n'ont décelé aucune différence dans la fréquence allélique de ce gène entre les participants présentant une obésité, une AM, ou une MC (Hinney et al. 1997). Quelques années plus tard, Bulik et Allison rapportent que beaucoup d'intérêt est porté à la question « pourquoi certaines personnes souffrent d'obésité », tandis que dans nos sociétés occidentales obésogènes actuelles, la question « pourquoi certaines personnes

ne souffrent pas d'obésité » pourrait également susciter de l'intérêt. Cette revue s'intéresse ainsi aux allèles pouvant contribuer à la résistance à l'obésité, et explore l'hérédité de la maigreur/minceur/résistance à l'obésité. Par cette approche épidémiologique de la génétique de la maigreur, il semble que la maigreur soit associée à un profil génétique particulier (Bulik and Allison 2001). La notion d'hérédité de la MC est également abordée par Bossu, qui s'intéresse à l'histoire familiale de la MC et de l'AM (Bossu et al. 2007). L'étude rapporte une observation simple : la fréquence moyenne des sujets maigres par famille est de 2.5 sur 3 générations dans les familles MC contre 0.5 dans les familles atteintes d'AM (Bossu et al. 2007; Estour et al. 2012). En effet, des familles où l'on retrouve des femmes mais également des hommes présentant une MC sur plusieurs générations ont été décrites (**Figure 11**). L'analyse de la cohorte STILTS révèlerait même que 74 % des individus MC présenterait une histoire familiale de MC, soulignant le caractère héréditaire de la MC (Riveros-McKay et al. 2019).



Le sujet en bleu est le propositus.

Issu de publication (Estour et al. 2012)

FIGURE 11 – ARBRE GENEALOGIQUE D'UNE FAMILLE ATTEINTE DE MAIGREUR CONSTITUTIONNELLE

Les analyses transcriptomiques d'échantillons musculaires montrent qu'un nombre important de gènes présenteraient des niveaux d'expression différents entre les sujets MC et T (Galusca et al. 2018). Il semblerait que beaucoup de gènes mitochondriaux soient moins exprimés chez les individus MC que

chez les individus T. L'étude montre également que certains gènes impliqués dans le stockage et le métabolisme des triglycérides seraient sous-exprimés dans la MC (Galusca et al. 2018). Une autre étude s'intéressant cette fois au tissu adipeux montre que 88 gènes, dont plusieurs gènes mitochondriaux, seraient différemment exprimés entre les sujets MC et les sujets T (Ling et al. 2019). L'analyse révèle également dans le tissu adipeux des sujets MC une surexpression des gènes du métabolisme oxydatif, notamment certains gènes de la β-oxydation des acides gras et de la biosynthèse des triglycérides (Ling et al. 2019). Cette même étude ne semble cependant pas révéler d'altération transcriptomique robuste en ce qui concerne le tissu musculaire (Ling et al. 2019). Peu après, l'équipe de Farooqi s'est interrogée sur le fait que certaines personnes sont plus susceptibles d'être obèses alors que d'autres restent très maigres malgré l'environnement obésogène (Riveros-McKay et al. 2019). Son équipe s'est spécifiquement intéressée à l'héritabilité de la maigreur et les résultats montrent une héritabilité comparable à celle de l'obésité (Riveros-McKay et al. 2019). L'étude d'association pangénomique de 1 471 MC contre 1 456 personnes atteintes d'obésité sévère a permis d'identifier 10 loci qui étaient auparavant uniquement associés avec l'obésité (Riveros-McKay et al. 2019). Ces travaux ont permis de nuancer la compréhension de l'architecture génétique de la régulation du poids corporel et apportent de nouveaux éclairages concernant l'identification de potentiels gènes cibles anti-obésité. En 2020, basé sur ce même constat de l'importance de la variabilité interindividuelle dans la propension à prendre du poids malgré un même environnement obésogène, un autre article a également rapporté les résultats d'une étude d'association pangénomique sur la MC (Orthofer et al. 2020). Le gène de l'« anaplastic lymphoma kinase » (ALK) est identifié comme gène potentiel pouvant expliquer le sous-poids et la résistance à la prise de poids de la MC (Orthofer et al. 2020).

La mention dans de nombreux articles du désir des sujets MC de prendre du poids et la suggestion de façon plus ou moins directe d'une résistance à la prise de poids chez ces sujets reposent essentiellement sur des résultats déclaratifs des sujets MC. Peu de travaux ont questionné expérimentalement les

réponses à une intervention à visée de prise de poids. À l'heure actuelle, seuls de rares travaux ont mis en place des programmes de type « surnutrition » chez des sujets MC afin de questionner expérimentalement la difficulté des sujets MC à prendre du poids.

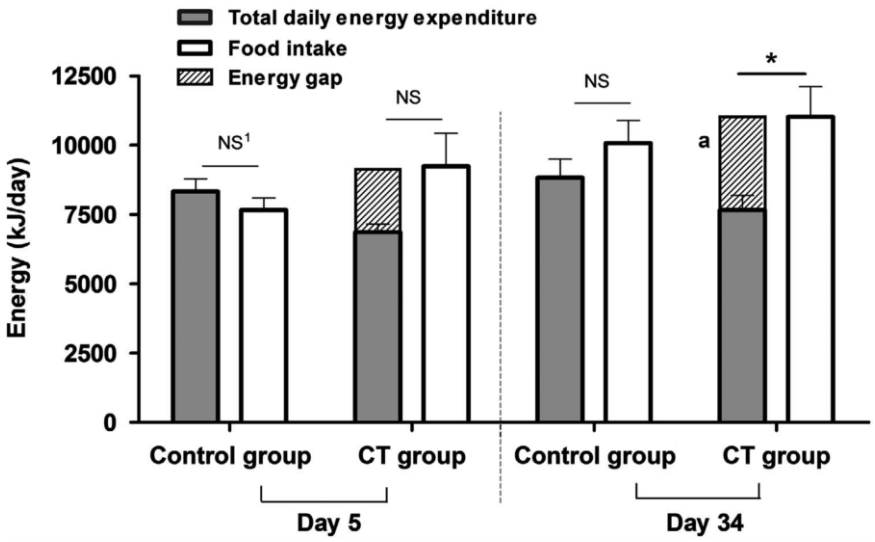
3.5. Effet de protocoles de surnutrition chez le sujet maigre constitutionnel

Ainsi que nous l'avons mentionné en *Partie 1.2 Approche historique*, la première étude ayant investigué l'effet d'une surnutrition chez le sujet MC daterait de 1955 (Passmore et al. 1955a, 1955b). Néanmoins, cette étude n'avait pas inclus de groupe T et ne concernait que 3 sujets dont le diagnostic de MC reste discutable. En 1992, une seconde étude a mené un protocole de surnutrition chez des sujets MC (Diaz et al. 1992), mais d'importants biais méthodologiques sont à noter : diagnostic de MC incorrect, absence de groupe T, faible nombre de participants et peu/pas de statistiques.

Finalement, la première étude clinique de surnutrition chez le sujet MC solide en termes de diagnostic et de méthodologie date de 2014 (Germain et al. 2014). Une surnutrition exclusivement lipidique a été effectuée auprès de 8 femmes MC (IMC<17.5 kg/m²) comparativement à 8 femmes normo-pondérées T (18.5 – 25 kg/m²) (Germain et al. 2014). La surnutrition consistait à ajouter 630 kcal/jour sous forme d'huile d'olive, cacahuètes, gruyère et beurre à la diète habituelle des participantes, pendant 4 semaines. À la suite de la surnutrition, les participantes reprenaient leur alimentation habituelle durant 4 semaines et étaient réévaluées. Cette étude a mis en évidence de manière expérimentale la résistance à la prise de poids décrite chez les sujets MC puisqu'elle montre une prise de poids chez les sujets T tandis que les sujets MC ne prennent pas de poids de façon significative (Germain et al. 2014). Il est également très intéressant de constater que sur les 4 semaines suivant la fin de la surnutrition, les participantes T conservent le poids acquis durant la surnutrition tandis que les sujets MC perdent du poids. Cette observation suggère que non seulement les sujets MC résistent à la prise de poids (très légère hausse du poids non significative), mais qu'en plus la surnutrition engendre une perte de poids significative sur

la phase suivant la surnutrition. De même qu'un régime restrictif engendre parfois un effet rebond chez le sujet obèse, un régime hypercalorique semble engendrer un effet rebond inverse chez le sujet MC. De façon très intéressante, la surnutrition engendre une augmentation de DER et de DER/MM chez les sujets MC seulement ; cela pourrait suggérer un rôle de la MM dans la résistance à la prise de poids des sujets MC. Pourtant, la DET ne semble pas augmenter significativement chez les sujets MC et T.

La notion de « gap » énergétique (différence entre apport et dépense énergétique) est également investiguée durant ce protocole de surnutrition. La **Figure 12** met en évidence cette notion de « gap » énergétique des sujets MC dont la prise alimentaire était significativement supérieure à la DET, sans pour autant observer une prise de poids avec la surnutrition.



Bilan énergétique avant le protocole de surnutrition (jour 5) et à la fin de la période de surnutrition de 2640 kJ (630 kcal) par jour (jour 34) dans le groupe CT (sujets maigres constitutionnels – n=8) et dans le groupe C (sujets témoins – n=8). La DET (barres grises) a été calculée comme suit : DET=DER×NAP (niveau d'activité physique). Le NAP a été évalué à l'aide d'un accéléromètre. L'apport alimentaire (barres blanches) a été évalué à l'aide d'un relevé alimentaire quotidien complété pendant 5 jours. Les données sont exprimées en moyenne ± SEM. Analyse statistique : * $p<0.05$ entre DET et apports alimentaires. NS : non significatif. « a » indique $p<0.05$ *vs.* jour 5.

Issu de publication (Germain et al. 2014)

FIGURE 12 – BALANCE ENERGETIQUE : MISE EN EVIDENCE D'UN « GAP » ENERGETIQUE CHEZ DES FEMMES PRESENTANT UNE MAIGREUR CONSTITUTIONNELLE

Ce constat témoigne d'une difficulté à expliquer, à l'aide des méthodes utilisées dans l'étude, la balance énergétique des sujets MC puisqu'une balance énergétique positive devrait logiquement être associée à une prise de poids qui n'est pas observée chez ces sujets. Ce « gap » paradoxal soulève des questions sur la précision des techniques d'évaluation de la balance énergétique utilisées dans cette étude et questionne également la balance énergétique des sujets MC.

À la suite de cette première étude, une surnutrition plus équilibrée en lipides, glucides et protéines a été mise en place durant 2 semaines auprès de 60 participants : 15 femmes MC, 15 hommes MC, 15 femmes T et 15 hommes T (Ling et al. 2016). La surnutrition est réalisée par la consommation d'une bouteille de Renutryl® Booster (600 kcal) par jour, en plus de leur régime alimentaire habituel. Une bouteille de Renutryl® Booster contient 30 g de protéines, 72 g de glucides et 21 g de lipides (**Tableau 5**). Vingt pour cent de l'énergie totale provient ainsi des apports protéiques, 48.5 % des apports glucidiques et 31.5 % des apports lipidiques (surnutrition légèrement hyper protéinée).

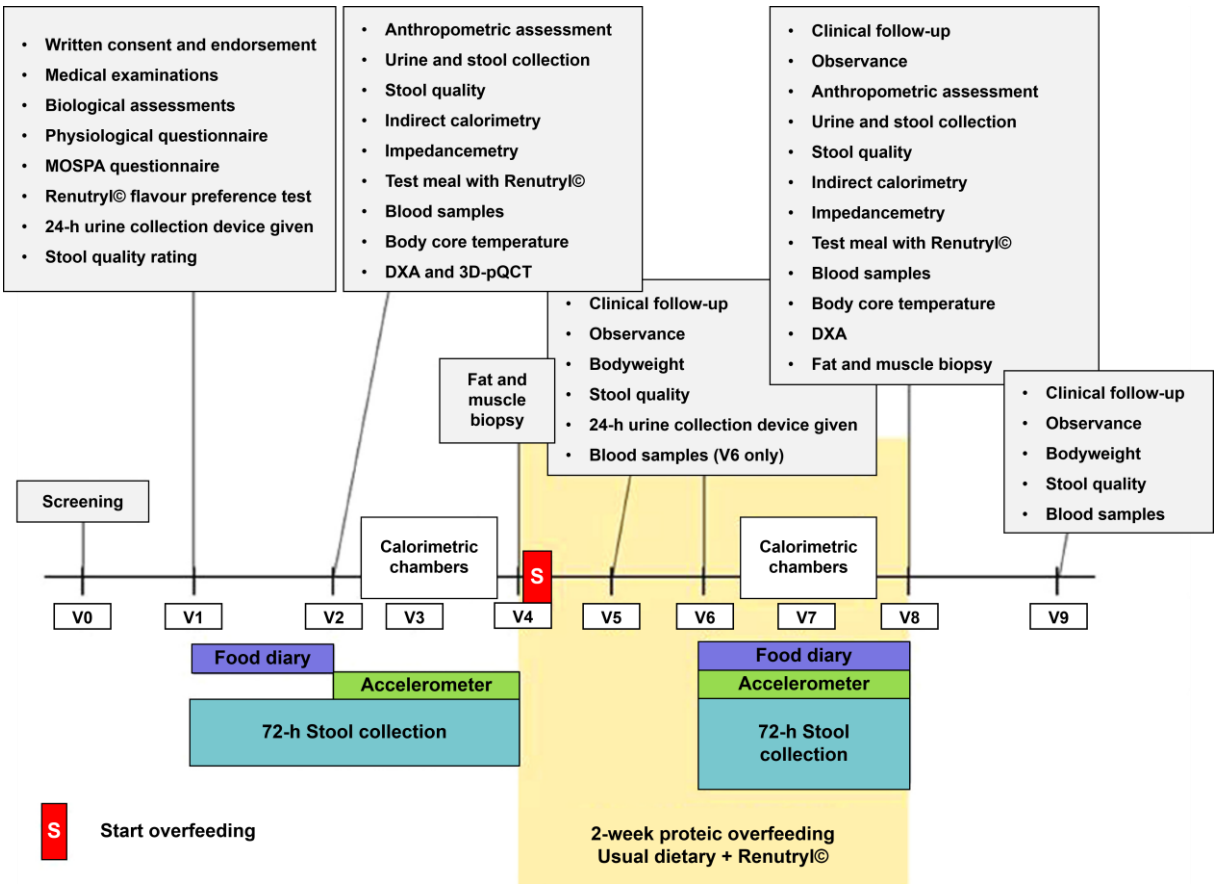
TABLEAU 5 – COMPOSITION D'UNE BOUTEILLE DE RENUTRYL® BOOSTER

Renutryl® Booster (300 ml)		
Energy	kcal	600
Fat 31.5% kcal	g	21
Carbohydrate 48.5 % kcal	g	72
of which sugars	g	21
of which lactose	g	<1.5
Protein 20 % kcal	g	30
Minerals		
Sodium	mg	285
Potassium	mg	720
Calcium	mg	687
Phosphate	mg	459

Renutryl® Booster est un complément nutritionnel oral hypercalorique (2 kcal/mL) fournissant des macronutriments et des micronutriments. Quatre saveurs (vanille, café, caramel et fraise) ont été proposées aux participants.

Adapté de la publication (Ling et al. 2016)

De nombreuses investigations ont été menées sur ce protocole de surnutrition : composition corporelle (DXA), qualité osseuse (DXA, tomodensitométrie quantitative périphérique haute résolution (HR-pQCT)), métabolisme de repos (calorimétrie indirecte : système portatif et chambres calorimétriques), dépense énergétique liée à l'activité physique (accéléromètre), DET (calculée : DER×NAP), questionnaire sur l'activité physique (MOSPA), évaluations psychologiques liées à la prise alimentaire (DEBQ, EDI, EDE, BSQ), apports énergétiques (carnet alimentaire), biopsies de tissu adipeux, biopsies de tissu musculaire, repas tests et suivi des paramètres biochimiques (glucose, insuline, ghréline, PYY, GLP-1), analyses métabolomiques et protéomiques, suivi de la température corporelle après le repas, et analyses des fèces (Ling et al. 2016). Le design de l'étude est présenté sur la **Figure 13.**

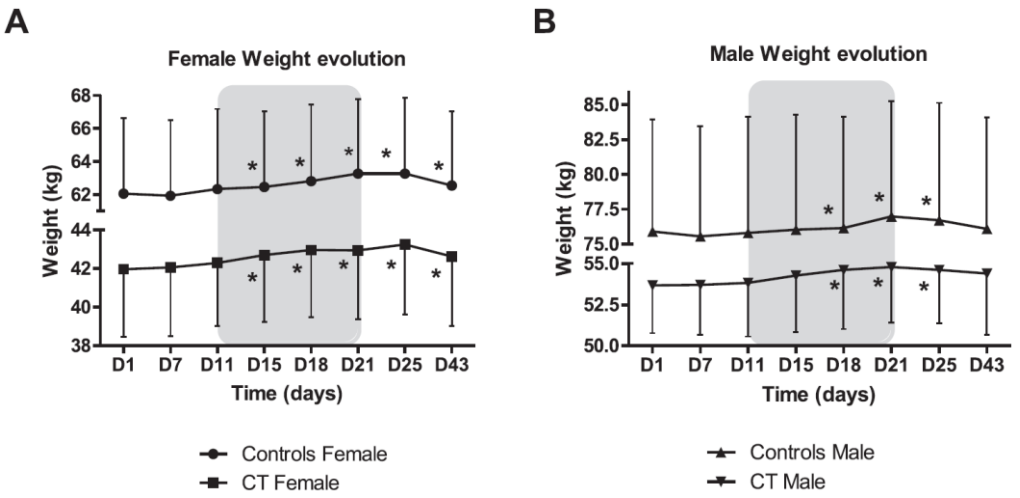


V : visite

Issu de la publication du design du protocole (Ling et al. 2016)

FIGURE 13 – DESIGN DE L'ETUDE DE SURNUTRITION

Ce protocole d'envergure a contribué à différents travaux de recherche. L'**étude 5** de ce travail de doctorat s'inscrit directement dans ce protocole et correspond aux travaux de recherche menés sur la composante musculaire de ce protocole. Outre l'investigation spécifique au tissu musculaire, deux études issues de ce programme de surnutrition ont été récemment publiées (Ling et al. 2019, 2020). La prise alimentaire des sujets a été évaluée de façon auto-déclarée (SUVIMAX) (Ling et al. 2016). Les participants étaient en contact régulier avec les investigateurs dans le but de vérifier la compliance et d'éviter d'éventuels comportements compensatoires. Les relevés alimentaires étaient vérifiés par un diététicien afin de s'assurer de la qualité des données collectées. La compliance a été définie comme suit : augmentation de la prise alimentaire d'au moins 450 kcal/jour et augmentation de l'azote urinaire (Ling et al. 2020). Ainsi qu'attendu, le protocole a effectivement engendré une augmentation significative des apports alimentaires totaux, mais également protéiques, glucidiques et lipidiques des sujets MC et T (Ling et al. 2020). Contrairement au protocole de surnutrition lipidique précédent (Germain et al. 2014), la surnutrition plus équilibrée de cette présente étude (Ling et al. 2020) montre une prise de poids légère mais significative chez les participants MC (**Figure 14**).



A – Évolution du poids corporel des femmes CT (maigres constitutionnelles) *vs.* C (témoins) ; **B** – Évolution du poids corporel des hommes CT *vs.* C; Données exprimées en tant que moyennes ± SD. *P < 0.05 *vs.* D1 (Jour 1) pour chaque groupe.

Issu de publication (Ling et al. 2020)

FIGURE 14 – ÉVOLUTION DU POIDS CORPOREL DE PARTICIPANTS MAIGRES CONSTITUTIONNELS ET NORMO-PONDERES TEMOINS EN REPONSE A UN PROTOCOLE DE SURNUTRITION

La surnutrition a entraîné une augmentation de la DET et de la DER chez les participants normo-pondérés T tandis que ces dépenses énergétiques sont restées stables chez les sujets MC (Ling et al. 2020) ; résultat qui contraste avec l'étude précédente (Germain et al. 2014). Certaines hormones régulatrices de l'appétit évolueraient différemment entre les sujets MC et T en réponse à la surnutrition tandis que d'autres semblent évoluer de la même manière (Ling et al. 2020). Les résultats sont difficiles à interpréter compte tenu des modalités (à jeun ou post-repas) et compte tenu de l'évolution parfois opposée d'hormones ayant un même effet anorexigène ou un même effet orexigène. Par exemple, le PYY anorexigène mesuré à jeun augmente avec la surnutrition chez les participants T mais reste stable chez les participants MC, tandis que la surnutrition entraîne une diminution du GLP-1 post-repas test chez les sujets T et une réponse au repas identique chez les sujets MC (Ling et al. 2020). La seconde publication s'est particulièrement intéressée à l'activité mitochondriale du tissu adipeux (Ling et al. 2019). L'évaluation de la respiration mitochondriale du tissu adipeux semble plus importante chez les sujets MC que T pré-

surnutrition (notamment au niveau du complexe II de la chaîne respiratoire) et la surnutrition tendrait à augmenter l'activité respiratoire mitochondriale du complexe II chez les sujets MC et T (Ling et al. 2019). Les analyses transcriptomiques du tissu adipeux ne semblent pas témoigner d'une réponse métabolique différente chez les participants MC de celle des sujets T en réponse à la surnutrition (Ling et al. 2019).

Ainsi, seuls deux protocoles de surnutrition ont été effectués auprès d'individus présentant une MC diagnostiquée de façon rigoureuse : une surnutrition lipidique (+ 630 kcal/jour – 4 semaines) ayant donné lieu à une publication (Germain et al. 2014) et une surnutrition plus équilibrée légèrement hyper protéinée (+600 kcal/jour – 2 semaines – design du protocole publié (Ling et al. 2016)) dont certains résultats ont déjà été publiés par deux articles scientifiques (Ling et al. 2019, 2020). Ce même protocole (Ling et al. 2016) a donné lieu à des analyses plus poussées dans le cadre de ce travail de thèse, concernant le métabolisme musculaire (**étude 5**).

3.6. Quel bilan ?

La revue de littérature centrée sur les publications relatives à la MC mène à plusieurs constats. Tout d'abord, les publications sont peu nombreuses et hétérogènes, tant dans les domaines d'études que dans les méthodes utilisées. Cette hétérogénéité favorise des résultats divergents, ce qui rend difficile toute interprétation et conclusion. Ce premier constat soulève la nécessité d'avoir une approche systématique et éventuellement méta-analytique de l'ensemble des résultats rapportés par les différentes investigations dans l'objectif d'augmenter la puissance statistique par accroissement des tailles d'échantillon, mais aussi d'apporter une analyse plus fine des méthodes et designs employés. L'analyse systématique et méta-analytique des résultats physiologiques relatifs à la MC fera l'objet de l'**étude 3** de ce travail de thèse.

Cette revue de littérature montre également que plusieurs études se sont spécifiquement intéressées à la balance énergétique des sujets MC dans le but de mieux comprendre leur physiologie potentiellement

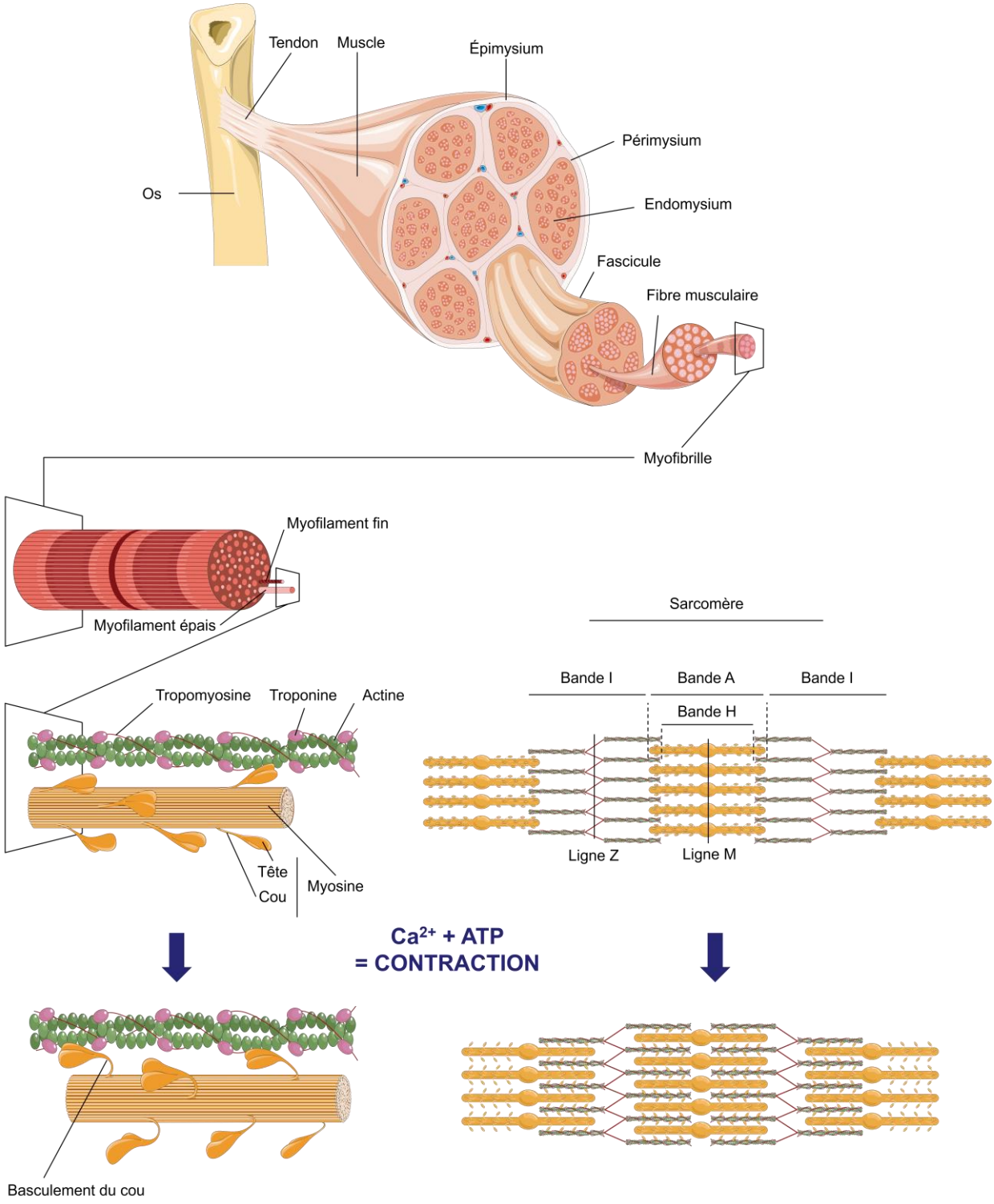
particulière. Ces différents travaux semblent unanimement indiquer des apports alimentaires similaires, voire même très légèrement supérieurs, chez les sujets MC *vs.* T. Néanmoins, la composante « dépense » de la balance énergétique est plus discutable. De plus, la plupart des études abordent cette notion de dépense énergétique uniquement par une estimation de la DER sans approche très explicative. Or, la MM est le principal facteur impactant la DER (Cunningham 1991). Le muscle strié squelettique, composant principal de la MM, est donc un déterminant majeur de la DER (Zurlo et al. 1990). Cela soulève donc un premier intérêt pour l'étude du tissu musculaire du sujet MC. D'une part, certaines études rapportent un ratio DER/MM supérieur chez les sujets MC par rapport aux sujets T (Bossu et al. 2007; Marra et al. 2019; Ling et al. 2020), suggérant une plus forte activité métabolique de leur tissu musculaire. D'autre part, certains gènes impliqués dans le stockage et le métabolisme des triglycérides du muscle ont été trouvés sous-exprimés dans la MC (Galusca et al. 2018), soulevant non seulement la question du métabolisme lipidique musculaire du sujet MC, mais également celui du métabolisme glucidique par extension.

4. MÉTABOLISME MUSCULAIRE ET CONTEXTE DE MAIGREUR CONSTITUTIONNELLE

4.1. Rappels de base sur le muscle strié squelettique

4.1.1. Organisation structurelle et fonctionnelle

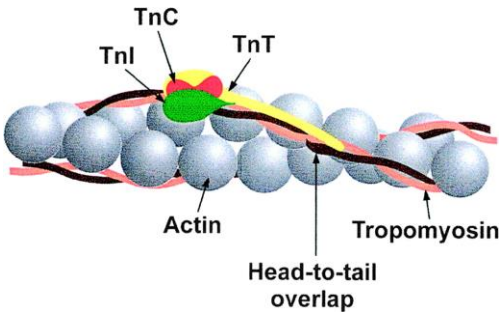
Le muscle strié squelettique s'organise en une disposition parallèle et régulière de myofilaments fins et épais, formant des myofibrilles, s'agrégeant ensemble pour former une fibre musculaire (Lépori 2005) (**Figure 15**). Ces cellules cylindriques de 50 à 100 µm de diamètre peuvent être extrêmement longues et parfois même parcourir toute la longueur d'un muscle (Hunter 2000). En raison de leur forme très allongée, ces cellules musculaires sont appelées « fibres ». Ces cellules possèdent plusieurs noyaux situés en périphérie (Hunter 2000). Les fibres musculaires sont entourées d'endomysium, une membrane de tissu conjonctif, et se regroupent sous la forme d'un fascicule musculaire entouré de périmysium. Ces fascicules s'agrègent pour former le muscle, lui-même entouré d'épimysium (**Figure 15**) (Lépori 2005). L'unité motrice, unité fonctionnelle du système moteur, est composée d'un motoneurone et de l'ensemble des fibres musculaires qu'il innerve, pouvant aller jusqu'à plusieurs centaines de fibres musculaires (Hunter 2000). Toutes les fibres musculaires innervées par un même motoneurone se contractent donc ensemble lorsqu'elles sont stimulées. Les propriétés structurelles et fonctionnelles des fibres musculaires d'une même unité motrice sont très proches. Le muscle résulte de l'assemblement de nombreuses unités motrices qui contribuent de façon spécifique et distincte à l'activité musculaire (Schiaffino and Reggiani 2011). Les myofilaments épais et fins qui constituent la fibre musculaire sont respectivement principalement composés de myosine et d'actine. Les myofilaments épais contiennent jusqu'à 200 molécules de myosine et font environ 16 nm de diamètre (Hunter 2000). Les myofilaments fins sont constitués de molécules d'actine G (globulaire, sous forme de monomère), se polymérisant en filament d'actine (actine F) de 6 nm de diamètre (Hunter 2000) et se structurant en brins formant finalement une double hélice.



Adaptée à partir du serveur médical d'images libres de droit ("Smart Servier Medical Art" n.d.)

FIGURE 15 – STRUCTURE DU MUSCLE STRIE SQUELETTIQUE

Les molécules de tropomyosine se polymérisent également sous la forme d'une double hélice s'enroulant légèrement autour de l'actine, masquant les sites de liaison actifs de l'actine avec la myosine (**Figure 16**) (Gordon et al. 2000). Trois sous-unités de la troponine constituent également le myofilament fin : la troponine T se liant à la tropomyosine, la troponine I pouvant avoir une activité inhibitrice, et la troponine C capable de fixer les ions calcium Ca^{2+}. La fixation de Ca^{2+} par la troponine C provoque un changement de conformation du complexe de la troponine entraînant avec elle la molécule de tropomyosine et démasquant ainsi le site de liaison actif de l'actine aux molécules de myosine (Gordon et al. 2000).

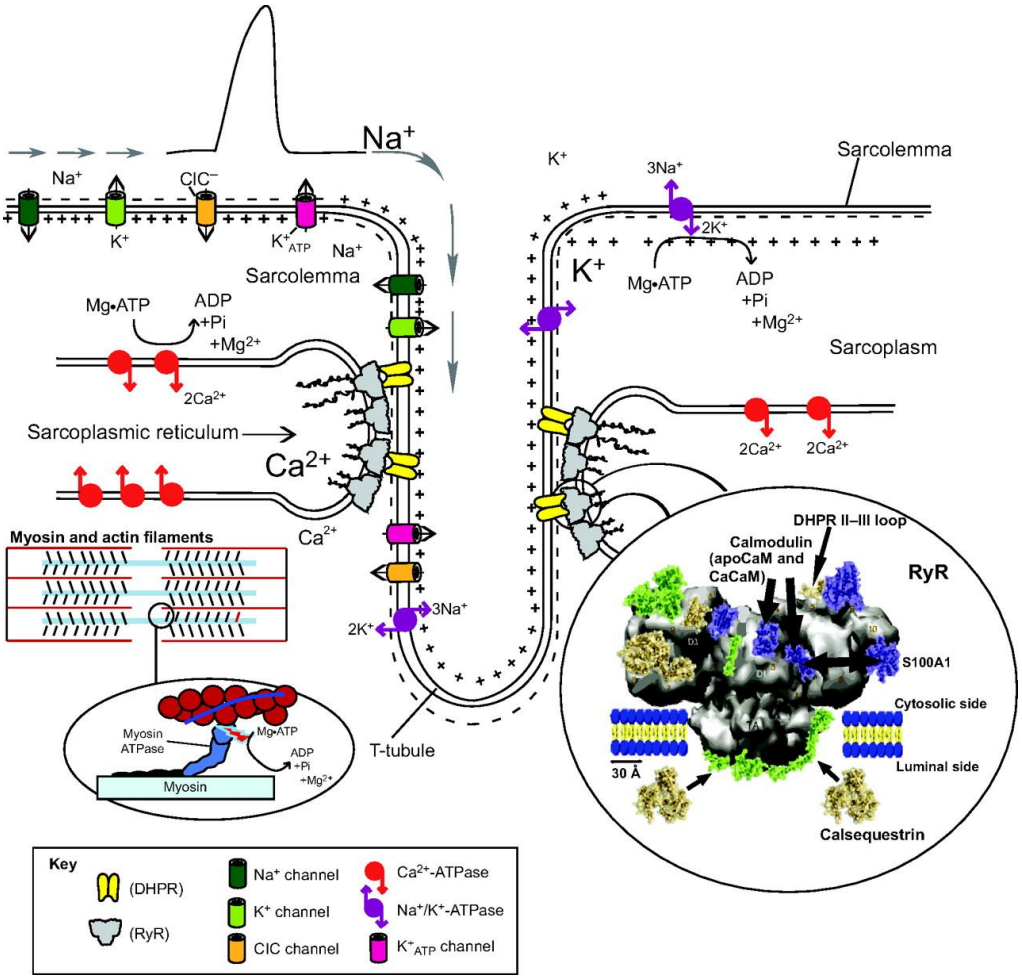


Issu de publication (Gordon et al. 2000)

FIGURE 16 – STRUCTURE PROTEIQUE DU MYOFILAMENT FIN

Les myofilaments fins et épais se lient sous l'effet de la présence de calcium intracellulaire et l'hydrolyse de l'adénosine triphosphate (ATP) par la tête de myosine engendre un changement de conformation au niveau du « cou », généralement décrit comme un « swinging lever arm » (Gordon et al. 2000). Ce « pivotement de bras de levier » entraînerait le « glissement » des myofilaments fins le long des myofilaments épais, raccourcissant ainsi la longueur du sarcomère (**Figure 15**). Les sarcomères ne mesurent environ que 2.2 µm dans leur forme étendue (Hunter 2000) ; ce qui implique que leur raccourcissement individuel est relativement infime à l'échelle musculaire. Néanmoins, ces sarcomères se répètent sur toute la longueur d'une fibre musculaire – 4 500 sarcomères environ se juxtaposent sur 1 cm de fibre musculaire. Ainsi, le raccourcissement est multiplié par le nombre de sarcomères juxtaposés et permet d'expliquer la contraction à l'échelle du muscle entier (**Figure 15**).

Le calcium et l'ATP sont indispensables à la contraction musculaire. L'ATP est fournie par différentes filières énergétiques, tandis que l'augmentation du taux de calcium cytosolique serait directement provoquée par l'excitation de la membrane plasmique de la cellule musculaire : le sarcolemme. Le potentiel d'action est transmis le long de la membrane du motoneurone, jusqu'à la jonction neuromusculaire, où l'acétylcholine prend le relai de la transmission du signal. La fixation de l'acétylcholine sur le récepteur nicotinique induit l'ouverture du canal du sarcolemme perméable aux cations Na^+ et Ca^{2+} (Fagerlund and Eriksson 2009). L'entrée cellulaire de cations engendre une dépolarisation à l'origine de la création d'un potentiel d'action post-synaptique. Ce potentiel d'action se transmet le long du sarcolemme jusqu'au tubule T, formé par une invagination de la membrane et entouré de deux réticulum sarcoplasmiques (triade) (**Figure 17**). Le récepteur à la dihydropyridine (DHPR) du tubule T voltage-sensible change de conformation sous l'effet du potentiel d'action (MacIntosh et al. 2012). Or, le DHPR interagit mécaniquement avec le récepteur à la ryanodine (RyR) du réticulum sarcoplasmique et son changement de conformation induit l'ouverture du RyR (Song et al. 2011), plus gros canal à Ca^{2+} connu à ce jour (Song et al. 2011) (**Figure 17**). L'ouverture du RyR permettrait ainsi le transport passif du Ca^{2+} vers le cytosol entraînant sa liaison avec la troponine C. Le calcium cytosolique est ensuite restocké activement par une pompe active Ca^{2+} – ATPase (pompe Calcium-ATPase du réticulum sarcoplasmique (SERCA)) (Song et al. 2011; MacIntosh et al. 2012).



DHRP : récepteur à la dihydropyridine ; RyR : récepteur à la ryanodine

Issu de publication (MacIntosh et al. 2012) ; adapté de (Song et al. 2011)

FIGURE 17 – COUPLAGE EXCITATION CONTRACTION ET RECEPTEURS

4.1.2. Notion de typologie

Les mammifères, et en particulier les êtres humains, font preuve d'une grande flexibilité permettant à un même muscle d'être différemment sollicité sur des efforts très divers, allant d'une activité de faible intensité telle que le maintien postural jusqu'à des contractions maximales, en passant par des contractions sous-maximales répétées telles que lors de la locomotion par exemple (Schiaffino and Reggiani 2011). Cette importante flexibilité est permise par la diversité et l'hétérogénéité des propriétés structurelles et fonctionnelles, généralement désignées sous le terme de phénotype, des fibres musculaires. De nombreuses études se sont portées sur les mécanismes moléculaires sous-tendant ces

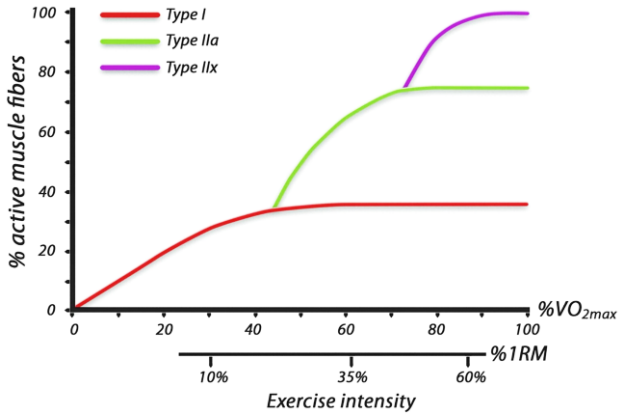
différents phénotypes de fibres musculaires (Schiaffino and Reggiani 2011). Deux grands types de fibres musculaires existent dans le muscle strié squelettique humain : les fibres de type I dites « lentes » et les fibres de type II dites « rapides » contenant les fibres de sous-types IIA dites « rapides oxydatives-glycolytique » et IIX dites « rapides glycolytiques » (Essén et al. 1975; Egan and Zierath 2013; Mukund and Subramaniam 2020). Le **Tableau 6** montre que les fibres de type I sont caractérisées par une forte résistance à la fatigue, une faible production de force, une capacité d'endurance élevée, une apparence rouge du fait d'un contenu élevée en myoglobine, une faible réactivité et un recrutement pour toutes les intensités, notamment les faibles intensités (Egan and Zierath 2013; Mukund and Subramaniam 2020). Au contraire, les fibres de types IIX ont une faible résistance à la fatigue, une forte production de force, une faible capacité d'endurance, une apparence blanche, un contenu faible en myoglobine, une forte réactivité et un recrutement pour toutes les intensités élevées (> 75 % VO_{2max} et/ou > 45 % de la force produite lors d'une répétition maximale (**Figure 18**) (Egan and Zierath 2013; Mukund and Subramaniam 2020). Les fibres de type IIA présentent un phénotype intermédiaire (**Tableau 6**).

TABLEAU 6 – CARACTERISTIQUES GENERALES DES DIFFERENTS TYPES DE FIBRES DU MUSCLE STRIE SQUELETTIQUE HUMAIN

		Type II	
	Type I	**Type IIA**	**Type IIX**
General properties			
Alternative nomenclature	SO, ST	FOG, FTa	FG, FTb
Myosin heavy-chain isoform	MHC1	MHC2A	MHC2X
Contractile and metabolic characteristics	Slow twitch, high oxidative, fatigue resistant	Fast twitch, oxidative-glycolytic, fatigue resistant	Fast twitch, glycolytic, fast fatigable
Force production (power output)	Weak	Intermediate	Strong
Endurance capacity	High	Intermediate	Low
Appearance/myoglobin content	Red/high	Red/intermediate	White/low
Time to peak tension [a]	80	30	
Ca^{2+} actomyosin ATPase activity [b]	0.16	0.48	
Mg^{2+} actomyosin ATPase activity [c]	0.30	0.84	
Recruitment threshold	All intensities	>40%VO_{2max}	>75%VO_{2max}

[a]msec ; [b]mmol min^{-1} mg $myosin^{-1}$; [c]mmol min^{-1} g $protein^{-1}$

Issu de publication (Egan and Zierath 2013) ; adapté de (Saltin and Gollnick 1983) – données issues du *vastus lateralis* d'hommes non entraînés



1RM : maximum sur une répétition, VO_{2max}: consommation maximale de dioxygène

Issu de publication (Egan and Zierath 2013)

FIGURE 18 – RECRUTEMENT DES FIBRES MUSCULAIRES EN FONCTION DE L'INTENSITE D'EXERCICE

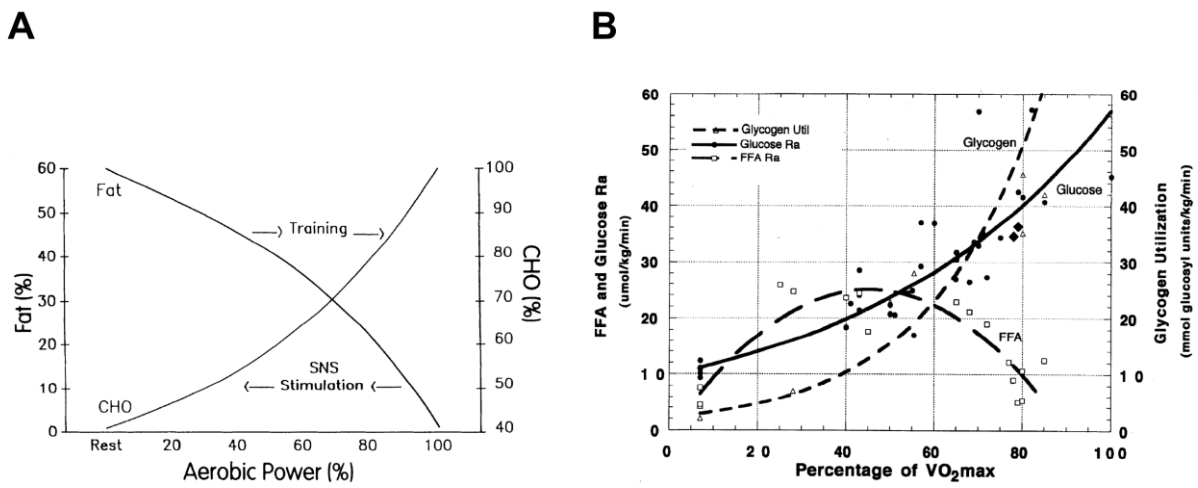
4.2. Fonctions métaboliques

Ainsi que précédemment évoqué, l'énergie sous forme d'ATP est indispensable au « swinging lever arm » (Gordon et al. 2000) à l'origine de l'activité musculaire. L'importante capacité métabolique du tissu musculaire est donc liée aux besoins élevés en énergie, nécessaire à la production de force et de mouvement. Le métabolisme oxydatif et glycolytique participe à cette fourniture d'énergie.

4.2.1. Utilisation des substrats à l'exercice

La **Figure 19A** illustre la proportion de la quantité de substrats oxydés provenant des lipides et des glucides au repos et à l'exercice (selon l'intensité d'exercice). Au repos, environ 60 % de la quantité de substrats oxydés provient des lipides et 40 % des glucides, avec un QR de l'ordre de 0.81 – 0.83 (Astrand and Rodahl 1986; Brooks and Mercier 1994). Au point de croisement, 30 % de la quantité de substrats oxydés provient des lipides et 70 % des glucides (**Figure 19A**). Étant donné qu'un gramme de lipides contient approximativement 9 kcal et un gramme de glucides 4 kcal ; le point de croisement correspond ainsi à l'intensité d'exercice à laquelle autant d'énergie (kcal) provient de l'oxydation des lipides que des glucides. Au-delà de cette intensité, l'énergie provenant des glucides prédomine sur celle provenant des lipides (Brooks and Mercier 1994). Les lipides sont donc majoritairement oxydés pour des intensités

d'exercice faibles à modérées, tandis que les glucides sont majoritairement oxydés pour des exercices de forte intensité (Brooks and Mercier 1994). Ce concept s'interprète au regard des différentes filières énergétiques et patterns de recrutement des différents types de fibres en fonction de la demande induite par l'intensité de l'exercice (Brooks and Mercier 1994). Différents facteurs influencent la contribution relative des glucides et des lipides à la fourniture d'énergie, tels que l'entraînement, le régime alimentaire, le statut pondéral, la composition corporelle, le sexe et les conditions environnementales (Romijn et al. 1993; Brooks and Mercier 1994; Pérez-Martin et al. 2001; Venables et al. 2005; Isacco et al. 2014; Mukund and Subramaniam 2020). La **Figure 19B** illustre l'oxydation absolue, et non relative, des lipides et glucides à l'exercice, en fonction de l'intensité de l'exercice. Avec l'augmentation de l'intensité de l'exercice, l'oxydation absolue des glucides augmente de façon exponentielle tandis que l'oxydation des lipides atteint un maximum avant de diminuer (Brooks and Trimmer 1996; Lazzer et al. 2010; Brun et al. 2011; Isacco et al. 2015). Ce point maximal d'oxydation des lipides, appelé « $Lipox_{max}$ » ou « Fat_{max} », est généralement obtenu pour des intensités d'exercice modérées (Brun et al. 2011).



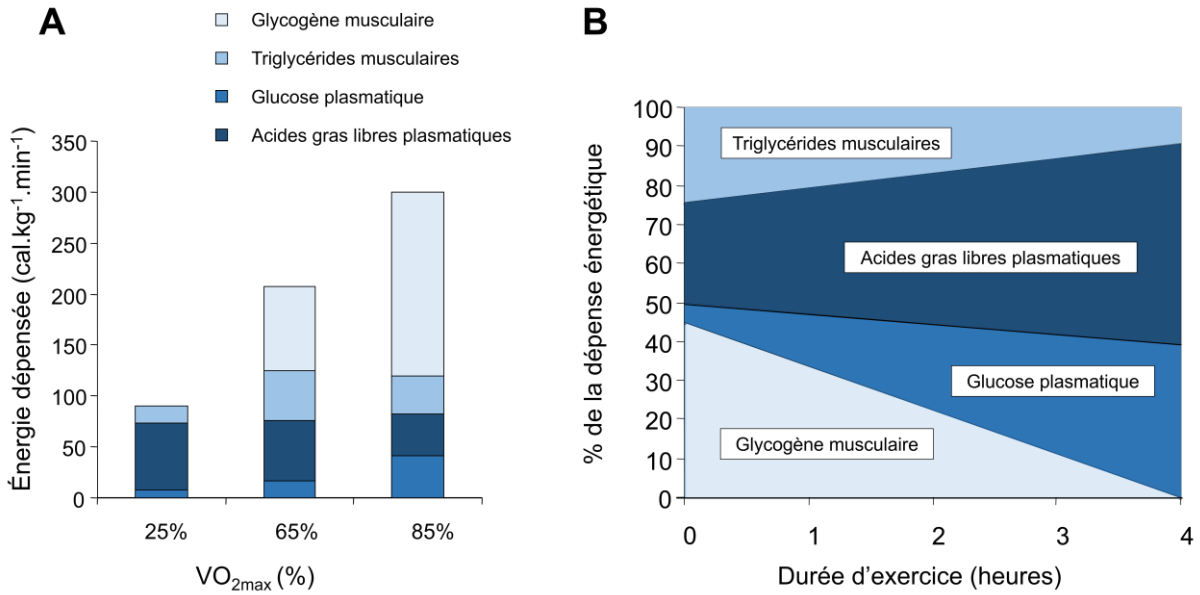
A. Augmentation relative de l'énergie provenant de l'utilisation des glucides (CHO) et diminution de l'énergie provenant de l'utilisation des lipides en fonction de la puissance relative produite. Au point de croisement, l'augmentation de l'intensité relative de l'exercice physique entraîne une dépendance de plus en plus élevée aux glucides et une moindre dépendance aux lipides. Même si, sur une échelle absolue, l'entraînement induit un déplacement des courbes vers la droite, sur une base relative, l'entraînement a probablement des effets minimes sur les courbes par rapport à la puissance aérobie. SNS : système nerveux sympathique. **B.** Résultats d'une recherche documentaire approfondie montrant les taux d'apparition (Ra) de glucose sanguin et d'acides gras libres (FFA) ainsi que la glycogénolyse musculaire nette, en fonction de l'intensité relative de l'exercice, telle que donnée par le pourcentage de consommation maximale d'O_2 (VO_{2max}). Cette analyse montre des augmentations exponentielles de la glycogénolyse musculaire et des taux d'apparition de glucose en fonction de l'intensité relative de l'exercice. En revanche, l'analyse montre une réponse polynomiale à multi-composante du taux d'apparition des acides gras libres plasmatiques, avec un exercice d'intensité légère à modérée (i.e. 25 – 40 % VO_{2max}) suscitant une forte augmentation du taux puis un croisement et une diminution à ~ 55 % VO_{2max}. Il est à noter que le taux d'apparition d'acides gras libres plasmatiques devrait atteindre des valeurs minimales à l'approche de VO_{2max}.

Issu de publications **A.** (Brooks and Mercier 1994) **B.** (Brooks and Trimmer 1996)

FIGURE 19 – CONCEPT DU POINT DE CROISEMENT

Les travaux des Romijn et Coyle se sont particulièrement intéressés à l'utilisation des substrats glucidiques et lipidiques selon leur provenance (musculaire ou plasmatique) en fonction de l'intensité de l'exercice (Romijn et al. 1993) et de sa durée (Coyle 1995). La **Figure 20A** montre la contribution absolue des différents substrats dans l'apport énergétique sur un exercice de durée fixe (30 min) à trois intensités différentes : 25 % de VO_{2max}, 65 % de VO_{2max}, 85 % de VO_{2max} (Romijn et al. 1993). À 25 % de VO_{2max}, l'énergie provient presque exclusivement des acides gras libres plasmatiques. À 65 % de VO_{2max},

le glycogène musculaire ainsi que les lipides musculaires et plasmatiques représentent les principales sources d'énergie tandis que le glucose plasmatique n'apporte qu'une faible contribution. À 85 % de VO_{2max}, l'énergie provient très majoritairement du glycogène musculaire (Romijn et al. 1993). La **Figure 20B** illustre la proportion de la dépense énergétique provenant des différents substrats énergétiques à intensité fixe (65-75 % de VO_{2max}) en fonction de la durée d'exercice (Coyle 1995). La proportion d'énergie provenant du tissu musculaire diminue avec la durée de l'exercice, tandis que la proportion d'énergie provenant des lipides et glucides plasmatiques augmentent avec le temps (Coyle 1995).



A. Contribution maximale à la dépense énergétique dérivée du glucose et des acides gras libres absorbés dans le sang et contribution minimale des réserves de triglycérides et de glycogène des muscles après 30 minutes d'exercice, exprimées en fonction de l'intensité de l'exercice. La quantité totale de calories (cal) disponibles à partir du plasma ne change pas en fonction de l'intensité de l'exercice. **B.** Pourcentage de l'énergie dérivée des quatre principaux substrats pendant un exercice prolongé à 65-75 % de VO_{2max}. Après 2 heures d'exercice, l'ingestion de glucides est nécessaire pour maintenir la concentration de glucose dans le sang et l'oxydation des glucides.

Issu de publications **A.** (Romijn et al. 1993) **B.** (Coyle 1995)

FIGURE 20 – CONTRIBUTION DES DIFFERENCES SOURCES D'ENERGIE AU COURS D'UN EFFORT PHYSIQUE

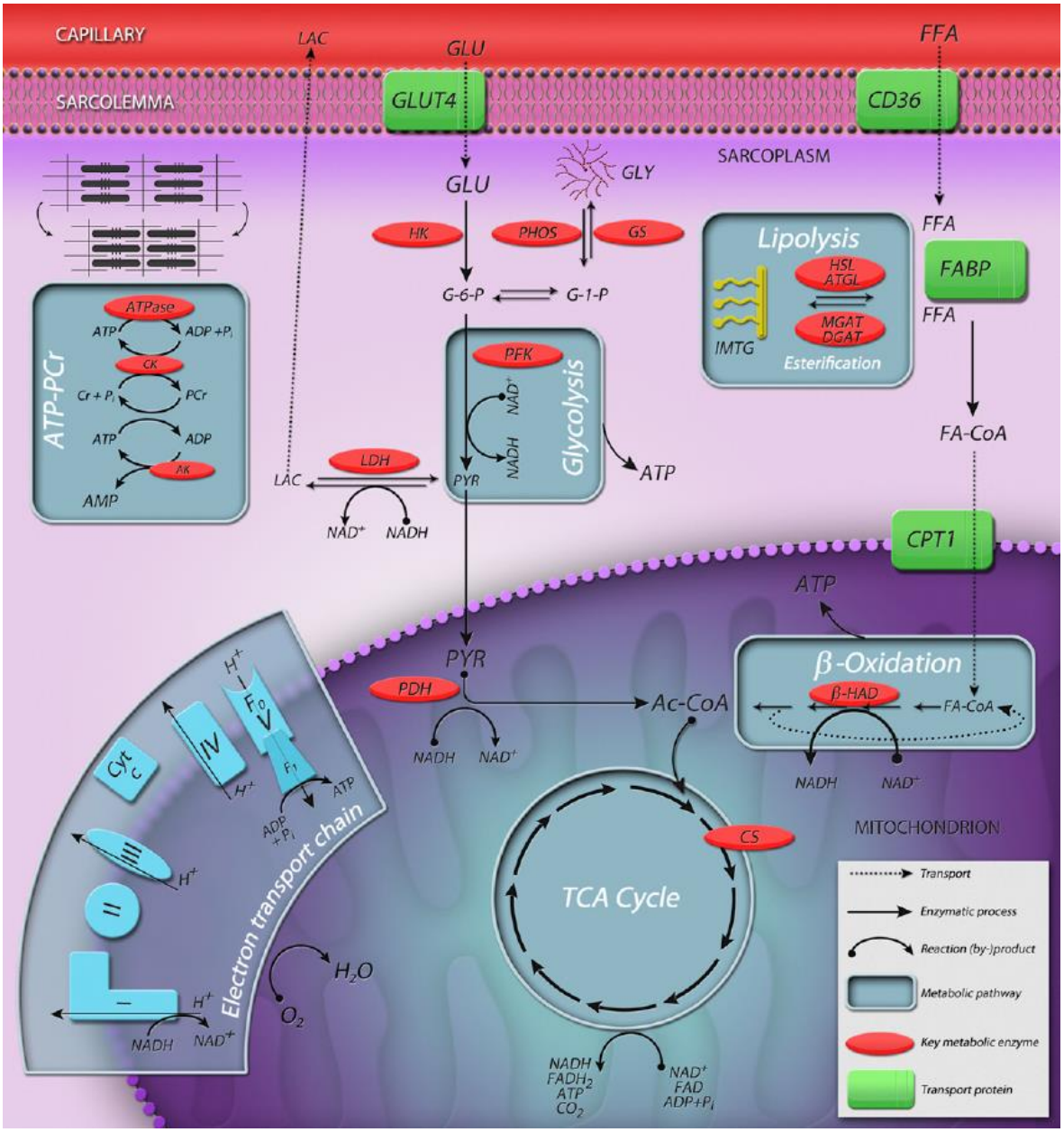
4.2.2. Métabolisme glycolytique et oxydatif à l'échelle cellulaire

Les substrats glucidiques sont essentiellement stockés sous la forme de glycogène, un polymère glucidique, au niveau hépatique et musculaire, tandis que les substrats lipidiques sont principalement stockés dans les tissus adipeux et musculaire (Purdom et al. 2018). Le glycogène musculaire est stocké dans le sarcoplasme sous forme de granules de 10 à 40 nm de diamètre et les lipides musculaires sont stockés sont forme de triglycérides dans des gouttelettes lipidiques du sarcoplasme.

La **Figure 21** représente les métabolismes glycolytique et oxydatif au niveau cellulaire. L'entrée du glucose dans le cytosol est permise par des transporteurs tels que glucose transporter type 4 (GLUT4) (Leto and Saltiel 2012). Le métabolisme glucidique débute par la glycolyse, responsable de la dégradation du glucose en 2 pyruvates, qui s'accompagne d'une production nette d'énergie de 2 ATP. En fonction des différentes conditions cellulaires, le pyruvate peut être réduit en lactate par la lactate déshydrogénase (filière anaérobie lactique), ou être transporté dans la mitochondrie par la pyruvate translocase pour être oxydé (filière aérobie) (Mukund and Subramaniam 2020). Dans ce dernier cas, le pyruvate est alors transformé en acétyl-coenzyme A (acétyl-CoA) par la pyruvate déshydrogénase. L'acétyl-CoA entre dans le cycle de réactions de décarboxylations oxydatives (cycle de Krebs) entraînant la formation des coenzymes oxydés par la chaîne respiratoire et associés à la création d'un gradient de protons H^+. La phosphorylation oxydative permet finalement la production d'ATP par l'ATP synthase en utilisant l'énergie du gradient protonique (Egan and Zierath 2013).

Les acides gras libres préalablement dissociés du glycérol entrent dans le cytosol par différents transporteurs tels que le transporteur des acides gras FAT/CD36 (Egan and Zierath 2013; Mukund and Subramaniam 2020) (**Figure 21**). L'acyl-CoA synthétase active les acides gras par la greffe de coenzyme A transformant ainsi les acides gras en acyl-CoA avant leur entrée dans la mitochondrie (Berg et al. 2002). La carnitine O-palmitoyltransferase 1 (CPT1) couple alors le transfert de l'acyl-CoA de la membrane

mitochondriale externe à la formation d'acyl-carnitine. La carnitine O-palmitoyltransferase 2 (CPT2) permet de coupler le transport dans la membrane mitochondriale interne avec la reconversion de l'acyl-carnitine en acyl-CoA, formant ainsi un système de navette (Mukund and Subramaniam 2020). L'acyl-CoA entre dans la β-oxydation (Eaton et al. 1996), aussi appelée hélice de Lynen. À chaque tour d'hélice, un acétyl-CoA est formé, deux carbones sont amputés à la molécule initiale d'acyl-CoA et des coenzymes destinés à être oxydés par la chaîne respiratoire sont produits. L'acétyl-CoA produit entre alors dans le cycle de Krebs, entraînant la formation de coenzymes ensuite oxydés dans la chaîne respiratoire et engendrant, ainsi que précédemment décrit, la production d'ATP (Egan and Zierath 2013; Mukund and Subramaniam 2020) (**Figure 21**).



Ac-CoA : acétyl-CoA ; AK : adénylate kinase (myokinase) ; ATGL : adipose triglycéride lipase ; CK : créatine kinase ; CPT1 : carnitine palmitoyltransférase 1 ; CS : citrate synthase ; Cyt c : cytochrome c ; DGAT : diacylglycérol acyltransférase ; FABPpm : protéine de liaison des acides gras ; FAT/CD36 : transporteur des acides gras ; FFA : acides gras libres ; GLU : glucose sanguin circulant ; GLUT4 : transporteur de glucose 4' ; GLY : glycogène musculaire, GS : glycogène synthase ; HK : hexokinase ; HSL : lipase hormonosensible ; IMTG : triglycérides intramusculaires ; LAC : lactate ; LDH : lactate déshydrogénase ; MGAT : monoacylglycérol acyltransférase ; PDH : complexe enzymatique pyruvate déshydrogénase ; PFK : phosphofructokinase ; PHOS : glycogène phosphorylase ; PYR : pyruvate ; TCA : cycle des acides tricarboxyliques

Issu de publication (Egan and Zierath 2013)

FIGURE 21 – METABOLISME ENERGETIQUE MUSCULAIRE

4.2.3. Métabolisme et typologie

La littérature montre que les différents types de fibres du muscle strié squelettique humain sont associés à différents profils métaboliques. Le **Tableau 7** présente les différences morphologiques et métaboliques en fonction de la typologie : fibres de type I, IIA ou IIX. Les fibres musculaires de type I sont associées à une forte capillarisation, une forte densité mitochondriale, de fortes activités enzymatiques oxydatives (citrate synthase, succinate déshydrogénase, 3-hydroxyl-CoA déshydrogénase), une faible concentration de phosphocréatine (filière anaérobie alactique) et de glycogène, une concentration élevée de triglycérides intramusculaires (IMTG) et jouent un rôle prépondérant dans les exercices de longues durées et de faibles intensités (Saltin and Gollnick 1983; Egan and Zierath 2013). Au contraire, les fibres musculaires de type IIX sont caractérisées par une faible capillarisation, une faible densité mitochondriale, de fortes activités enzymatiques glycolytiques (phosphofructokinase, glycogène phosphorylase, lactate déshydrogénase), une forte concentration de phosphocréatine et de glycogène et jouent un rôle important dans les exercices de courtes durées et fortes intensités (**Tableau 7**) (Essén et al. 1975; Saltin and Gollnick 1983; Egan and Zierath 2013).

4.2.4. Réseau micro vasculaire

Comme illustré dans le **Tableau 7**, la typologie musculaire est associée à des différences de capacité oxydative. La vascularisation, ainsi que le contenu mitochondrial et enzymatique, sont liés à la capacité oxydative de la fibre musculaire. Les muscles striés squelettiques sont oxygénés et fournis en nutriments par un réseau élaboré d'artères dont la densité varie selon les types musculaires. Les artères se divisent ensuite en un réseau de petites artères (artérioles) situées dans le périmysium qui se ramifient en un fin réseau capillaire (Mukund and Subramaniam 2020).

TABLEAU 7 – CARACTERISTIQUES MORPHOLOGIQUES ET METABOLIQUES DES DIFFERENTS TYPES DE FIBRES DU MUSCLE STRIE SQUELETTIQUE HUMAIN

		Type II	
	Type I	**Type IIA**	**Type IIX**
Morphological Properties			
Capillary density (capillaries per fiber)	4.2	4.0	3.2
Mitochondrial density	High	Intermediate	Low
Fiber size (cross-sectional area) [a]	5310	6110	5600
Percent distribution in whole muscle	54.0 ± 12.2 (50–55)	32.3 ± 9.1 (30–35)	13.0 ± 7.6 (10–20)
Myonuclear domain size	Small	Intermediate	Large
Glycolytic and Oxidative Enzyme Activities[b]			
Creatine kinase	13.1	16.6	
Phosphofructokinase	7.5	13.7	17.5
Glycogen phosphorylase	2.8	5.8	8.8
Lactate dehydrogenase	94	179	211
Citrate synthase	10.8	8.6	6.5
Succinate dehydrogenase	7.1	4.8	2.5
3-hydroxyl-CoA dehydrogenase	14.8	11.6	7.1
Metabolic and Substrate Properties			
Oxidative potential	High	Intermediate-high	Low
Glycolytic potential	Low	Intermediate-high	High
[Phosphocreatine] [c]	12.6	14.5	14.8
[Glycogen] [c]	77.8	83.1	89.2
[IMTG] [c]	7.1	4.2	
Exercise-type dominance	Prolonged low intensity	Moderate duration, high intensity	Short duration, maximal effort

[a] mm^2 ; [b] $mmol\ kg^{-1}\ min^{-1}$ except creatine kinase in $mmol\ min^{-1}\ g^{-1}$; [c] $mmol\ kg^{-1}$ wet weight, IMTG: intramuscular triglycerides

Issu de publication (Egan and Zierath 2013); adapté de (Saltin and Gollnick 1983) – données issues du *vastus lateralis* d'hommes non entraînés

L'étude de la densité du système vasculaire et de l'épaisseur des capillaires permet d'estimer la perfusion et représente donc un indicateur important du tissu musculaire, reflétant généralement sa capacité oxydative. Différents indices capillaires ont ainsi été élaborés afin d'estimer le flux des capillaires sanguins (Harris 2005). Dans cette revue, Harris distingue les indices globaux non fibre-dépendants des indices locaux fibre-dépendants (Harris 2005). Parmi les indices globaux se référant à une unité de surface de section musculaire, la densité capillaire est un indice fréquemment utilisé. Elle représente le nombre de capillaires sur une unité de surface musculaire (Harris 2005; Vincent et al. 2010). Ainsi qu'illustré sur la **Figure 22**, la densité capillaire peut être calculée sans repérage des fibres musculaires,

ce qui est la principale limite de cet indice. Les fibres musculaires n'étant pas considérées, deux surfaces musculaires peuvent contenir le même nombre de capillaires, mais avec des nombres de fibres très différents. L'indice de la densité capillaire ne traduirait alors aucune différence malgré une capillarisation individuelle des fibres différente. Contrairement à la densité capillaire, l'indice du ratio C/F tient indirectement compte de la taille des fibres puisqu'il consiste à calculer le rapport entre le nombre de capillaires et le nombre de fibres musculaires sur une même surface de tissu musculaire (**Figure 22**) (Harris 2005). Néanmoins, tenir compte de la taille des fibres peut également représenter une limite. Par exemple, dans le cas d'une fonte musculaire sans modification du réseau microvasculaire, la densité capillaire augmenterait tandis que le ratio C/F resterait identique. Pour autant, un nombre de capillaires par unité de surface augmenté pourrait être profitable pour l'irrigation de cette surface. Ainsi, ces différents indices présentent différents avantages et limites. L'analyse approfondie du réseau microvasculaire nécessite généralement les informations croisées obtenues à partir de différents indices.

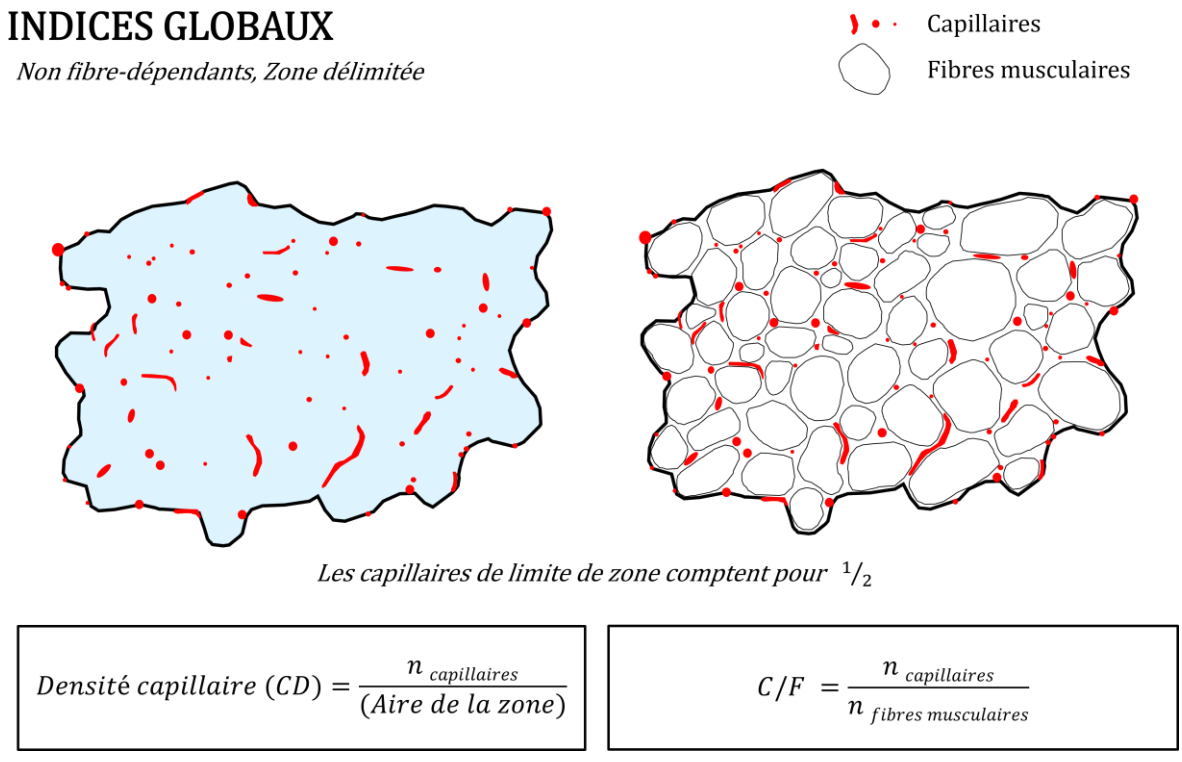


FIGURE 22 – INDICES GLOBAUX DES CAPILLAIRES DU TISSU MUSCULAIRE

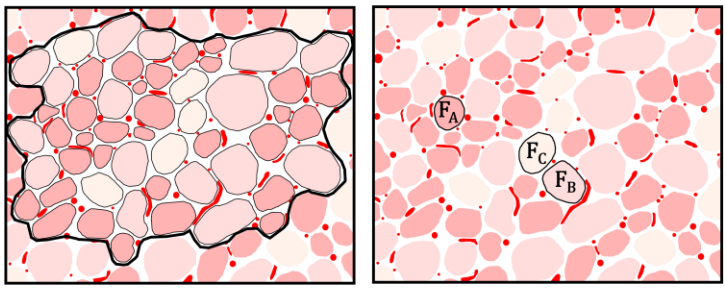
Contrairement aux indices globaux, les indices locaux se réfèrent à la fibre musculaire plutôt qu'à une unité de surface de section musculaire quelconque. Le fait de se référer à l'unité « fibre musculaire » permet généralement de proposer des analyses en fonction des différents types de fibres musculaires. L'indice des « capillaires au contact » (CC) rapporte le nombre de capillaires au contact avec la fibre musculaire considérée (Plyley and Groom 1975; Hepple et al. 1997; Harris 2005), ainsi qu'illustré sur la **Figure 23**. Cet indice ne donne pas d'indication sur la taille de la fibre ou sur le facteur de partage des capillaires. En effet, un capillaire peut être en contact avec un nombre de fibres musculaires plus ou moins élevé, ce qui diminuera l'apport en dioxygène et nutriments d'autant plus que le nombre de fibres musculaires irriguées par ce même capillaire sera élevé. Le facteur de partage d'un capillaire désigne le nombre de fibres en contact avec ce capillaire (Plyley and Groom 1975; Hepple et al. 1997; Harris 2005). Le CC de chaque fibre musculaire peut ainsi être pondéré par ce facteur de partage appliqué à chacun des capillaires entourant la fibre analysée (**Figure 23**). D'autres indices capillaires prennent en considération la taille des fibres musculaires, en ramenant le nombre de capillaires au contact de la fibre musculaire analysée à son périmètre (Hepple 1997) ou à sa surface de section transversale (Charifi et al. 2004). Une analyse plus poussée des caractéristiques morphologiques des micro-vaisseaux peut également être effectuée par l'analyse du diamètre des capillaire, de leur périmètre et de leur surface de section transversale (Vincent et al. 2010; Ravelojaona et al. 2015; Merlet et al. 2019). Il est également possible d'estimer la surface capillaire par unité de surface transversale musculaire en multipliant la densité capillaire par le périmètre des capillaires ; ce qui reflète la surface d'échange cumulée des capillaires avec le tissu musculaire (Vincent et al. 2010). De même, le produit de la densité capillaire par la surface de section transversale des capillaires fournit une estimation du volume sanguin irrigant la section musculaire (Vincent et al. 2010; Merlet et al. 2019). D'autre part, la tortuosité des capillaires influence particulièrement la perfusion musculaire et n'est pourtant pas prise en compte par les indices qui ne considèrent qu'un « nombre » de capillaires. Certains indices permettent cependant d'estimer la tortuosité, tel que l'indice LC/PF calculant le rapport entre la longueur cumulée des capillaires en contact

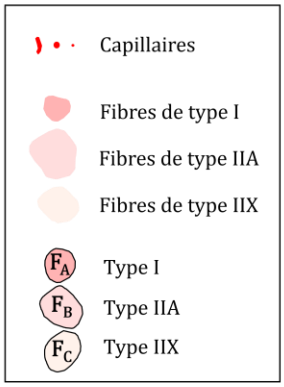
avec la fibre (LC) sur le périmètre de la fibre musculaire (PF) (**Figure 23**) (Charifi et al. 2004; Charles et al. 2006). En 2010, un nouvel indice de mesure de la tortuosité des capillaires (CapTor) est inventé (Vincent et al. 2010). Cet indice évalue le rapport entre la surface du capillaire tortueux ramené à la surface qu'il occuperait s'il n'était pas tortueux mais disposé de façon parallèle aux fibres musculaires, et donc coupé transversalement (Vincent et al. 2010; Ravelojaona et al. 2015; Merlet et al. 2019) (**Figure 23**). La critique est faite par Vincent et ses collaborateurs que l'indice LC/PF ne serait fiable pour estimer la tortuosité capillaire que dans le cas où les surfaces des fibres et/ou capillaires resteraient inchangées entre les points de comparaison. Pour cette équipe, l'indice CapTor constituerait ainsi un indice particulièrement adapté d'estimation de la tortuosité capillaire, même si cette équipe attribue toujours à l'indice LC/PF un rôle fonctionnel majeur d'indice « d'échange » et donc de « perfusion » de la fibre.

Ainsi, l'analyse du réseau microvasculaire du tissu musculaire est complexe et de nombreux indices reflétant différents aspects de la perfusion sanguine musculaire existent, présentant chacun des avantages et des limites. Le choix des indices capillaires nécessite d'être adapté au rationnel et à l'objectif de l'étude.

INDICES LOCAUX

Fibre-dépendants



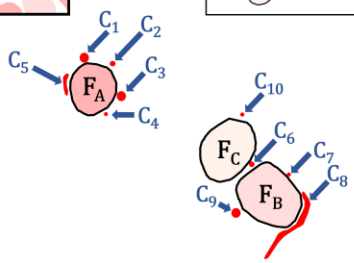


CAPILLAIRES AU CONTACT (CC ou CAF)

$$CC\ (F_{A\ \_\ TYPE\ I}) = n\ (C_1 + C_2 + C_3 + C_4 + C_5) = 5$$

$$CC\ (F_{B\ \_\ TYPE\ IIA}) = n\ (C_6 + C_7 + C_8 + C_9) = 4$$

$$CC\ (F_{C\ \_\ TYPE\ IIX}) = n\ (C_6 + C_{10}) = 2$$



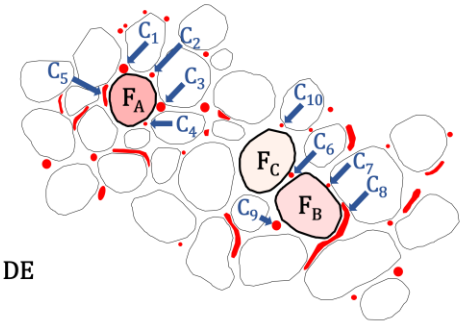
FACTEUR DE PARTAGE (SF)

$SF\ (C_1) = 3$ $SF\ (C_5) = 4$ $SF\ (C_9) = 3$

$SF\ (C_2) = 3$ $SF\ (C_6) = 2$ $SF\ (C_{10}) = 2$

$SF\ (C_3) = 3$ $SF\ (C_7) = 2$

$SF\ (C_4) = 3$ $SF\ (C_8) = 4$



CAPILLAIRES AU CONTACT TENANT COMPTE DU FACTEUR DE PARTAGE (CC avec SF)

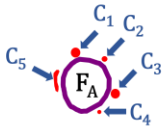
$$CC\ avec\ SF\ (F_{A\ \_\ TYPE\ I}) = \left(\frac{1}{SF\ (C_1)}\right) + \left(\frac{1}{SF\ (C_2)}\right) + \left(\frac{1}{SF\ (C_3)}\right) + \left(\frac{1}{SF\ (C_4)}\right) + \left(\frac{1}{SF\ (C_5)}\right) = \left(\frac{1}{3}\right) + \left(\frac{1}{3}\right) + \left(\frac{1}{3}\right) + \left(\frac{1}{3}\right) + \left(\frac{1}{4}\right)$$

$$CC\ avec\ SF\ (F_{B\ \_\ TYPE\ IIA}) = \left(\frac{1}{SF\ (C_6)}\right) + \left(\frac{1}{SF\ (C_7)}\right) + \left(\frac{1}{SF\ (C_8)}\right) + \left(\frac{1}{SF\ (C_9)}\right) = \left(\frac{1}{2}\right) + \left(\frac{1}{2}\right) + \left(\frac{1}{4}\right) + \left(\frac{1}{3}\right)$$

$$CC\ avec\ SF\ (F_{C\ \_\ TYPE\ IIX}) = \left(\frac{1}{SF\ (C_6)}\right) + \left(\frac{1}{SF\ (C_{10})}\right) = \left(\frac{1}{2}\right) + \left(\frac{1}{2}\right)$$

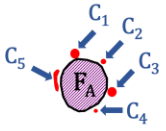
INDICE D'ÉCHANGE CAPILLAIRES – PÉRIMÈTRE DE FIBRE (Indice CFPE)

$$CFPE\ (F_{A\ \_\ TYPE\ I}) = \frac{CC\ (F_A)}{PE\ (F_A)}$$



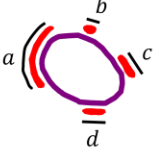
CAPILLAIRES PAR SURFACE DE SECTION TRANSVERSALE (CSA) DE FIBRE (Indice CAFA)

$$CAFA\ (F_{A\ \_\ TYPE\ I}) = \frac{CC\ (F_A)}{CSA\ (F_A)}$$



Indice LC/PF

$$LC/PF = \frac{\sum Longueurs\ des\ capillaires}{Périmètre\ de\ la\ fibre} = \frac{(a+b+c+d)}{PF_{violet}}$$



Indice CapTor

$$CapTor = \frac{Surface\ réelle\ du\ capillaire\ tortueux\ (aire\ rouge)}{Surface\ théorique\ du\ capillaire\ transversal\ (aire\ violette)} = \frac{Aire\ mesurée}{\pi \times R^2} = \frac{Aire\ mesurée}{\pi \times (D/2)^2}$$

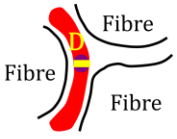


FIGURE 23 – INDICES LOCAUX DES CAPILLAIRES DU TISSU MUSCULAIRE

4.3. Approche technique de l'histologie musculaire

Le tissu musculaire est un tissu complexe et son exploration a nécessité le développement de différentes techniques d'étude, notamment en histologie. L'hétérogénéité typologique reflétant l'hétérogénéité métabolique et fonctionnelle du tissu musculaire est connue maintenant depuis longtemps. La première observation d'une différence de couleur dans le tissu musculaire aurait été rapportée par Lorenzini dès 1678 : "In uno stesso piede di coniglio io ho osservato i muscoli e bianchi e rossi" (« Dans la même patte de lapin, je voyais dans les muscles et le blanc et le rouge ») (Appell and Hammersen 1979). En 1873, l'histologiste français Louis-Antoine Ranvier décrit pour la première fois les différences de propriétés et de structures entre les « muscles rouges » et les « muscles blancs » (Ranvier 1873). Plus tard, la découverte des différentes propriétés histochimiques de l'ATPase myofibrillaire (Bárány et al. 1965) a longtemps été utilisée pour différencier les types de fibres musculaires (Brooke and Kaiser 1970; Johnson et al. 1973; Essén et al. 1975; Denis et al. 1986; Proctor et al. 1995; Gavin et al. 2015). Plus récemment, les propriétés immunologiques de la myosine ont été exploitées pour mettre en évidence la typologie musculaire en lumière blanche (Schiaffino et al. 1989; Gorza 1990; Kadi et al. 1998) ou en utilisant la fluorescence ces dernières années (Bloemberg and Quadrilatero 2012; Nederveen et al. 2016; Merlet et al. 2019). Les premières techniques d'identification des capillaires reposaient quant à elles sur l'association de l'amylase à une coloration à l'acide périodique de Schiff (Andersen 1975). Différents marqueurs des capillaires ont ensuite été utilisés (Qu et al. 1997) jusqu'à être progressivement remplacés par des approches immunologiques (Charifi et al. 2004; Charles et al. 2006; Ravelojaona et al. 2015; Nederveen et al. 2016).

L'analyse du réseau microvasculaire par des indices fibre-dépendants nécessite une identification typologique. Bien que la nécessité d'une identification simultanée de la typologie musculaire et du réseau microvasculaire ait été soulevée depuis longtemps (Rosenblatt et al. 1987; Paljärvi and Naukkarinen 1990; Eržen and Maravić 1993), peu de solutions techniques optimales semblent avoir été

proposées depuis. Pourtant, le recours à l'utilisation de coupes sériées présente de nombreuses limitations, et plus encore sur le tissu musculaire humain. Ainsi qu'illustré par la **Figure 24**, l'utilisation de coupes sériées multiplie l'utilisation du tissu musculaire par le nombre de lames nécessaire pour les différentes techniques de colorations. Or, le prélèvement de tissu est un acte invasif, ce qui implique d'un point de vue éthique, d'utiliser ce tissu de façon optimale dans nos approches méthodologiques. Plus le tissu est « rentabilisé » lors de l'expérimentation, moins, les prélèvements seront nécessaires en termes de fréquence et de quantité. De plus, les coupes sériées nécessitent le repérage des mêmes fibres musculaires entre les différentes lames (**Figure 24**), ce qui pose deux limitations majeures : une perte de temps et un manque de précision. L'identification d'une fibre musculaire parmi des milliers d'autres est très chronophage et peut également être particulièrement complexe en cas de difficultés techniques à la coupe. En effet, lorsque la coupe du tissu est difficile, les coupes peuvent être mal orientées et plus éloignées dans l'espace en cas de déchets à la coupe. La reconnaissance d'une même fibre entre les différentes coupes est alors rendue encore plus lente et difficile. Par ailleurs, la fibre musculaire évoluant en quelques dizaines ou centaines de micromètres, la perte de précision est réelle si ces micromètres sont perdus entre les différentes coupes sériées. À plus long terme, l'aspect très chronophage de ce type d'analyse peut ralentir les travaux de recherche.

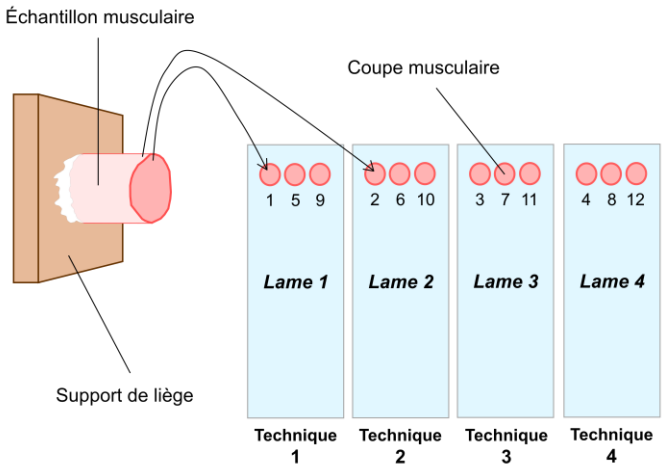


FIGURE 24 – PRINCIPE DES COUPES SERIEES

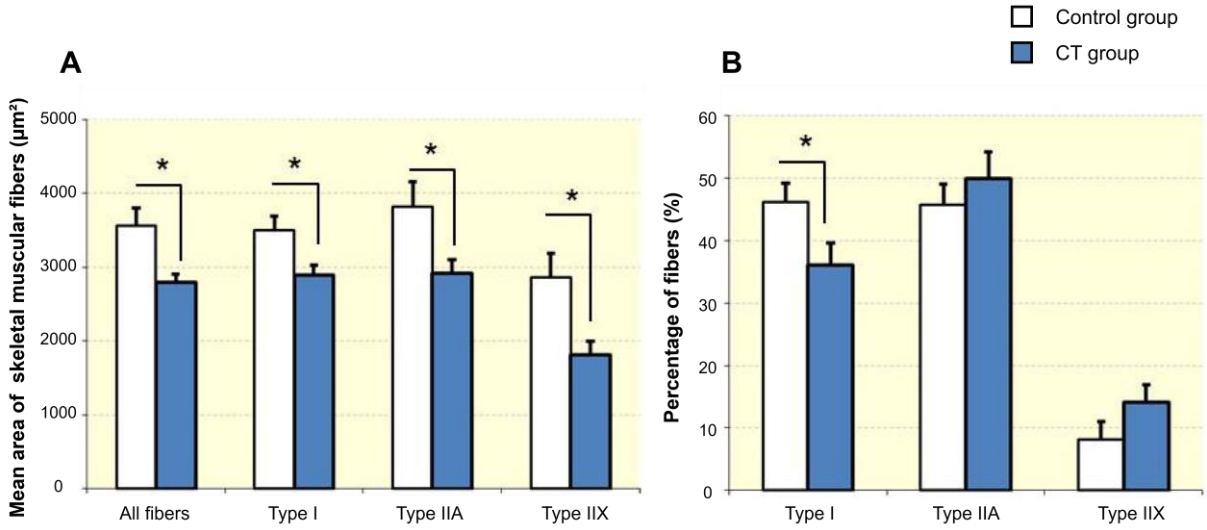
Certaines publications ont mis au point des techniques immunohistologiques permettant de colorer, sur une même coupe de tissu musculaire, les fibres de type I et II, les capillaires, et la lame basale des fibres musculaires (Snijders et al. 2016; Nederveen et al. 2016; Tan et al. 2018). Néanmoins, ces techniques ne permettent pas la distinction des fibres IIX et des fibres IIA, dont les propriétés métaboliques et fonctionnelles sont pourtant très différentes (**Tableau 6** et **Tableau 7**). À notre connaissance, seule une étude a réalisé une coloration simultanée des trois types de fibres, des capillaires, et de la lame basale sur une même coupe musculaire (Al-Shammari et al. 2019), en utilisant la lectine *Ulex Europaeus* Agglutinin (UEA 1). Aucune étude ne semble cependant avoir réalisé une coloration simultanée des trois types de fibres, de la lame basale, et des capillaires en utilisant l'anticorps CD31, reconnu pour être un marqueur très sensible et spécifique des cellules endothéliales (Duscha et al. 1999) puisqu'il agit en se fixant à la molécule d'adhésion des cellules endothéliales plaquettaires-1 (platelet endothelial cell adhesion molecule (PECAM-1)). Le développement de nouvelles méthodes de coloration immunohistochimiques permettant la coloration simultanée de la typologie, de la capillarisation, et de la lame basale fera l'objet de l'**étude 4** de ce travail de thèse.

4.4. Cas de la maigreur constitutionnelle

4.4.1. Analyse du tissu musculaire dans la maigreur constitutionnelle

À notre connaissance, seule une publication a investigué le tissu musculaire de personnes MC (Galusca et al. 2018), ce qui témoigne d'une quasi-absence de littérature concernant les caractéristiques structurelles et fonctionnelles du muscle strié squelettique dans un contexte de MC. Cette étude publiée en 2018 n'incluait que des femmes, à savoir 10 participantes MC (IMC < 17.5 kg/m²) et 10 participantes T (Galusca et al. 2018). Les critères d'inclusion des sujets MC vérifiaient notamment l'absence de trouble alimentaire, une stabilité pondérale et l'absence de maladie, aiguë, chronique ou congénitale, telle qu'une myopathie, pouvant altérer le tissu musculaire. L'analyse histologique des biopsies musculaires montre une surface de section transversale des fibres musculaires de type I, IIA, et IIX plus faible chez

les participantes MC *vs.* T (Galusca et al. 2018) (**Figure 25**). Les participantes MC présenteraient ainsi des fibres de petites tailles. La proportion des fibres de type I est plus faible chez les participantes MC que chez les participantes T, tandis qu'aucune différence significative n'apparaît pour la proportion des fibres de type IIA et IIX (**Figure 25**). Néanmoins, ce résultat n'est plus significatif lorsque la proportion des fibres de type I est exprimée en pourcentage de l'aire occupée par ces fibres (meilleure estimation du volume occupé par ces fibres dans le muscle) (Galusca et al. 2018).



C (sujets témoins), CT (sujets maigres constitutionnels)

Issu de publication (Galusca et al. 2018)

FIGURE 25 – SURFACES DE SECTIONS TRANSVERSALES DES DIFFERENTS TYPES DE FIBRES MUSCULAIRE ET TYPOLOGIE CHEZ DES FEMMES PRESENTANT UNE MAIGREUR CONSTITUTIONNELLE

Concernant le réseau microvasculaire, le ratio C/F est inférieur chez les participantes MC (**Tableau 8**). Les indices locaux fibre-dépendants investigués dans cette étude révèlent également une plus faible capillarisation musculaire chez les participantes MC. Les indices du CC (en tenant compte ou non du facteur de partage) et du ratio LC/PF montrent des résultats plus faibles chez les sujets MC *vs.* T pour tous les types de fibres (I, IIA, et IIX) (Galusca et al. 2018). L'activité de la cytochrome *c* oxydase de la chaîne respiratoire, évaluée par coloration histochimique (diaminobenzidine), apparaît plus faible chez les participantes MC *vs.* T pour les fibres de type I uniquement (**Tableau 8**). Le seuil de significativité

n'est pas atteint pour les fibres de type IIA et IIX. L'analyse des activités enzymatiques de la créatine kinase, la phosphofructokinase, la citrate synthase et l'hydroxyacyl-CoA dehydrogenase ne semble cependant pas montrer de différences entre les participantes MC et T (Galusca et al. 2018).

TABLEAU 8 – RESEAU MICRO VASCULAIRE DU MUSCLE STRIE SQUELETTIQUE DE FEMMES PRESENTANT UNE MAIGREUR CONSTITUTIONNELLE

	CT (n = 10)	Controls (n = 10)	*P*-value
Capillary global indexes			
Capillary Density (capillaries/mm^2)	395 ± 10	420 ± 14	.22
C/F (capillaries/fibre)	1.27 ± 0.06	1.73 ± 0.11	<.01
Fibre-type-specific indexes			
Type I fibres			
CC (capillaries/fibre)	2.75 ± 0.12	3.61 ± 0.13	<.01
C/F$_i$ (capillaries/fibre)	1.02 ± 0.05	1.36 ± 0.06	<.01
LC/PF (%)	12 ± 0.7	15 ± 0.8	<.01
Type IIA fibres			
CC (capillaries/fibre)	2.62 ± 0.15	3.27 ± 0.21	<.05
C/F$_i$ (capillaries/fibre)	0.97 ± 0.05	1.22 ± 0.08	<.05
LC/PF (%)	11 ± 0.4	13 ± 0.6	<.05
Type IIX fibres			
CC (capillaries/fibre)	1.59 ± 0.08	2.32 ± 0.16	<.01
C/F$_i$ (capillaries/fibre)	0.57 ± 0.03	0.84 ± 0.07	<.01
LC/PF (%)	8 ± 0.4	10 ± 0.7	<.05
COX activity			
Type I (AU)	42.0 ± 2.1	50.6 ± 2.2	<.01
Type IIA (AU)	29.4 ± 2.3	31.9 ± 2.8	.46
Type IIX (AU)	14.7 ± 2.1	19.9 ± 3.0	.14

CT (sujets maigres constitutionnels), Controls (sujets témoins), AU: unité arbitraire, CC: capillaires au contact, C/Fi: CC tenant compte du facteur de partage, COX: cytochrome *c* oxydase, LC/PF: longueur cumulée de contact des capillaires avec la fibre musculaire sur le périmètre de la fibre musculaire

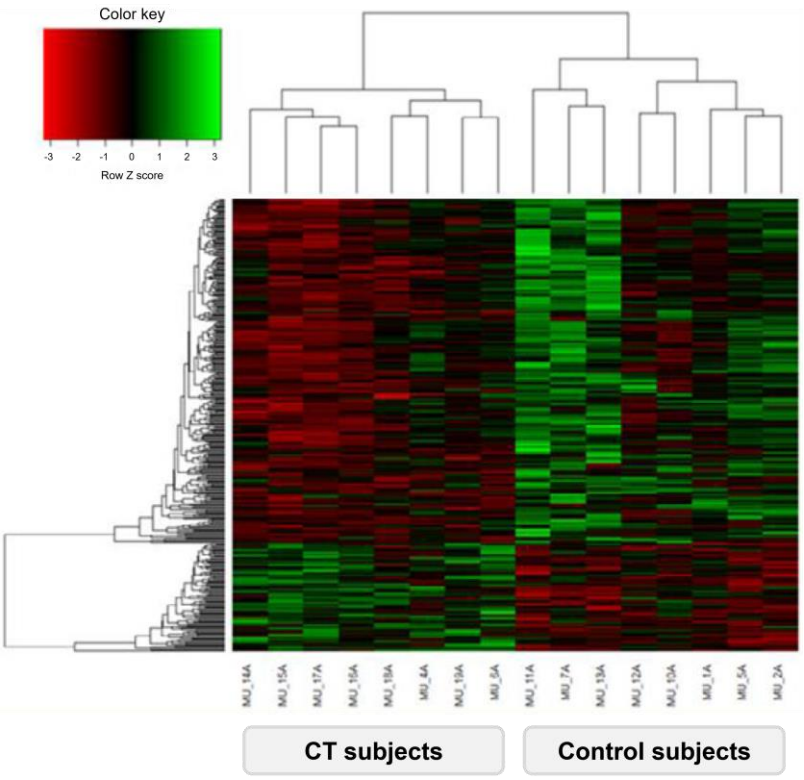
Issu de publication (Galusca et al. 2018)

Finalement, l'analyse du muscle strié squelettique de 10 participantes MC *vs.* 10 participantes T a mis en évidence des fibres musculaires de petite taille, une proportion plus faible de fibres oxydatives, une activité diminuée de la cytochrome *c* oxydase dans les fibres de type I, ainsi qu'une capillarisation musculaire plus faible (Galusca et al. 2018). Cette unique étude ayant porté sur l'analyse du tissu

musculaire dans un contexte de MC rapporterait ainsi un profil musculaire faiblement oxydatif chez cette population (Galusca et al. 2018).

4.4.2. Approches transcriptomiques

Les analyses transcriptomiques du tissu musculaire des participantes MC *vs.* T révèlent un phénotype spécifique dans le cas d'une MC. L'analyse montre que 386 gènes du tissu musculaire ont un niveau d'expression significativement différent entre les participantes MC et T, dont 293 seraient sous-exprimés chez les participantes MC. Une approche par regroupement hiérarchique de ces profils de gènes révèle effectivement une classification en deux groupes discriminants : MC et T (**Figure 26**).



Analyse de regroupement hiérarchique des sondes à régulation différentielle entre les sujets témoins (n=10) (control subjects) et les sujets maigres constitutionnels (CT subjects) (n=10).

Issu de publication (Galusca et al. 2018)

FIGURE 26 – ANALYSES TRANSCRIPTOMIQUES DU TISSU MUSCULAIRE DE FEMMES PRESENTANT UNE MAIGREUR CONSTITUTIONNELLE

L'étude montre qu'environ 10 % des gènes différemment exprimés entre les individus MC et T serait directement liés à l'activité mitochondriale et seraient sous-exprimés dans la MC. De façon intéressante, plusieurs gènes impliqués dans le stockage et le métabolisme des triglycérides apparaissent sous-exprimés dans le tissu musculaire des participantes MC. C'est en particulier le cas pour les gènes « fat storage-inducing transmembrane 1 » (FITM1) et « fat storage-inducing transmembrane 2 » (FITM2) (Galusca et al. 2018). FITM1 est une protéine transmembranaire spécifique du muscle squelettique tandis que FITM2 est exprimée dans de nombreux tissus (Kadereit et al. 2008). Ces deux protéines transmembranaires jouent un rôle important dans le stockage des triglycérides nouvellement synthétisés sous forme de gouttelettes lipidiques (Kadereit et al. 2008). Si ces observations tendent à suggérer un stockage d'IMTG potentiellement amoindri dans le cadre d'une MC, aucune étude n'a cependant directement évalué le stockage lipidique musculaire dans la MC.

4.4.3. Vers de nouveaux intérêts

L'analyse de la littérature portant sur le tissu musculaire du sujet MC est extrêmement faible et se limite, à notre connaissance, à une étude réalisée chez 10 femmes MC *vs.* 10 femmes normo-pondérées T (Galusca et al. 2018). Tant par ses approches histologiques que transcriptomiques, cette étude a soulevé plusieurs caractéristiques atypiques du tissu musculaire des participantes MC suggérant des propriétés structurelles, fonctionnelles et métaboliques musculaires particulières chez cette population. Malgré de premiers résultats prometteurs, cette étude a été réalisée sur un échantillon relativement faible et constitué uniquement de femmes et sans évaluation des stockages énergétiques musculaires (IMTG et glycogène).

PARTIE II – CONTRIBUTION PERSONNELLE

OBJECTIFS DE LA THÈSE

La revue de la littérature a tout d'abord mis en exergue le manque de connaissance et de reconnaissance de la MC. Il a été reporté une absence de consensus et une ambiguïté à propos de la définition même de la MC et de son diagnostic, dont les critères apparaissent multiples et hétérogènes. Les enjeux sont pourtant considérables, tant sur la considération médicale de la MC en tant que diagnostic différentiel de l'AM, que sur la possibilité de comparer les différentes études cliniques menées auprès de cette population. Au-delà de l'hétérogénéité des critères de diagnostic et d'inclusion des participants MC, les études semblent également rapporter des résultats équivoques mais également divers dans leur domaine d'étude. La revue de littérature a notamment soulevé d'importantes dissemblances entre les études concernant les questions de composition corporelle, de balance énergétique et de bilans biochimiques dans le cadre de la MC. Finalement, les différents éléments de la revue de littérature ont soulevé la nécessité clinique de s'intéresser de façon systématique au diagnostic et à la physiologie de la MC.

Un premier axe : « **Diagnostic et physiologie de la maigreur constitutionnelle : approche systématique et méta-analytique** » est ainsi tout d'abord développé dans ce travail de doctorat. Cet axe est composé de 3 études :

- **Étude 1 : Définition et diagnostic de la maigreur constitutionnelle : revue systématique**
- **Étude 2 : Composition corporelle atypique de la maigreur constitutionnelle**
- **Étude 3 : Physiologie de la maigreur constitutionnelle : revue systématique et méta-analyse**

De façon intéressante, l'auteur P. Maffetone a soulevé dans sa lettre à l'éditeur concernant l'**étude 1** l'importance de considérer le métabolisme lié à la masse grasse et du muscle squelettique dans la MC

(**publication en annexe**). Le *British Journal of Nutrition* nous a invités à une réponse à ces éléments intéressants soulevé par P. Maffetone. Notre réponse a ainsi pu être également publiée (**publication en annexe**).

D'autre part, la revue de littérature a rapporté différents résultats soulevant des questions quant aux aspects structurels et métaboliques du tissu musculaire chez le sujet MC. L'apport de la littérature a ainsi soulevé l'intérêt d'explorer le muscle strié squelettique chez des femmes mais également chez des hommes présentant une MC, notamment en termes de typologie, de taille des fibres musculaires, de capillarisation, de capacité oxydative mitochondriale mais également de stockage des substrats énergétique. D'un point de vue méthodologique, la littérature témoigne de différentes limitations inhérentes aux techniques de colorations immunohistochimiques des capillaires et typologiques actuellement utilisées.

Un second axe : « **Phénotypage du tissu musculaire de personnes présentant une maigreur constitutionnelle** » est ensuite développé dans ce travail de doctorat. Cet axe est composé de 2 études :

- **Étude 4 : Développement et validation de deux nouvelles méthodes d'analyse en histologie musculaire (étude préliminaire)**
- **Étude 5 : Caractérisation du phénotype musculaire chez des femmes et des hommes présentant une maigreur constitutionnelle et effet d'une surnutrition sur leur phénotype musculaire**

AXE 1 : Diagnostic et physiologie de la maigreur constitutionnelle : approche systématique et méta-analytique

ÉTUDE 1 : DÉFINITION ET DIAGNOSTIC DE LA MAIGREUR CONSTITUTIONNELLE : REVUE SYSTÉMATIQUE

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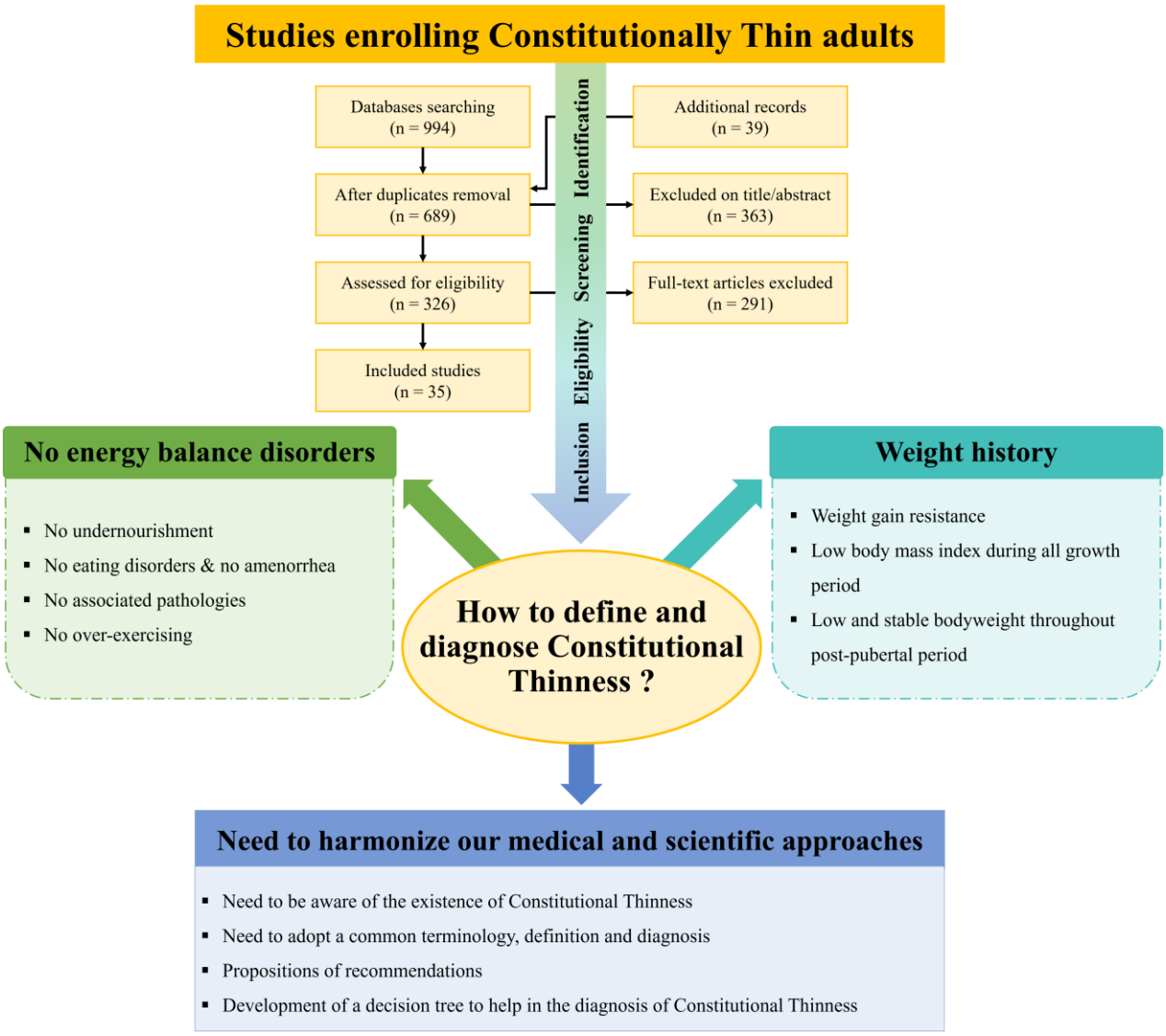

Graphical abstract

Definition and diagnosis of constitutional thinness: a systematic review

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Keywords: Constitutional thinness; Constitutional leanness; Diagnosis; Weight gain resistance; Underweight

Abstract

The existing literature about the definition and diagnostic criteria of constitutional thinness appears equivocal. The present work systematically reviewed the criteria used in the diagnosis of adult individuals with constitutional thinness (PROSPERO registration number: CRD42019138236). Five electronic bibliographic databases were searched between December 2018 and November 2019: MEDLINE, EMBASE, CENTRAL (Cochrane Library), Google Scholar and CLINICAL TRIALS. Search terms were combined with Medical Subject Headings (MeSH) terms. The search strategy included any clinical trials that enrolled adults with constitutional thinness. Studies were systematically excluded if the state of thinness was not due to a well-identified constitutional origin. From the 689 references after duplicates removal, 199 studies were excluded based on title and 164 based on abstract. According to the inclusion and exclusion criteria, 291 other studies were removed. Finally, 35 studies remained at the end of the process. The analysis of these studies showed high heterogeneity in the diagnostic criteria of constitutional thinness. It emerged the real need to adopt a common terminology and to systematically exclude potential non-constitutional origins of thinness such as eating disorders, associated pathology or over-exercising, with validated tools. Weight history, physiological menses, and weight gain resistance are also important criteria to consider. The present systematic review revealed that our medical and scientific approaches of constitutional thinness need to be harmonized in terms of terminology and diagnostic criteria. Although further studies are needed, we finally proposed recommendations and a decision tree to help in the recognition and diagnosis of constitutional thinness.

Introduction

As early as 1933, the existence of constitutional thinness (CT) had already been mentioned by Erich Grafe [1], followed by the first observations of Passmore et al. [2] and Genest et al. [3] in 1955. In a French publication from 1953 [4], Bernard Wissmer wondered why CT and its treatment had raised so little consideration contrary to obesity. This remark is still valid about sixty years later with obesity and its treatment being widely investigated while CT remains poorly studied [5]. Although there is a growing preoccupation for CT among clinicians due to an increasing number of individuals presenting thinness and seeking to gain weight without apparent criteria of anorexia nervosa (AN); the prevalence of CT remains difficult to determine [5], but would be less than 0.4% for males and less than 2.7% for females (underweight from all causes) [6]. Despite a large proportion of concerned individuals, a lot of them do not consult because of a lack of recognition and diagnosis of this condition. Given this lack of interest in literature, CT is poorly described, which can favor its misunderstanding and misdiagnosis [5], mainly with AN. Although CT and AN are both characterized by a low body mass index (BMI), people with CT do not present eating disorders, food restriction, psychological disorders, hormonal signs of undernutrition but present an equilibrated energy metabolism, stable bodyweight within lower percentiles of growth curve and physiological menses for females [7–11]. Despite these clinical differences, the distinction between AN and CT remains difficult. Guy-Grand & Badevant proposed a first decision tree to diagnose CT in the early eighties [12], but its diagnosis is still debated; especially with the removal of amenorrhea criterion from the definition of AN in the Diagnostic and Statistical Manual of Mental Disorder V (DSM-V) [8,13]. In our modern societies, individuals with CT have to face social stigmatization similar to that of anorectic patients [14], due to their low bodyweight and corpulence. Unlike patients with AN, people with CT show an important desire to gain weight, which is the main reason for medical consultation [5]. As already noted in 1982 [15], the demand of individuals with CT for clinical examination is stereotyped; they are concerned about their thinness and dissatisfied with their morphology usually

judged for its lack of femininity for women or virility for men. CT seems then to be a natural state of underweight leading to a high self-dissatisfaction and whose causes remain unclear. While absolute resting energy expenditure (REE) was found lower [7,8,10] or similar [16–18] in CT individuals *vs* normal-weight control subjects, REE-to-fat-free mass (FFM) ratio was found higher in CT *vs* control subjects in some studies [7,18], but not significantly higher in some other studies [10,17,19]. Other evidence seems to indicate a more pronounced brown fat activity in CT [20]. Despite an apparently similar energy intake (quantitatively as well as qualitatively) as normal-weight people [5,7,9,10,19], specific physiological control of appetite has been suggested in individuals with CT [9–11,21–23], with for instance an earlier and higher satiety onset during meals leading to reduced but more frequent intakes (more in-between meal snacking) [10]. CT subjects present no eating disorder-related traits and even have lower food restrictive behaviors compared to normal-weight people [8,10]. Despite their low BMI, they present a non-blunted fat mass (FM) percentage [7,8,10,11,17,19,23–27]. However, CT people display impairments in their bone quality: small bone sizes, low bone mass, low calculated breaking strength [28], low bone mineral density [19,24,26,28], but however apparent normal bone turnover [28]. Even if the potential increased risk of osteoporosis with aging in CT remains to be robustly demonstrated, these bone impairments could be considered as the main comorbidity associated with CT. This public health concern might not be the only one, but issues in the recognition and diagnosis of CT likely lead to a lack of knowledge. With 2.5 thin subjects per family in CT *vs* 0.5 in AN, CT is strongly suggested to be a heritable trait likely attributable to genetic factors [7,29,30]. Moreover, the exploration of the genetic architecture of thinness demonstrated the polygenic component of CT: genome-wide association studies revealed evidence of loci that could confer susceptibility of CT and also be informative in the identification of potential anti-obesity targets [30]. While there is a growing scientific and clinical interest to better understand and characterize CT, the used inclusion and exclusion criteria remain highly heterogeneous in-between studies, making any comparison and conclusion difficult. This high variability in CT diagnosis underlines today a clear need for a common definition of CT and harmonized criteria that should be used for CT detection. According

to the recent literature [8,10,18,23,30,31], parameters such as the terminology used, the characterization and fluctuation of the level of thinness, the consideration of psychological or physical illnesses, the weight gain resistance, or the level of physical activity appear, *a priori*, to be the main parameters to focus on in this systematic review. Thus, the present paper proposed a systematic analysis of all the parameters used so far as inclusion criteria of CT individuals in the available studies, trying to suggest a clear definition and diagnostic method of CT.

Materials and Methods

The systematic literature search was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and was registered in the International Prospective Register of Systematic Reviews (PROSPERO registration number: CRD42019138236).

Search strategy

The search was conducted on CT and aimed to include any clinical trials enrolling a group of adults with CT. Five electronic bibliographic databases were searched between December 2018 and November 2019: MEDLINE, EMBASE, CENTRAL (Cochrane Library), GOOGLE SCHOLAR and CLINICAL TRIALS. Relevant keywords were discussed and selected between the co-authors. Search terms were also combined with MeSH terms. The following syntax was finally used to search on the MEDLINE database: ((constitution[TI] OR constitutional[TI] OR constitutionally[TI]) AND (thinness[TI] OR leanness[TI] OR thin[TI] OR lean[TI])) OR "constitutional thinness"[TW] OR "constitutional leanness"[TW] OR (((resistance[TI] OR resistant[TI]) AND "weight gain"[TI]) NOT "insulin resistance"[TI]) OR ("thinness/physiology"[Mesh] OR ((physiological[TI] OR physiologically[TI] OR physiology[TI]) AND (thinness[TI] OR leanness[TI] OR thin[TI] OR lean[TI])) NOT "obesity"[Mesh]) AND ("humans"[Mesh] OR "humans"[TW] OR "human"[TW]). Searches were carried out on articles published from 1950. Adapted syntaxes were used to perform the

search on the other databases. The authors collectively discussed any discrepancies. All the selected references were then extracted to Zotero Software (5.0.21, CHNM, GMU, USA).

Study eligibility

Inclusion criteria

Clinical trials had to be published in English or French languages and had to enroll constitutional thin/lean adult females or males. Any fields of study could be included in the analysis. However, experiments on animals and clinical trials on children were not eligible for the systematic review. In addition, studies were not included if not enough data were available: letters to the Editor, reviews, abstracts alone, or case studies. Only thinness due to a "constitutional" origin was considered. To do this, papers had to mention at least one of these criteria: "constitutional thin/lean" keywords, state of thinness confirmed by measurements, absence of eating disorders, no over-exercising, no associated pathology, physiological menstruations, stable bodyweight, and/or weight gain resistance/desire.

Exclusion criteria

Studies were excluded if thinness was not due to a well-identified constitutional origin, such as associated diseases, undernourishment, eating disorders, over-exercising, or any "non-constitutional" origins causing a state of thinness. Specific attention was given to the large number of studies that wrongly named their normal-weight control groups as "lean" groups. Normal-weight "lean" control groups were not considered as "constitutional lean" groups and were therefore excluded from the systematic review.

Data extraction and synthesis of results

After the removal of duplicates, a first selection was performed on titles and abstracts of studies to assess eligibility of identified records through databases searching. Full-text articles were then screened and included according to the aforementioned inclusion and exclusion criteria. At each step of this process, a second screener assessed independently the identification, eligibility, and inclusion of papers. Any disagreements about the eligibility and inclusion of papers or about the appraisal of methodological quality were solved by discussing with a third reviewer until a consensus was reached. Potentially relevant references cited in full-text read articles were also added to the initial search. Computer files containing the selected papers at each stage of the selection process were developed and made available to all the co-authors. At the end of the process, 35 studies were collectively included in the analysis. The flow diagram of identification, screening, eligibility and inclusion process is provided in Figure 1. Data extraction of the 35 selected papers was performed using a standardized extraction spreadsheet to collect relevant information. As presented in Table 1, relevant information was summarized on established parameters chosen collectively by the authors: reference, population characteristics, definition of thinness, consideration of the absence of eating disorders, consideration of other main parameters, and areas of study. We mean by "presence of terminology" (Table 1) the explicit mention of "constitutional(ly) thin(ness)/lean(ness)" keywords. Outcome variables were not assessed in the present work: only the inclusion criteria of the selected studies were considered. Parameters such as food questionnaires or nutritional markers do not appear in Table 1 if these parameters were used as outcomes after the constitution of groups and not as inclusion criteria. Studies were listed in Table 1 according to the publication year, from the oldest to the most recent. Since this systematic review focuses on diagnostic criteria, it was not considered appropriate to retain studies from the same cohorts (recorded as duplicates).

Risks of bias

The Cochrane Collaboration's tool [32] was used to assess the risks of bias; as presented in Table 2. Two authors estimated independently the risks of bias in each included study. The following criteria were assessed: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias) and selective reporting (reporting bias). Any disagreements were discussed with a third co-author until a consensus was reached. No study was excluded based on the risks of bias.

Results

The initial database search yielded a total of 994 studies and 39 additional studies were also identified. In total, 689 studies remained after the removal of duplicates. After the review of titles and abstracts, 363 studies were excluded: 199 based on title and 164 based on abstract. Thus, 326 full-text articles were scrutinized for eligibility according to inclusion and exclusion criteria. Finally, 35 studies were considered for analysis (Figure 1). The risks of bias were estimated with the Cochrane Collaboration's tool [32] as presented in Table 2.

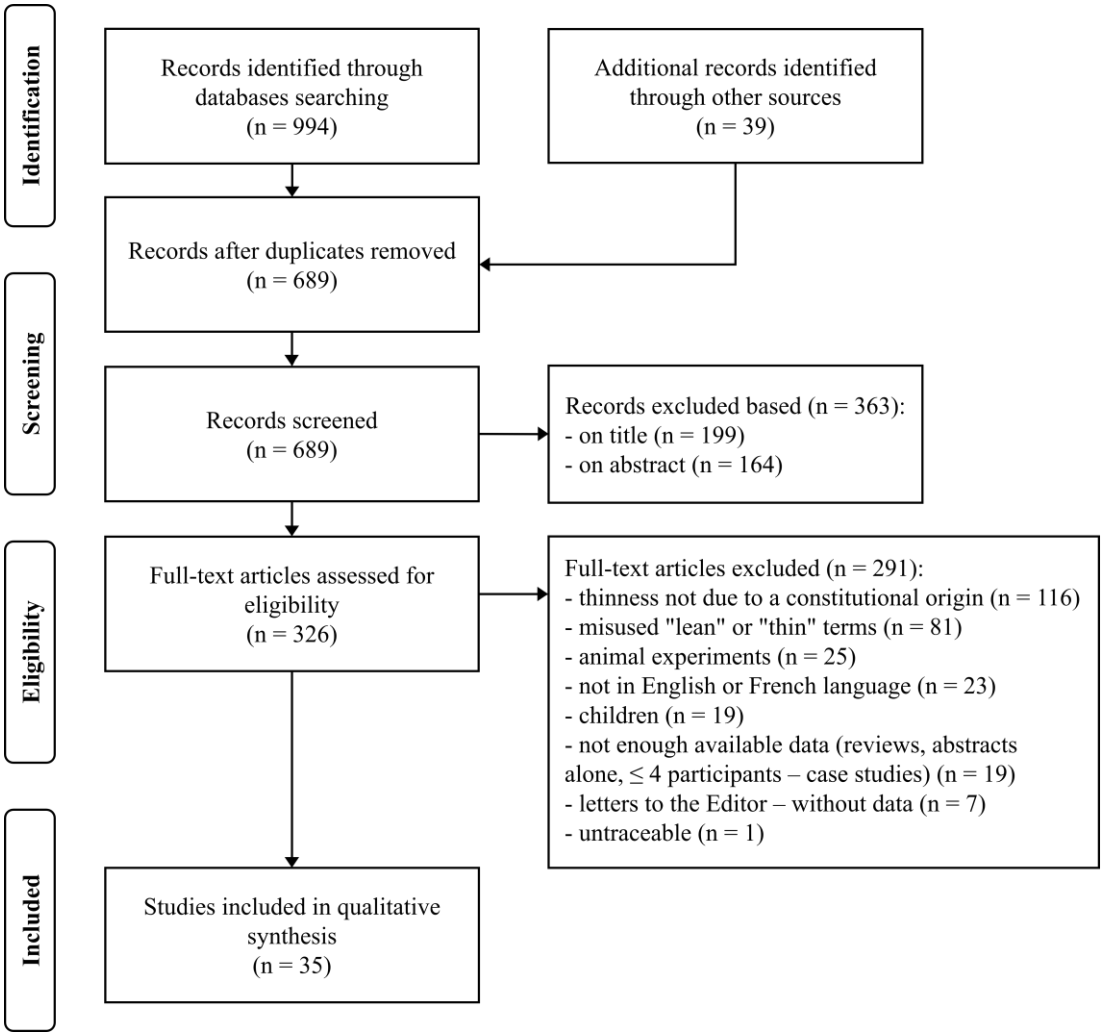

Figure 1. Flow diagram of the description of the screening, selection and inclusion process

Population characteristics

Of the 35 studies selected in the systematic review, 26 [7–11,16,17,19–28,33–41] enrolled females exclusively, 3 [18,42,43] enrolled males exclusively and 6 [30,31,44–47] enrolled both females and males (Table 1). Of these 35 studies, 32 [7–11,16–28,30,31,33–37,39–42,45–47] included a normal-weight control group and 23 [7–9,11,16–18,20–25,27,28,30,34,35,37,38,41,44,46] included a group of individuals with AN (18 [7–9,11,16–18,20–24,27,28,35,37,41,46] of restrictive type, 2 [38,44] of both restrictive and binge eating/purging type and 3 [25,30,34] did not report the type of AN). Selected studies included sample sizes ranging from 6 [37] to 1 622 [30] (both genders) in individuals with CT, from 7 [7,9] to 10 433 [30] (both genders) in normal-weight control people and from 6 [7] to 96 [44]

(both genders) in patients with AN. Studies enrolled participants from 19.4 [25] to 42.4 [36] years old in people with CT, from 19.3 [24] to 52.3 [30] years old (both genders) in normal-weight people and from 15.3 [44] to 26.4 [41] years old in patients with AN. BMI ranged from 15.7 [9,11] to 22.5 [42] $kg.m^{-2}$ in individuals with CT, from 20.3 [37] to 27.6 [42] $kg.m^{-2}$ in normal-weight controls and from 12.0 [38] to 17.1 [18] $kg.m^{-2}$ in patients with AN.

Definition of thinness

The "constitutional(ly) thin(ness)/lean(ness)" keywords were mentioned in 28 [7–11,17–23,25–28,30,31,33,35–38,41–43,46,47] of the 35 studies and were therefore not mentioned in the 7 remaining studies [16,24,34,39,40,44,45]. Of the 35 included studies, thinness threshold was reported through absolute BMI value in 21 studies [7–10,16,19,21–28,30,31,35,37,41,45,47] (ranging from 16.5 [7,9,21–23,28] to 20.0 $kg.m^{-2}$ [35]), through BMI percentile in one study (≤ 15th BMI percentile) [44], through percentage of ideal bodyweight in 2 studies (at least 25% lower than the average ideal bodyweight [33] or 80-90% of ideal bodyweight [34]), through silhouette ratings (1: very thin, 9: very large) in 2 studies (ranging from 1 to 3 for thin females [36] and from 1 to 4 for thin males [42]), through FM percentage in one study [43] (body fat ≤ 20% and low or normal weight), and through both BMI (< 20 $kg.m^{-2}$) and FM percentage (between 10 and 20%) in one study [40]. Thinness threshold was not clearly reported in 7 [11,17,18,20,38,39,46] of the 35 studies. Weight history was considered in 25 [7–10,16–19,21–23,27,28,30,31,35,36,38–40,42,44–47] of the 35 studies: 4 studies [16,39,40,45] reported a stable bodyweight for a certain period of time before the experiment (ranging from one week [45] to 2 years [16,39,40]) and 21 studies [7–10,17–19,21–23,27,28,30,31,35,36,38,42,44,46,47] reported it for a longer period throughout the growth period and/or the post-pubertal period. Weight history was not considered in the 10 [11,20,24–26,33,34,37,41,43] remaining studies.

Consideration of the absence of eating disorders in individuals with CT

Of the 35 studies, 32 [7–11,16–28,30,31,33–38,41–46] considered the absence of eating disorders in the inclusion criteria of CT and 3 [39,40,47] did not consider it. The absence of eating disorders was implicitly confirmed by the presence of a group of patients with AN in 23 studies [7–9,11,16–18,20–25,27,28,30,34,35,37,38,41,44,46]. This absence of eating disorders was confirmed using questionnaires in 5 studies [10,30,31,35,41], interviews in 3 studies [36,42,46], and both in one study [44]. Different questionnaires and thresholds were used: the three-factor eating questionnaire (TFEQ) [48] for 2 studies [41,44] with a cognitive restraint score ≤ 5 [44] or ≥ 13 [41] using their respective version of the TFEQ, a food questionnaire with normal scores not further defined for one study [35], the dutch eating behavior questionnaire (DEBQ) [49] and the eating disorder examination questionnaire (EDE) [50] without reported thresholds for 2 studies [10,31], the eating disorder inventory questionnaire (EDI) [51] and the body shape questionnaire (BSQ) [52] without reported thresholds for one study [31], and the SCOFF questionnaire [53] without reported thresholds for one study [30]. The Composite International Diagnostic Interview (CIDI) [54] was used for one study [44], the Structured Clinical Interview for DSM-III-R was used for 2 studies [36,42], and an interview to detect potential lifetime eating disorders in accordance with the criteria of the DSM-IV was used for one study [46]. The 26 remaining studies [7–9,11,16–28,33,34,37–40,43,45,47] did not mentioned the use of questionnaires or interviews. Three studies [10,19,31] presented the following criteria as inclusion criteria: normal insulin-like growth factor-1 (IGF-1), estradiol and free triiodothyronine (FT_3). Among them, 2 studies [10,31] also added normal mean cortisol and non-blunted leptin as inclusion criteria. Under nutritional markers were not assessed in the 32 remaining studies [7–9,11,16–18,20–28,30,33–47].

Consideration of other important parameters in individuals with CT

Of the 35 studies, 26 [7–11,16,17,20–28,31,34,35,37–41,46,47] mentioned the presence of menses in their group of CT, 6 [19,30,33,36,44,45] did not mention it and 3 studies [18,42,43] did not enroll females but only males (not

applicable criterion). Weight gain resistance/desire was taken into consideration in 14 articles [7–10,16,21–23,27,28,31,43,46,47] and was not reported in the 21 other selected studies [11,17–20,24–26,30,33–42,44,45]. Among them, 12 studies [7–10,21–23,27,28,31,46,47] specifically referred to the idea of a "desire" to gain weight, one study [16] reported a complaint about being chronically underweight, and one study [43] identified a difficulty in gaining weight. No studies used the term "resistance" to weight gain. The absence of associated pathology was considered in 28 [7–10,16–23,25–28,30,31,33,34,37,39–41,43,44,46,47] of the 35 studies but was not reported in the 7 remaining studies [11,24,35,36,38,42,45]. Physical activity was reported in 13 studies [7,10,16,17,22,23,25,28,30,31,39,40,47] and was consequently not reported in the 22 remaining studies [8,9,11,18–21,24,26,27,33–38,41–46]. Ten articles [7,16,17,22,25,28,30,39,40,47] just mentioned the absence of over-exercising without questionnaire-based assessment. Among them, 2 articles [39,40] specified that participants did not spend more than one hour per week on sport activities and one article [30] excluded all participants who stated that they exercised more than 3 times a week or with an intensity exceeding 6 metabolic equivalents (METs) for any duration or frequency [55]. Three articles [10,23,31] used the Monica Optional Study of Physical Activity (MOSPA) questionnaire [56] to assess the absence of over-exercising and one [31] of them added intensive physical activity (more than 3 sessions of physical activity per week) as an exclusion criterion.

Areas of study

Various fields of study were investigated in the selected articles. Of the 35 studies included in the systematic review, 21 [7–11,17–28,31,37,43,45] investigated body composition, 19 [7–11,19,21–24,26–28,31,34,35,37,38,40] assessed hormonal or biochemical parameters and 15 [7–10,16–20,26,31,35,40,43,45] studied energy balance of individuals with CT. Investigations were carried out in a total of 8 studies [9–11,21–23,31,38] on appetite-regulating hormones, 6 studies [8,19,24,28,31,45] on bone tissue or bone markers, 7 studies [7,8,10,31,36,41,42] on psychological profile, 5 studies [10,19,30,31,44] on genetics or omics approaches, 4 studies [19,31,39,40] on muscle tissue or muscle function, 2 studies [16,20] on thermogenesis or brown adipose tissue, one study [47] on

ophthalmology, one study [33] on pregnancy, one study [35] on cardiology, one study [46] on functional dyspepsia and one study [41] on neurology.

Table 1. Inclusion criteria used for diagnosis of constitutional thinness in the clinical trials selected in the systematic review

Reference	Population characteristics (sample size, age; BMI) (means ± SD)	Definition of thinness	Consideration of the absence of eating disorders in individuals with CT	Consideration of other main parameters in individuals with CT	Areas of study
Schneider et al., 1979 [33]	Females: CT: n=53; 25.3 ± 5.2§ yr; NR C: n=100; 25.8 ± 4.2§ yr; NR	Presence of terminology Thinness threshold: at least 25% lower than the average ideal weight defined for the height at the first prenatal consultation (first trimester of pregnancy) No apparent consideration of weight history	Considered No group of AN Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	Amenorrhea: NR Weight gain resistance/desire: NR Healthy, absence of associated pathology Physical activity: NR	9
van Binsbergen et al, 1990 [34]	Females: CT: n=10; 26.4§ yr; 18.4§ kg.m^{-2} C: n=10; 25.1§ yr; 20.8§ kg.m^{-2} AN: n=20; 24.8§ yr; 14.3§ kg.m^{-2} (AN type: NR)	Absence of terminology Thinness threshold: 80-90% of ideal bodyweight No apparent consideration of weight history	Considered Implicitly confirmed by the presence of a group of AN (DSM-III) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: NR Healthy, absence of associated pathology Physical activity: NR	3
Diaz et al., 1992 [43]	Males: CT: n=7; 26.3 ± 4.5 yr; 21.7 ± 1.3 kg.m^{-2}	Presence of terminology Thinness threshold: body fat ≤ 20% (and low or normal weight) No apparent consideration of weight history	Considered No group of AN Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	Criterion of amenorrhea: NA (males) Weight gain resistance/desire: mentioned (they declared themselves to be good eaters and claimed to have difficulty gaining weight) Healthy, absence of associated pathology Physical activity: NR	1, 2
Scalfi et al., 1992 [16]	Females: CT: n=7; 28.6 ± 5.6 yr; 16.8 ± 0.8 kg.m^{-2} C: n=8; 28.5 ± 3.4 yr; 22.5 ± 2.5 kg.m^{-2} AN: n=7; 21.3 ± 3.7 yr; 15.3 ± 2.1 kg.m^{-2} (AN: restrictive-type)	Absence of terminology Thinness threshold: BMI < 18.5 kg.m^{-2} Consideration of personal weight history (stable in the 2 years before the experiment ± 1.5 kg by interview)	Considered Implicitly confirmed by the presence of a group of AN (DSM-III) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR (but no clinical or biochemical evidence of hyperthyroidism)	No amenorrhea Weight gain resistance/desire: mentioned (they complained of being chronically underweight and perceived themselves as normal eaters or large eaters) Healthy, absence of associated pathology Absence of over-exercising	1, 10
Hinney et al., 1997 [44]	Females: CT: n=48; 24.7 ± 3.9 yr; 17.6 ± 0.8 kg.m^{-2}	Absence of terminology Thinness threshold: ≤ 15th BMI percentile	Considered (DSM-IV) Implicitly confirmed by the presence of a group	Amenorrhea: NR Weight gain resistance/desire: NR	7

	AN: n=92; 16.6 ± 3.4 yr; 14.5 ± 1.5 kg.m^{-2} (AN: restrictive and binge eating/purging type) Males: CT: n=64; 26.1 ± 4.1 yr; 19.0 ± 1.0 kg.m^{-2} AN: n=4; 15.3 ± 0.9 yr; 13.9 ± 2.0 kg.m^{-2} (AN: restrictive and binge eating/purging type)	Consideration of personal weight history (semi-structured interview to assess weight history up to age 18 - at ages 10, 15 and 18)	of AN (DSM-IV) Confirmed by questionnaire and interview (TFEQ with a cognitive restraint score ≤ 5 and CIDI in accordance with DSM-IV) Under nutritional markers: NR	Healthy, absence of associated pathology Physical activity: NR	
Petretta et al., 1997 [35]	Females: CT: n=10; 22 ± 3 yr; 16.6 ± 1.1 kg.m^{-2} C: n=10; 21 ± 3 yr; 23.4 ± 2.4 kg.m^{-2} AN: n=13; 20 ± 2 yr; 15.7 ± 2.4 kg.m^{-2} (AN: restrictive-type)	Presence of terminology Thinness threshold: BMI < 20 kg.m^{-2} Consideration of personal weight history (history of leanness throughout life)	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Confirmed by questionnaire (normal scores on food questionnaire – not further defined) Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: NR Healthy, associated pathology: NR Physical activity: NR	1, 3, 12
Slof et al., 2003 [36]	Females: CT: n=80; 42.4 ± 7.2 yr; 20.3 ± 1.5 kg.m^{-2} C: n=881; 43.0 ± 7.7 yr; 26.8 ± 6.2 kg.m^{-2}	Presence of terminology (but "persistent thinness" preferentially used) Thinness threshold: 1-3 (1: very thin, 9 very large) on silhouette ratings Consideration of personal weight history (persistent thinness with consideration of childhood, adolescence and adulthood)	Considered (DSM-Ⅲ-R and DSM-IV) No group of AN Confirmed by interview (Structured Clinical Interview for DSM-Ⅲ-R by trained interviewers - 40 hours of training) Under nutritional markers: NR	Amenorrhea: NR Weight gain resistance/desire: NR Healthy, associated pathology: NR Physical activity: NR	11
Tolle et al., 2003 [11]	Females: CT: n=8; 23.3 ± 3.1[§] yr; 15.7 ± 0.4[§] kg.m^{-2} C: n=10; 23.2 ± 1.1[§] yr; 21.5 ± 0.7[§] kg.m^{-2} AN: n=9; 17.2 ± 0.9[§] yr; 14.6 ± 0.4[§] kg.m^{-2} (AN: restrictive-type)	Presence of terminology Thinness threshold: NR (but BMI similar to the AN group before renutrition) No apparent consideration of weight history	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: NR Healthy, associated pathology: NR Physical activity: NR	2, 3, 4
Bosy-Westphal et al., 2004 [45]	CT (12 females): n=12; 26.4 ± 6.8 yr; 16.9 ± 0.9 kg.m^{-2} C (12 females and 13 males): n=25; 25.4 ± 2.4 yr; 22.3 ± 2.0 kg.m^{-2}	Absence of terminology Thinness threshold: BMI < 18.5 kg.m^{-2} Consideration of personal weight history (stable for at least one week)	Considered (DSM-IV) No group of AN Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR (but blood glucose and lipid profile assessed)	Amenorrhea: NR Weight gain resistance/desire: NR Healthy, associated pathology: NR Physical activity: NR	1, 2, 5

Mazzeo et al., 2004 [42]	Males: CT: n=158; NR but probably 29-69 yr; 22.5 ± 2.1 $kg.m^{-2}$ C: n=915; NR but probably 29-69 yr; 27.6 ± 4.2 $kg.m^{-2}$	Presence of terminology (but "persistent thinness" preferentially used) Thinness threshold: 1-4 (1: very thin, 9 very large) on silhouette ratings Consideration of personal weight history (persistent thinness with consideration of childhood, adolescence and adulthood)	Considered (DSM-III-R) No group of AN Confirmed by interview (Structured Clinical Interview for DSM-III-R) Under nutritional markers: NR	Criterion of amenorrhea: NA (males) Weight gain resistance/desire: NR Healthy, associated pathology: NR Physical activity: NR	11
Tagami et al., 2004 [37]	Females: CT: n=6; 27.5 ± 4.2 yr; 17.7 ± 0.5 $kg.m^{-2}$ C: n=16; 25.7 ± 2.9 yr; 20.3 ± 1.5 $kg.m^{-2}$ AN: n=31; 25.5 ± 8.1 yr; 14.0 ± 2.5 $kg.m^{-2}$ (AN: probably restrictive-type)	Presence of terminology Thinness threshold: BMI < 18.0 $kg.m^{-2}$ No apparent consideration of weight history	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: NR Healthy, absence of associated pathology Physical activity: NR	2, 3
Miljic et al., 2006 [38]	Females: CT: n=10; 22.5 ± 4.4 yr; 17.6 ± 1.3 $kg.m^{-2}$ AN: n=9; 25.1 ± 5.1 yr; 12.0 ± 1.2 $kg.m^{-2}$ (AN: restrictive and binge eating/purging type)	Presence of terminology Thinness threshold: NR (but subnormal bodyweight 51.4 ± 7.6 kg (45-60 kg) and BMI 17.6 ± 1.3 $kg.m^{-2}$ (16.6-19.3 $kg.m^{-2}$)) Consideration of personal weight history (without history of weight loss)	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: NR Healthy, associated pathology: NR Physical activity: NR	3, 4
Bossu et al., 2007 [7]	Females: CT: n=7; NR but 18-26 yr; 16.1 ± 0.6 $kg.m^{-2}$ C: n=7; NR but 18-26 yr; 21.2 ± 0.8 $kg.m^{-2}$ AN: n=6; NR but 18-26 yr; 15.8 ± 0.8 $kg.m^{-2}$ (AN: restrictive-type)	Presence of terminology Thinness threshold: BMI: 14.5-16.5 $kg.m^{-2}$ Consideration of personal weight history (stable throughout the post-pubertal period and weight history retrospectively reconstituted from birth to 18 years)	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: mentioned (desire for weight gain as a main reason for medical consultation) Healthy, absence of associated pathology Absence of over-exercising	1, 2, 3, 11
Germain et al., 2007 [9]	Females: CT: n=10; 20.2 ± 3.8 yr; 15.7 ± 0.6 $kg.m^{-2}$ C: n=7; 23 ± 2.1 yr; 20.4 ± 0.8 $kg.m^{-2}$ AN: n=12; 20.7 ± 4.2 yr; 15.2 ± 1.4 $kg.m^{-2}$ (AN: probably restrictive-type)	Presence of terminology Thinness threshold: BMI: 14.5-16.5 $kg.m^{-2}$ Consideration of personal weight history (stable throughout the post-pubertal period)	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: mentioned (desire for weight gain as a main reason for medical consultation) Healthy, absence of associated pathology Physical activity: NR	1, 2, 3, 4

Marra et al., 2007 [17]	Females: CT: n=20; 22.5 ± 5.8 yr; 17.2 ± 1.0 kg.m^{-2} C: n=20; 22.0 ± 3.7 yr; 21.7 ± 2.4 kg.m^{-2} AN: n=20; 18.8 ± 3.4 yr; 15.1 ± 1.6 kg.m^{-2} (AN: restrictive-type)	Presence of terminology Thinness threshold: NR Consideration of personal weight history (bodyweight that has always been in the lower percentiles for age, gender and ethnicity)	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR (but normal thyroid functions seem to be assessed)	No amenorrhea Weight gain resistance/desire: NR Healthy, absence of associated pathology Absence of over-exercising	1, 2
Galusca et al., 2008 [28]	Females: CT: n=25; 23.1 ± 6.0 yr; 15.8 ± 0.5 kg.m^{-2} C: n=28; 23.9 ± 7.4 yr; 20.7 ± 2.1 kg.m^{-2} AN: n=44; 23.4 ± 8.0 yr; AN: 15.5 ± 0.7 kg.m^{-2} (AN: restrictive-type)	Presence of terminology Thinness threshold: BMI: 12.0-16.5 kg.m^{-2} Consideration of personal weight history (stable throughout the growth period until the age of 18)	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: mentioned (desire for weight gain as a main reason for medical consultation) Healthy, absence of associated pathology Absence of over-exercising	2, 3, 5
Fernández-García et al., 2009 [24]	Females: CT: n=22; 19.7 ± 5.3 yr; 16.7 ± 1.0 kg.m^{-2} C: n=20; 19.3 ± 1.6 yr; 22.3 ± 1.6 kg.m^{-2} AN: n=25; NR for restrictive-type; 16.1 ± 1.5 kg.m^{-2} (AN: restrictive-type)	Absence of terminology Thinness threshold: BMI < 18.5 kg.m^{-2} No apparent consideration of weight history (but after 5 years of follow-up, none presented any criteria for eating disorders)	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: NR Healthy, associated pathology: NR Physical activity: NR	2, 3, 5
Germain et al., 2009 [21]	Females: CT: n=9; 24.1 ± 3.6 yr; 16.1 ± 0.3 kg.m^{-2} C: n=10; 23.1 ± 4.4 yr; 20.5 ± 1.3 kg.m^{-2} AN: n=15; 20.4 ± 5.0 yr; 14.8 ± 0.4 kg.m^{-2} (AN: restrictive-type)	Presence of terminology Thinness threshold: BMI < 16.5 kg.m^{-2} Consideration of personal weight history (stable throughout the growth period)	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: mentioned (a stated desire for weight gain) Healthy, absence of associated pathology Physical activity: NR	2, 3, 4
Marra et al., 2009 [25]	Females: CT: n=10; 19.4 ± 2.4 yr; 16.8 ± 1 kg.m^{-2} C: n=30; 20.0 ± 2.1 yr; 22.5 ± 2.8 kg.m^{-2} AN: n=30; 19.0 ± 2.0 yr; 16.7 ± 0.5 kg.m^{-2} (AN type: NR)	Presence of terminology Thinness threshold: BMI < 18.5 kg.m^{-2} No apparent consideration of weight history	Considered Implicitly confirmed by the presence of a group of AN Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: NR Healthy, absence of associated pathology Absence of over-exercising	2

Hasegawa et al., 2011 [26]	Females: CT: n=20; 23.2 ± 2.3 yr; 17.6 ± 0.8 kg.m^{-2} C: n=20; 23.1 ± 2.1 yr; 21.9 ± 1.2 kg.m^{-2}	Presence of terminology (but "lean" term preferentially used) Thinness threshold: BMI < 18.5 kg.m^{-2} No apparent consideration of weight history	Considered No group of AN Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: NR Healthy, absence of associated pathology Physical activity: NR	1, 2, 3
Galusca et al., 2012 [22]	Females: CT: n=14; 23.7 ± 6[§] yr; 16.0 ± 0.4[§] kg.m^{-2} C: n=10; 23.1 ± 5[§] yr; 20.8 ± 0.6[§] kg.m^{-2} AN: n=19; 23.2 ± 8[§] yr; 15.3 ± 0.4[§] kg.m^{-2} (AN: restrictive-type)	Presence of terminology Thinness threshold: BMI < 16.5 kg.m^{-2} Consideration of personal weight history (stable throughout the growth period)	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: mentioned (a stated desire for weight gain) Healthy, absence of associated pathology Absence of over-exercising	2, 3, 4
Santonicola et al., 2012 [46]	Females and males (not clearly reported): CT: n=9; 24.9 ± 6.6 yr; NR C: n=22; 23.7 ± 3.3 yr; NR AN: n=20; 22.5 ± 4.2 yr; NR (AN: probably restrictive-type)	Presence of terminology Thinness threshold: NR (but severely underweight) Consideration of personal weight history (stable throughout the post-pubertal period)	Considered (DSM-IV) Implicitly confirmed by the presence of a group of AN (DSM-IV) Confirmed by interview (to detect potential lifetime eating disorders in accordance with the criteria of the DSM-IV) Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: mentioned (desire for weight gain as a main reason for medical consultation) Healthy, absence of associated pathology Physical activity: NR	13
Pasanisi et al., 2013 [20]	Females: CT: n=7; 21.7 ± 3.6 yr; 16.2 ± 0.9 kg.m^{-2} C: n=20; 25.6 ± 3.9 yr; 21.7 ± 2.4 kg.m^{-2} AN: n=7; 23.4 ± 4.5 yr; 15.3 ± 0.8 kg.m^{-2} (AN: restrictive-type)	Presence of terminology Thinness threshold: NR No apparent consideration of weight history	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR (but normal thyroid function)	No amenorrhea Weight gain resistance/desire: NR Healthy, absence of associated pathology Physical activity: NR	1, 2, 10
Paschalis et al., 2013 [39]	Females: CT: n=8; 21.4 ± 1.1 yr; 17.3 ± 0.6 kg.m^{-2} C: n=12; 20.2 ± 1.4 yr; 22.0 ± 1.0 kg.m^{-2}	Absence of terminology Thinness threshold: NR (but groups constituted according to BMI) Consideration of personal weight history (stable at their anthropometric characteristics for at least the last 2 years)	NR No group of AN Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: NR Healthy, absence of associated pathology Absence of over-exercising (≤ 1 hour per week on sport activities)	6

Germain et al., 2014 [10]	Females: CT: n=8; 21.6 ± 5.4 yr; 17.1 ± 0.8 $kg.m^{-2}$ C: n=8; 22.1 ± 2.3 yr; 22.1 ± 0.8 $kg.m^{-2}$	Presence of terminology Thinness threshold: BMI: 13-17.5 $kg.m^{-2}$ Consideration of personal weight history (stable throughout the post-pubertal period)	Considered No group of AN Confirmed by questionnaires (DEBQ and EDE – no reported thresholds) Normal nutritional markers (normal IGF-1, estradiol, FT_3, mean cortisol and non-blunted leptin)	No amenorrhea Weight gain resistance/desire: mentioned (recruited among outpatients consulting for bodyweight gain desire) Healthy, absence of associated pathology Absence of over-exercising (according to the MOSPA questionnaire)	1, 2, 3, 4, 7, 11
Galusca et al., 2015 [23]	Females: CT: n=22; 23.2 ± 2.3 yr; 15.9 ± 0.5 $kg.m^{-2}$ C: n=14; 22.6 ± 6.0 yr; 21.6 ± 1.1 $kg.m^{-2}$ AN: n=23; 22.5 ± 6.2 yr; 14.6 ± 2.4 $kg.m^{-2}$ (AN: restrictive-type)	Presence of terminology Thinness threshold: BMI < 16.5 $kg.m^{-2}$ Consideration of personal weight history (stable throughout the growth period)	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: mentioned (a stated desire for weight gain) Healthy, absence of associated pathology Absence of over-exercising (according to the MOSPA questionnaire)	2, 3, 4
Germain et al., 2016 [27]	Females: CT: n=10; 20.6 ± 6.6 yr; 15.9 ± 0.9 $kg.m^{-2}$ C: n=10; 22.7 ± 1.6 yr; 21.4 ± 1.6 $kg.m^{-2}$ AN: n=10; 21.6 ± 4.7 yr; 15.1 ± 2.5 $kg.m^{-2}$ (AN: restrictive-type)	Presence of terminology Thinness threshold: BMI < 17 $kg.m^{-2}$ Consideration of personal weight history (stable throughout the growth period)	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: mentioned (a stated desire for weight gain) Healthy, absence of associated pathology Physical activity: NR	2, 3
Gunes et al., 2016 [47]	CT (16 females, 8 males): n=24; 22.1 ± 3.7 yr; 17.4 ± 1.2 $kg.m^{-2}$ C (9 females, 15 males): n=24; 23.5 ± 4.0 yr; 22.1 ± 2.4 $kg.m^{-2}$	Presence of terminology Thinness threshold: BMI < 18.5 $kg.m^{-2}$ Consideration of personal weight history (stable during the post-pubertal period)	NR No group of AN Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: mentioned (desire for weight gain as a main reason for medical consultation) Healthy, absence of associated pathology Absence of over-exercising	8
Ling et al., 2016 [31]	Females: CT: n=15; NR (design) but 18-35 yr; NR (design) 13-17.5 $kg.m^{-2}$ C: n=15; NR (design) but 18-35 yr; NR (design) but 20-25 $kg.m^{-2}$ Males: CT: n=15; NR (design) but 18-35 yr; NR (design) 13-18.5 $kg.m^{-2}$	Presence of terminology Thinness threshold: BMI: 13-17.5 $kg.m^{-2}$ (females), 13-18.5 $kg.m^{-2}$ (males) Consideration of personal weight history (stable for post-pubertal and at least 3 months)	Considered (DSM-IV) No group of AN Confirmed by questionnaires (DEBQ, EDE, EDI, and BSQ – no reported thresholds) Normal nutritional markers (normal IGF-1, estradiol, FT_3, mean cortisol and non-blunted leptin)	No amenorrhea Weight gain resistance/desire: mentioned (recruited among outpatients consulting for bodyweight gain desire) Healthy, absence of associated pathology Absence of over-exercising (according to the MOSPA questionnaire and ≤ 3 sessions per week)	1, 2, 3, 4, 5, 6, 7, 11

	C: n=15; NR (design) but 18-35 yr; NR (design) but 20-25 kg.m^{-2}				
Estour et al., 2017 [8]	Females: CT: n=56; 26.9 ± 7.6 yr; 16.5 ± 0.9 kg.m^{-2} C: n=54; 23.4 ± 4.1 yr; 20.9 ± 2.2 kg.m^{-2} AN: n=40; 25.0 ± 6.5 yr; 16.0 ± 0.8 kg.m^{-2} (AN: restrictive-type)	Presence of terminology Thinness threshold: BMI < 17.5 kg.m^{-2} Consideration of personal weight history (when available (26/56 CT), weight history from birth to at least 18 years old was retrospectively reconstituted)	Considered Implicitly confirmed by the presence of a group of AN (DSM-IV) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: mentioned (desire for weight gain as a main reason for medical consultation) Healthy, absence of associated pathology Physical activity: NR	1, 2, 3, 5, 11
Galusca et al., 2018 [19]	Females: CT: n=10; 22.1 ± 5.1 yr; 17.0 ± 0.9 kg.m^{-2} C: n=10; 22.2 ± 2.5 yr; 21.7 ± 1.3 kg.m^{-2}	Presence of terminology Thinness threshold: BMI < 17.5 kg.m^{-2} Consideration of personal weight history (stable throughout the post-pubertal period)	Considered No group of AN Not explicitly confirmed by questionnaire or interview Normal nutritional markers (normal IGF-1, 17β estradiol, FT_3)	Amenorrhea: NR Weight gain resistance/desire: NR Healthy, absence of associated pathology Physical activity: NR	1, 2, 3, 5, 6, 7
Florent et al., 2019 [41]	Females: CT: n=10; 22.4 ± 2.5 yr; 17.1 ± 0.9 kg.m^{-2} C: n=10; 21.8 ± 2.2 yr; 21.9 ± 1.3 kg.m^{-2} AN: n=10; 26.4 ± 6.0 yr; 15.3 ± 1.9 kg.m^{-2} (AN: restrictive-type)	Presence of terminology Thinness threshold: BMI < 18.5 kg.m^{-2} No apparent consideration of weight history	Considered (DSM-IV) Implicitly confirmed by the presence of a group of AN (DSM-IV) Confirmed by questionnaire (TFEQ with a cognitive restraint score ≥ 13) Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: NR Healthy, absence of associated pathology Physical activity: NR	11, 14
Margaritelis et al., 2019 [40]	Females: CT: n=12; 21.2 ± 1.4 yr; 17.8 ± 0.8 kg.m^{-2} C: n=14; 20.4 ± 1.8 yr; 22.4 ± 1.1 kg.m^{-2}	Absence of terminology Thinness threshold: BMI < 20 kg.m^{-2} and body fat: 10-20% Consideration of personal weight history (bodyweight did not change more than ± 3 kg the last 2 years prior to participation in the study)	NR No group of AN Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	No amenorrhea Weight gain resistance/desire: NR Healthy, absence of associated pathology Absence of over-exercising (≤ 1 hour per week on sport activities)	1, 3, 6
Marra et al., 2019 [18]	Males: CT: n=15; 23.3 ± 5.2 yr; 17.9 ± 0.6 kg.m^{-2} C: n=18; 22.3 ± 3.7 yr; 22.3 ± 1.7 kg.m^{-2} AN: n=17; 22.3 ± 5.3 yr; AN: 17.1 ± 1.2 kg.m^{-2} (AN: probably restrictive-type)	Presence of terminology Thinness threshold: NR Consideration of personal weight history (stable on time)	Considered Implicitly confirmed by the presence of a group of AN (DSM-V) Not explicitly confirmed by questionnaire or interview Under nutritional markers: NR	Criterion of amenorrhea: NA (males) Weight gain resistance/desire: NR Healthy, absence of associated pathology Physical activity: NR	1, 2

Riveros-McKay et al., 2019 [30]	Females: CT: n=1 325; 36.6 ± 14.3 yr; 17.6 ± 0.9 $kg.m^{-2}$ C: n=5 837; 52.0 ± 16.7 yr; 27.0 ± 7.9 $kg.m^{-2}$ AN type: NR Males: CT: n=297;35.2 ± 14.5 yr; 17.6 ± 1.1 $kg.m^{-2}$ C: n=4 596; 52.7 ± 17.3 yr; 26.9 ± 7.8 $kg.m^{-2}$ AN type: NR	Presence of terminology (but "persistent/healthy thinness" preferentially used) Thinness threshold: BMI < 18 $kg.m^{-2}$ (but a small number of individuals with a BMI of 19.0 $kg.m^{-2}$ were included as they had a strong family history of thinness) Consideration of personal weight history (persistently thin/always thin throughout life)	Considered Implicitly confirmed by the presence of a group of AN Confirmed by questionnaire (SCOFF questionnaire – no reported thresholds) Under nutritional markers: NR	Amenorrhea: NR Weight gain resistance/desire: NR Healthy, absence of associated pathology Absence of over-exercising (excluded if they exercised more than 3 times a week or with an intensity exceeding 6 METs for any duration or frequency)	7

Abbreviations: BMI: body mass index, SD: standard deviation, CT: constitutional thinness, NR: not reported, C: control subjects, AN: anorexia nervosa, BSQ: body shape questionnaire [52], DSM: diagnostic and statistical manual of mental disorder, NA: not applicable, TFEQ: three-factor eating questionnaire [48], CIDI: composite international diagnostic interview [54], DEBQ: dutch eating behavior questionnaire [49], EDE: eating disorder examination questionnaire [50], IGF-1: insulin-like growth factor-1, FT_3: free triiodothyronine, MOSPA: monica optional study of physical activity [56], EDI: eating disorder inventory questionnaire [51], SCOFF questions: Do you make yourself **S**ick because you feel uncomfortably full? Do you worry you have lost **C**ontrol over how much you eat? Have you recently lost more than **O**ne stone in a 3-month period? Do you believe yourself to be **F**at when others say you are too thin? Would you say that **F**ood dominates your life? [53], METs: metabolic equivalents

Areas of study: 1: Energy balance, 2: Body composition, 3: Hormonal, biochemical assays, 4: Appetite-regulating hormones, 5: Bone tissue / Bone markers, 6: Muscle tissue / Muscle function, 7: Genetics or omics approaches, 8: Ophthalmology, 9: Pregnancy, 10: Thermogenesis / Brown adipose tissue, 11: Psychological profile, 12: Cardiology, 13: Functional dyspepsia, 14: Neurology

"Terminology presence" means the mention of "constitutional(ly) thin(ness)/lean(ness)" crucial keywords

§: Type of values dispersion (SD or SEM) not clearly reported

Table 2. Risks of bias

Reference	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)
Schneider et al., 1979 [33]	Moderate risk	NR	Low risk	Moderate risk	Moderate risk	Moderate risk
van Binsbergen et al, 1990 [34]	Low risk	NR	High risk	Low risk	NR	Low risk
Diaz et al., 1992 [43]	Low risk	NR	High risk	High risk	High risk	Low risk
Scalfi et al., 1992 [16]	Low risk	NR	High risk	Moderate risk	NR	Low risk
Hinney et al., 1997 [44]	Low risk	NR	High risk	Low risk	Low risk	Low risk
Petretta et al., 1997 [35]	Low risk	NR	High risk	Low risk	NR	Low risk
Slof et al., 2003 [36]	Moderate risk	NR	High risk	High risk	Low risk	Low risk
Tolle et al., 2003 [11]	Moderate risk	NR	High risk	Low risk	Moderate risk	Low risk
Bosy-Westphal et al., 2004 [45]	Low risk	NR	High risk	Low risk	High risk	Low risk
Mazzeo et al., 2004 [42]	Moderate risk	NR	High risk	High risk	Low risk	Low risk
Tagami et al., 2004 [37]	Low risk	NR	High risk	Low risk	Moderate risk	Low risk
Miljic et al., 2006 [38]	Moderate risk	NR	High risk	Low risk	Moderate risk	Low risk
Bossu et al., 2007 [7]	Low risk	NR	High risk	Moderate risk	NR	Low risk
Germain et al., 2007 [9]	Low risk	NR	High risk	Low risk	NR	Low risk
Marra et al., 2007 [17]	Moderate risk	NR	High risk	Low risk	NR	Low risk
Galusca et al., 2008 [28]	Low risk	NR	High risk	Low risk	NR	Low risk
Fernández-García et al., 2009 [24]	Low risk	NR	High risk	Low risk	Moderate risk	Low risk
Germain et al., 2009 [21]	Low risk	NR	High risk	Low risk	Moderate risk	Low risk
Marra et al., 2009 [25]	Low risk	NR	High risk	Low risk	NR	Low risk
Hasegawa et al., 2011 [26]	Low risk	NR	High risk	Low risk	NR	Low risk
Galusca et al., 2012 [22]	Low risk	NR	High risk	Low risk	NR	Low risk

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Reference	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)
Santonicola et al., 2012 [46]	Moderate risk	NR	High risk	High risk	NR	Low risk
Pasanisi et al., 2013 [20]	Moderate risk	NR	High risk	Low risk	Moderate risk	Low risk
Paschalis et al., 2013 [39]	Moderate risk	NR	High risk	Moderate risk	NR	Low risk
Germain et al., 2014 [10]	Low risk	NR	High risk	Moderate risk	NR	Low risk
Galusca et al., 2015 [23]	Low risk	NR	High risk	Low risk	NR	Low risk
Germain et al., 2016 [27]	Low risk	NR	High risk	Low risk	NR	Low risk
Gunes et al., 2016 [47]	Low risk	NR	High risk	Low risk	NR	Low risk
Ling et al., 2016 [31]	Low risk	NR	High risk	Moderate risk	NA	NA
Estour et al., 2017 [8]	Low risk	NR	High risk	Low risk	NR	Low risk
Galusca et al., 2018 [19]	Low risk	NR	High risk	Low risk	NR	Low risk
Florent et al, 2019 [41]	Low risk	NR	High risk	Moderate risk	Moderate risk	Low risk
Margaritelis et al., 2019 [40]	Low risk	NR	High risk	Moderate risk	NR	Low risk
Marra et al., 2019 [18]	Moderate risk	NR	High risk	Low risk	NR	Low risk
Riveros-McKay et al., 2019 [30]	Low risk	NR	High risk	Low risk	Low risk	Low risk

Discussion

The literature shows a growing number of clinical trials enrolling underweight participants without apparent disorders in their energy balance, suggesting a constitutional origin of thinness. These studies, however, reveal a high heterogeneity when it comes to the employed definition and diagnosis of CT; as well as a high diversity in the fields of study. In that context, we proposed here a systematic analysis of the clinical trials that enrolled participants with CT in order to propose a better definition and diagnosis of CT.

The need for a clear terminology

The lack of consensus and visibility concerning CT is probably due to the lack of common terminology. Among the 35 studies considered in the present systematic review, 7 [16,24,34,39,40,44,45] did not used the key terms "constitutional thinness" or "constitutional leanness". This makes highly probable that people might not detect those references while conducting simple scientific or systematic researches. For example, Farooqi and her research team who conducted a very interesting genetic research on CT [30] preferentially used the "persistent/healthy thinness" expression even if "constitutional thinness" is still found once [30]. In addition, studies enrolling "lean" or "underweight" groups need to be particularly screened. Most of the time, the "lean" term refers to normal-weight individuals and "underweight" term to undernourishment, but confusingly, these terms also remain found in the literature to designate CT individuals. Thus, we would privilege a common terminology, such as "constitutional thinness" or "constitutional leanness" designations. Since CT individuals do not seem to be characterized by a very low body fat percentage despite their low BMI [8,10,11,17,19,23–27], we would favor the terminology of "constitutional thinness" which therefore seems more appropriate than "constitutional leanness". A

common terminology would drastically facilitate the referencing of CT in research databases and increase its visibility.

Thinness threshold

As underlined in different studies, dealing with thinness first requires to properly set a threshold for this thinness [8,15,57]. The World Health Organization (WHO) defines different thresholds, based on BMI cut-offs: grade 1 - mild thinness (17.00 – 18.49 $kg.m^{-2}$), grade 2 - moderate thinness (16.00 – 16.99 $kg.m^{-2}$), and grade 3 - severe thinness (< 16.00 $kg.m^{-2}$) [57,58]. Thus, the WHO uses the BMI measurement to provide demarcation points. Of the 35 included studies, 22 [7–10,16,19,21–28,30,31,35,37,40,41,45,47] also used BMI cut-offs and one study [44] used a threshold of BMI percentile (≤ 15th BMI percentile). BMI cut-offs ranged from 16.5 [7,9,21–23,28] to 20.0 [35,40] $kg.m^{-2}$ for studies using a BMI threshold and mean BMI ranged from 15.7 [9,11] to 22.5 [42] in individuals with CT; revealing a high heterogeneity in BMI values. Two studies [40,43] used percentages of FM to define a thinness cut-off. From an etymological point of view, "leanness" defines a low body fat content and interestingly, Maffetone et al. proposed the use of the "underfat" term instead of "underweight" [59]. Nevertheless, Maffetone et al. proposed this terminology considering thinness due to chronic illness or eating disorders, not thinness due to a constitutional origin [59]. Despite their low BMI, CT individuals have been suggested to present a non-blunted FM percentage [7,8,10,11,17,19,23–27], unlike AN individuals whose FM seems significantly lower compared to CT people [7,8,11,17,23,24]. The use of a body fat percentage threshold does not seem yet adequate to diagnose CT and could on the contrary lead to misdiagnosis. While we therefore suggest that "underfat" might not be an appropriate term in the context of CT, further studies using similar inclusion criteria and methodologies are required to provide more evidence about body composition in CT. Two studies [33,34] focused their definition of thinness on a percentage of ideal bodyweight and two studies [36,42] argued that silhouette ratings were

a better choice to base their definition of thinness. Nevertheless, silhouette ratings led to the inclusion of individuals with a relatively high BMI of 20.3 $kg.m^{-2}$ [36] in females and 22.5 $kg.m^{-2}$ [42] in males; whose CT diagnosis was therefore highly debatable. Seven studies [11,17,18,20,38,39,46] did not clearly report any threshold for their definition of thinness. Thus, the systematic review revealed that studies do not systematically point out a cut-off to define thinness. In addition, large variability in both the used criteria and cut-off values was observed. In that context, it seems complex to propose specific recommendations concerning a thinness threshold. However, given the BMI cut-offs of the WHO [57,58], we would recommend not to enroll CT individuals with a BMI exceeding 18.49 $kg.m^{-2}$.

Weight history

Weight fluctuation and duration of fluctuations are other important parameters that should accompany consideration of the thinness degree. The present systematic review showed that weight history was well taken into consideration with 25 studies [7–10,16–19,21–23,27,28,30,31,35,36,38–40,42,44–47] reporting this criterion. However, there was a high heterogeneity in modalities: 4 studies [16,39,40,45] reported a stable weight for a certain period of time before the experiment (ranging from one week [45] to 2 years [16,39,40]) and 21 studies [7–10,17–19,21–23,27,28,30,31,35,36,38,42,44,46,47] reported it for a longer period throughout the growth period and/or the post-pubertal period. In 1982, Apfelbaum and Sachet already stressed the need to consider the weight history of CT patients and to differentiate between slimness and slimming [15]. Indeed, weight history opposes CT from AN [5,7,8]. Contrary to AN that is characterized by a curve break at the onset of anorexic tendencies, the diagnosis of CT should be supported by a low BMI (approximately the 3rd percentile) during all the growth period and by a stable bodyweight throughout the post-pubertal period [5,7,8]. In addition, CT seems to be a heritable trait [29,30], leading to CT families [7]. For three

generations, an average of 2.5 thin subjects per family is found CT for only 0.5 per family in AN [7]. Thus, the presence of other thin individuals in familial history can also reinforce a CT diagnosis.

Absence of eating disorders, associated pathology, and over-exercising

Potential eating disorders and associated diseases, as well as an energy imbalance caused by a high energy expenditure through physical activity, need to be taken into account to properly identify CT [5,8,10,19,21,28,31]. In the 35 included papers, the absence of eating disorders was well-considered: only 3 papers [39,40,47] did not consider this criterion. Although well-considered, this absence of eating disorders is most of the time simply mentioned or implicitly suggested without any details regarding its assessment. Only 26% [10,30,31,35,36,41,42,44,46] of the included studies used specific tools, like questionnaires or interviews, to confirm the absence of eating disorders and only 2 studies [10,31] associated questionnaires with the assessment of the following nutritional biomarkers: normal IGF-1, estradiol, FT_3, mean cortisol, and non-blunted leptin. In addition, questionnaires and interviews used were highly heterogeneous, using different versions, rarely reporting thresholds, and if so, with different thresholds. This observation shows the real need to adopt harmonized and common methods to robustly detect eating disorders. Concerning the absence of associated pathology, this criterion was well-considered in the selected papers: only 7 studies [11,24,35,36,38,42,45] did not report it. Since some studies may have taken into account some diagnostic parameters without explicitly detailing them in their inclusion process, we assume that some diagnostic parameters may have been slightly underestimated. Regarding physical activity, 63% [8,9,11,18–21,24,26,27,33–38,41–46] of the included studies did not report any physical activity level in their inclusion criteria. Ten articles [7,16,17,22,25,28,30,39,40,47] simply mentioned the absence of high physical activity level and only 3 articles [10,23,31] actually assessed physical activity level, using the MOSPA questionnaire [56]. Importantly, the relevance of the MOSPA questionnaire [56] should be discussed. This

questionnaire has been validated [56] among 50 pregnant women only, and several limitations in the methodological approaches of its validation need to be recognized [56]. The thresholds used to define the different physical activity levels differ: 2 articles [39,40] specified that participants did not spend more than one hour per week in sport activities, one article [31] considered the practice of more than 3 sessions of physical activity per week as an exclusion criterion and one article [30] excluded all participants who stated that they exercised more than 3 times a week or with an intensity exceeding 6 METs for any duration or frequency [55]. Altogether, these observations raised a real need to precisely describe the population in terms of type, duration, frequency, and intensity of physical activity with validated questionnaires but also with a more objective method such as accelerometry. Interestingly, spontaneous repeated muscle contractions in daily life, like fidgeting, were also suggested to be a relevant parameter to evaluate in CT for future studies [10,17,20,31].

Weight gain resistance/desire

Of the included articles, less than half of them [7–10,16,21–23,27,28,31,43,46,47] mentioned weight gain resistance/desire in their inclusion criteria of CT people, and most of these articles have been written by members of the same research team [7–10,21–23,27,28,31]. Of the 14 articles [7–10,16,21–23,27,28,31,43,46,47] mentioning this weight gain resistance/desire, 12 [7–10,21–23,27,28,31,46,47] used the idea of a "desire" to gain weight, one study [16] mentioned a complaint about being chronically underweight, another study [43] reported difficulty in gaining weight, and no studies used the term "resistance" to weight gain. Even if the desire to gain weight is actually, most of the time, the main reason for medical consultation in CT [5] and definitely differentiates CT from AN, we suggest here that an individual with CT might not present a strong desire to gain weight despite a physiological weight gain resistance, to the same extent that obesity is not defined as the subject's "willingness" to lose weight. In the case of CT, it may seem more

accurate to define it as a "resistance" to gain weight; that can result in a desire to gain weight – but not necessarily. Indeed, CT was found to be the first human model of physiological weight gain resistance [10] and several publications proposed supplements and treatments to help CT people gain weight, a few decades earlier [3,4,15,60] or more recently [10,31]. Bulik and Allison even proposed the following definition of CT: "constitutional protection against the need to diet in order to maintain a low body weight" [29].

Female gender predominance and amenorrhea

A female gender predominance was observed with 26 studies [7–11,16,17,19–28,33–41] that were conducted among females exclusively, 6 studies [30,31,44–47] on both genders and 3 studies [18,42,43] in males exclusively. As the systematic review was performed on clinical studies, it seems to us that this observation probably only illustrates the lower consultation rate in men and we encourage further researches in both genders; as CT is not a sex-specific condition. The presence of menses in the diagnosis of CT was widely taken into account: only 6 studies [19,30,33,36,44,45] did not mention this criterion of the 32 studies [7–11,16,17,19–28,30,31,33–41,44–47] enrolling females. Although the absence of amenorrhea was well-considered in the studies, the removal of this criterion from the revised DSM-V [13] can lead to new difficulties in the differential diagnosis between AN and CT [8]. It seems however relevant to us to verify the absence of amenorrhea in the diagnosis of CT.

Recommendations in the diagnosis of CT

The systematic review of clinical trials that enrolled participants with CT definitely revealed the real need to adopt both a common terminology and a well-defined diagnosis of CT. Based on the present results, we collectively propose here the key term "constitutional thinness" to be used. Using the "constitution" term to refer to the innate and natural cause of thinness seems of particular interest since it also helps

clarify the distinction with other behavioral or pathological origins of thinness. In this respect, it seems essential to systematically exclude energy imbalance caused by inappropriately low energy intake (eating disorders) and/or an inappropriately high exercise-induced energy expenditure, using validated tools. Ideally, eating behavior should be evaluated not only with common validated questionnaires or interviews using specific thresholds, but also with the assessment of nutritional biomarkers. If possible, the absence of over-exercising should not only be declarative but also measured with robustly validated questionnaires or even by accelerometry technique. Although amenorrhea has been removed from the definition of AN in the DSM-V [13], it seems relevant to consider the presence of physiological menstruations in the diagnosis of CT. In addition, weight gain resistance and weight history also need to be taken into consideration in the diagnosis. Finally, the question of defining a strict threshold for thinness remains complex and arbitrary. Even though BMI assessment is associated with various limitations [59], we would tend to favor this measurement as long as it is very common and simple to perform. Conversely, we recommend not to use the percentage of body fat as a maximal threshold since CT does not seem to be characterized by a low body fat percentage [8,10,11,17,19,23–27]. Given the BMI cut-offs of the WHO [57,58], we propose that CT should not be discussed with a BMI exceeding the value of 18.49 $kg.m^{-2}$. Beyond these essential criteria for CT diagnosis, some studies seem to suggest certain common characteristics in CT groups. In comparison to people with AN, CT individuals might display higher REE and REE-to-FFM [7,17,18] (although it does not seem significant in two studies [8,16]), non-blunted FM percentages despite their low BMI [7,8,11,17,23,24], and different profiles of appetite-regulating hormones [9,11,21–23]). If these types of results were supported by a substantial number of studies and clinical evidence, they could be used as new criteria for the distinction of CT from AN in the future, which remains to be robustly demonstrated. In order to visually synthesize the potential actual recommendations in CT diagnosis, based on this systematic analysis, a decision tree is proposed in Figure 2.

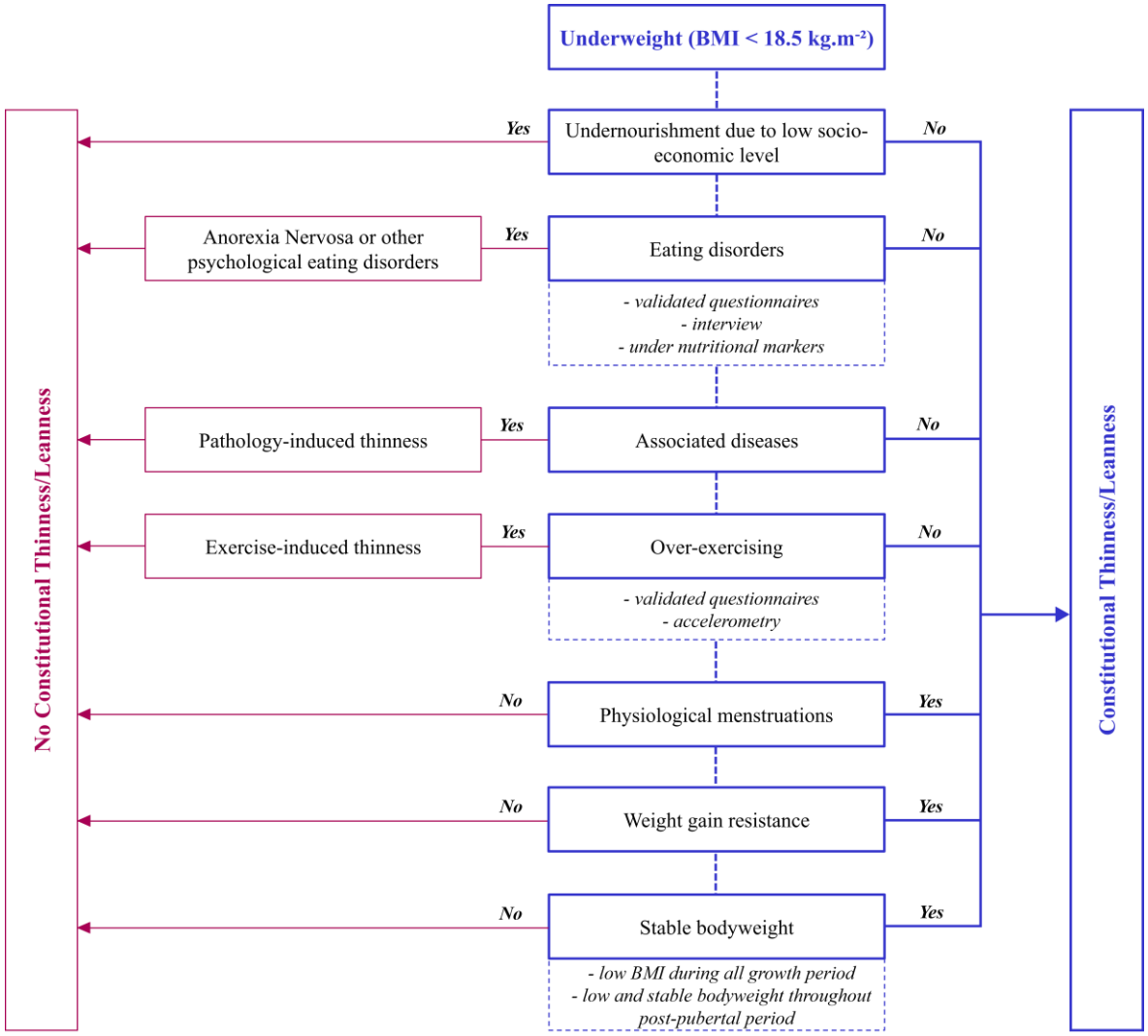

Figure 2. Decision tree in the diagnosis of constitutional thinness

On top of the inclusion criteria used by the selected studies, their methodologies must also be considered when interpreting our results as analyzed and presented in our Risks of Bias table (Table 2). Indeed, as reported in Table 2, 34 out of the 35 included studies present a high risk for the "blinding of participants and personnel", which might affect the obtained results when it comes for instance to the evaluation of energy intake, eating profiles or physical activity that could be affected by the non-blinding of participants or personnel. This interpretation of our analyses must also consider the high proportion of studies presenting a moderate-to-high risk regarding the attrition bias, or even unreported data.

Conclusion

The present review used a systematic approach to identify any clinical trials that enrolled individuals with CT, particularly focusing on the methods used to define and diagnose CT. The employed methodology led us to identify 35 clinical trials enrolling a group of participants with CT. This clearly pointed out a relatively reduced number of studies interested in this condition. In addition, the definition and the diagnostic features of CT were found highly heterogeneous in these studies. Terminology and thinness thresholds do not reach consensus and a high heterogeneity was also observed regarding the assessment of weight history, weight gain resistance and the presence of physiological menses. The absence of eating disorders, associated pathology or over-exercising was not systematically verified and if so, with various methodological approaches. This systematic review points out the essential need not only to be aware of the existence of CT but also to harmonize our medical and scientific practices in the definition and diagnosis of CT. Altogether, the present results led us to propose a decision tree that could help practitioners and researchers better define and diagnose constitutional thinness, in a potentially more harmonized way. Importantly, not only the proposed decision tree has been elaborated based on clinically relevant indicators that have to be considered for the diagnosis of CT, but it also proposes different evaluation alternatives (from self-reported eating questionnaires to undernutrition physiological makers for instance); guaranteeing its clinical feasibility and applicability.

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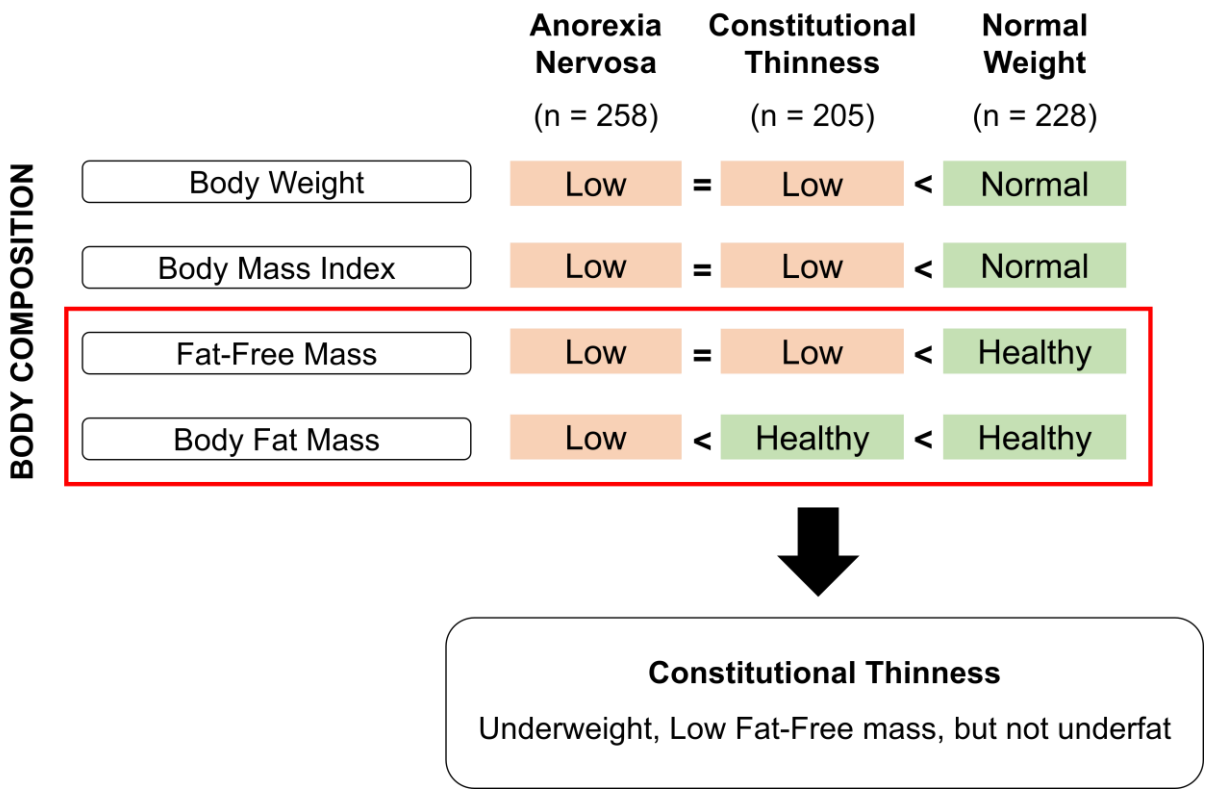
ÉTUDE 2 : COMPOSITION CORPORELLE ATYPIQUE DE LA MAIGREUR CONSTITUTIONNELLE



Graphical abstract

Underweight but not underfat: is fat-free mass a key factor in constitutionally thin women?

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Highlights: •Constitutionally thin people are as underweight as anorectic patients but not underfat; •Constitutional thinness: a fat-free mass as low as in anorexia nervosa; •New interests for lean-fat partitioning in constitutional thinness

Keywords: Constitutional thinness; Constitutional leanness; Underweight; Weight gain resistance; Review

List of abbreviations: AN: anorexia nervosa, BMI: body mass index, CI: confidence interval, CT: constitutional thinness, FFM: fat-free mass, FM: fat mass, NW: normal-weight, REE: resting energy expenditure, SMD: standardized mean difference

Abstract

Background/Objectives: Constitutional thinness is defined as a state of severe underweight with a body mass index similar to anorectic patients (BMI < 17.5 kg/m²), in the absence of any eating disorders or other obvious disruptive factors impacting energy balance. The analysis of body composition is essential as a first approach to characterize constitutional thinness and might help identify new discriminating differences between constitutional thinness and anorexia nervosa.

Subjects/Methods: A meta-analytical approach was performed to compare body composition of constitutionally thin, anorectic, and normal-weight subjects from all available studies found in the literature. The statistical analysis was carried out on large sample sizes: n=205 females with constitutional thinness, n=228 normal-weight control females, and n=258 females with anorexia nervosa.

Results: Despite being as underweight as anorectic patients, constitutionally thin participants paradoxically presented higher percentages of fat mass than anorectic patients (18.9% *vs.* 11.4% respectively; SMD [95% CI]: 1.62 [1.16 – 2.08]), even found in the normal healthy ranges. Constitutionally thin people however display as low fat-free mass as anorectic patients.

Conclusions: These observations question the use of high-fat diets in this population and bring new insights for nutrition and/or training strategies directed towards muscle mass gain. The present results give new elements to further distinguish constitutional thinness from anorexia nervosa and reinforce the need to better investigate the atypical phenotype of constitutional thinness.

Introduction

Interests for constitutional thinness. In our modern obesogenic societies, overweight and its many health issues have given rise to a wide range of researches on both overweight prevention and management, mainly through multidisciplinary approaches. Although the various consequences of overweight and obesity are largely evidenced today; underweight remains underexplored and is usually associated with anorexia nervosa (AN) in the common beliefs. However, a reduced part of the scientific literature but an important number of clinicians also describe the existence of non-pathological thinness characterized by the willingness to gain body mass along with weight gain resistance. The first scientific publications as early as the 1930s [1] described cases of individuals resistant to weight gain who consulted for medical advice. More than 80 years later, less than 50 scientific papers have been interested in this issue, often naming this weight gain resistance associated with a low BMI (<17.5 kg/m²) [2–6] as 'constitutional thinness (CT)' [6–15] or 'constitutional leanness' [16–20]. This state of thinness does not seem to be mainly explained by obvious environmental or behavioural causes since individuals with CT do not present any eating disorders, undernourishment, over-exercising, or associated pathology [2,4,10,21]. Therefore, CT is likely to be in one way to another the result of a particular physiology [15].

Relevance of body composition analysis. While the use of the terminology 'leanness' suggests a high proportion of lean tissue, most of the studies conducted so far considered fat mass (FM) as a way to discriminate individuals with CT from normal-weight ones, suggesting a state of underweight and underfat similar to AN [2,5,6,8,15,22], even if some studies also suggested higher FM percentages (%FM) in individuals with CT compared with AN [11,23]. In order to properly diagnose CT and clarify its distinction from AN [4,5,24], there is today a clear lack of consideration of the physiology of CT itself, compared with normal-weight (NW) but also with AN. While there are controversial findings in the

literature on body composition, and since it plays a crucial role in the regulation of energy metabolism and then body mass [25,26], it seems necessary today to better question its repartition and implication in individuals with CT, potentially revealing further differences between CT, AN, and NW when it comes to corpulence, body weight, FM and fat-free mass (FFM). The present work proposes then a systematic analysis and meta-analytic comparison of the available evidences regarding body composition between CT, NW and AN individuals.

Materials and Methods

Search strategy. Recommendations from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement were followed in the search process. Bibliographical research was carried out on 5 electronic databases between December 2018 and June 2020: PubMed-Medline, Embase, the Cochrane Library, Google Scholar, and Clinical Trials. The co-authors discussed the search strategy and selected the following keywords: 'constitutional', 'thinness', 'leanness', 'weight gain resistance', 'physiology', and 'human'.

Study selection. Selected clinical trials were published in English or French languages. Studies enrolling at the same time CT, AN, and control participants and assessing body weight, BMI, and body composition were screened. The inclusion of studies was based on a reliable diagnosis of CT beyond the state of underweight: absence of eating disorders, associated diseases, over-exercising but presence of stable bodyweight within lower percentiles of growth curve and physiological menses [24]. Since only one study including CT, NW and AN groups was conducted among males [20], the choice was made to perform the analysis in females exclusively in order to avoid sex-related bias. Studies were not included if: (a) thinness was not due to a well-identified 'constitutional' origin, (b) studies used 'lean' word to refer to

NW participants instead of a state of underweight, (c) no enough data was available: letters to the Editor, reviews, abstracts alone, or case studies. Two authors independently selected clinical trials based on title and abstract. The full texts remaining were screened based on the eligibility criteria. Disagreements were resolved by discussion and if needed, by the opinion of a third author. The flow diagram of the selection process is displayed in Figure 1. Statistical analyses were finally performed on 13 studies [5,7,8,10,11,16–18,23,27–30] selected against the aforementioned inclusion and exclusion criteria. References with the population's characteristics, methods of body composition measurements, and main results of each selected study are detailed in Table 1.

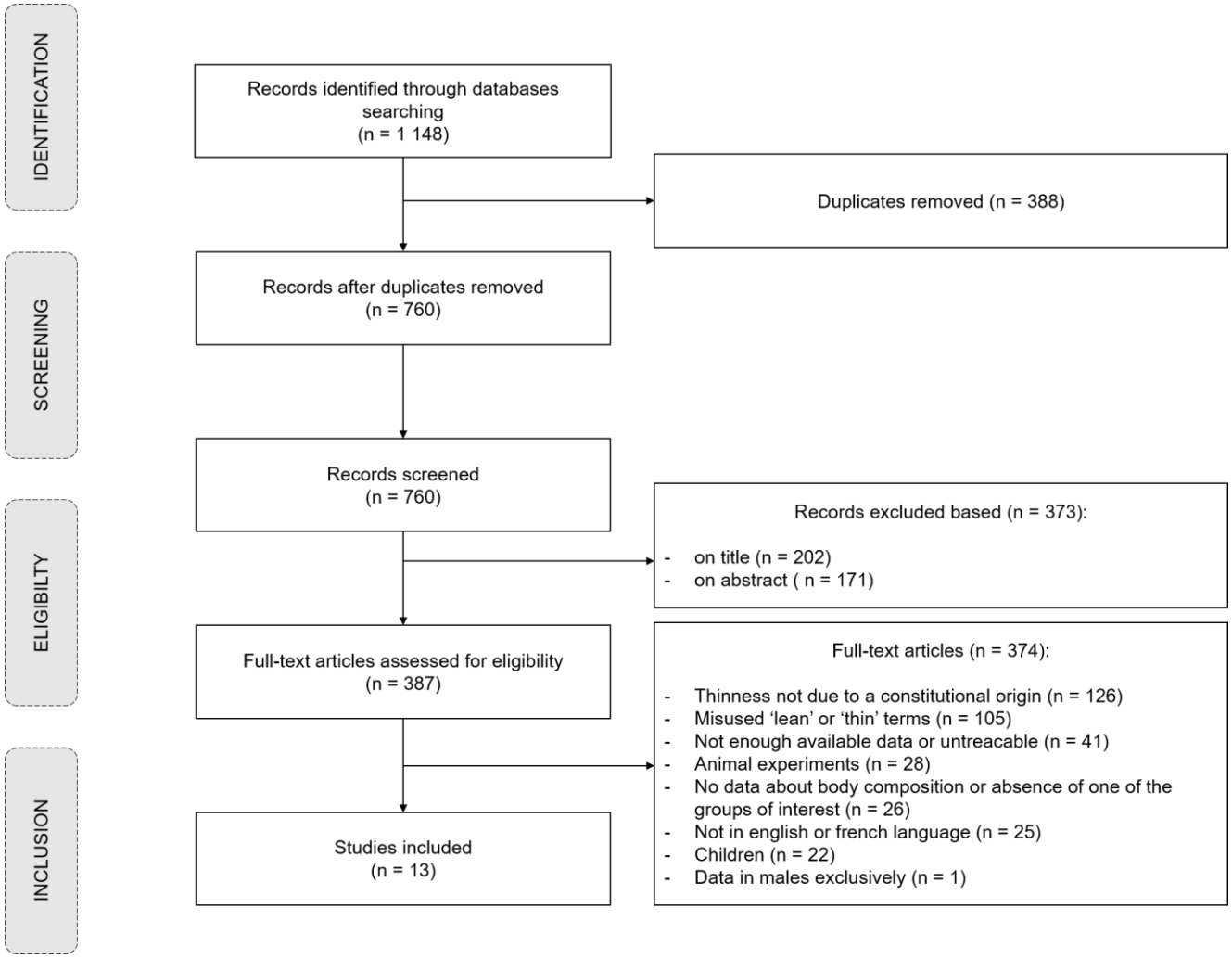

Figure 1. Flow diagram of the inclusion process

Table 1: Characteristics of constitutionally thin, control and anorectic female participants, methodology and results

Reference	Design of the study	Characteristics of the participants (means ± SD)	Investigated parameters (methods used)	Results
Tolle *et al.*, 2003 [7]	Cross-sectional trial	CT: n=8; 23.3 ± 8.8 [a] yr; 15.7 ± 1.1 [a] kg.m^{-2} C: n=10; 23.2 ± 3.5 [a] yr; 21.5 ± 2.2 [a] kg.m^{-2} AN-R: n=9; 17.2 ± 2.7 [a] yr; 14.6 ± 1.2 [a] kg.m^{-2}	BW, BMI, FM (BIA – Analicor 2)	BW: AN=CT<C BMI: AN=CT<C %FM: AN<CT=C
Tagami *et al.*, 2004 [8]	Cross-sectional analysis	CT: n=6; 27.5 ± 4.2 yr; 17.7 ± 0.5 kg.m^{-2} C: n=16; 25.7 ± 2.9 yr; 20.3 ± 1.5 kg.m^{-2} AN: n=31; 25.5 ± 8.1 yr; 14.0 ± 2.5 kg.m^{-2} (AN: probably restrictive-type)	BMI, FM (BIA – Tanita, analyser TBF-110)	BMI: AN, CT<C %FM: AN, CT<C (statistical tests *vs.* C only)
Bossu *et al.*, 2007 [10]	Cross-sectional analysis	CT: n=7; NR but 18-26 yr; 16.1 ± 0.6 kg.m^{-2} C: n=7; NR but 18-26 yr; 21.2 ± 0.8 kg.m^{-2} AN-R: n=6; NR but 18-26 yr; 15.8 ± 0.8 kg.m^{-2}	BW, BMI, FM, FFM (DXA – LUNAR)	BW, BMI: AN=CT<C %FM: AN<CT<C FFM: CT=AN<C
Germain *et al.*, 2007 [11]	Cross-sectional trial	CT: n=10; 20.2 ± 3.8 yr; 15.7 ± 0.6 kg.m^{-2} C: n=7; 23 ± 2.1 yr; 20.4 ± 0.8 kg.m^{-2} AN: n=12; 20.7 ± 4.2 yr; 15.2 ± 1.4 kg.m^{-2} (AN: probably restrictive-type)	BMI, FM, FFM (DXA – LUNAR)	BMI: AN=CT<C %FM: AN<CT<C FFM: NR
Marra *et al.*, 2007 [16]	Cross-sectional analysis	CT: n=20; 22.5 ± 5.8 yr; 17.2 ± 1.0 kg.m^{-2} C: n=20; 22.0 ± 3.7 yr; 21.7 ± 2.4 kg.m^{-2} AN-R: n=20; 18.8 ± 3.4 yr; 15.1 ± 1.6 kg.m^{-2}	BW, BMI, FM, FFM (single frequency BIA – Akern)	BW, BMI: AN=CT<C %FM: AN<CT<C FFM: AN=CT; CT=C; AN<C
Galusca *et al.*, 2008 [23]	Cross-sectional analysis	CT: n=25; 23.1 ± 6.0 yr; 15.8 ± 0.5 kg.m^{-2} C: n=28; 23.9 ± 7.4 yr; 20.7 ± 2.1 kg.m^{-2} AN-R: n=44; 23.4 ± 8.0 yr; AN: 15.5 ± 0.7 kg.m^{-2}	BMI, FM, FFM (DXA – HOLOGIC)	BMI: AN=CT<C %FM: AN<CT<C FFM: NR
Germain *et al.*, 2009 [27]	Cross-sectional trial	CT: n=9; 24.1 ± 3.6 yr; 16.1 ± 0.3 kg.m^{-2} C: n=10; 23.1 ± 4.4 yr; 20.5 ± 1.3 kg.m^{-2} AN-R: n=15; 20.4 ± 5.0 yr; 14.8 ± 0.4 kg.m^{-2}	BMI, FM, FFM (DXA – LUNAR)	BMI: AN=CT<C %FM: AN<CT<C FFM: NR

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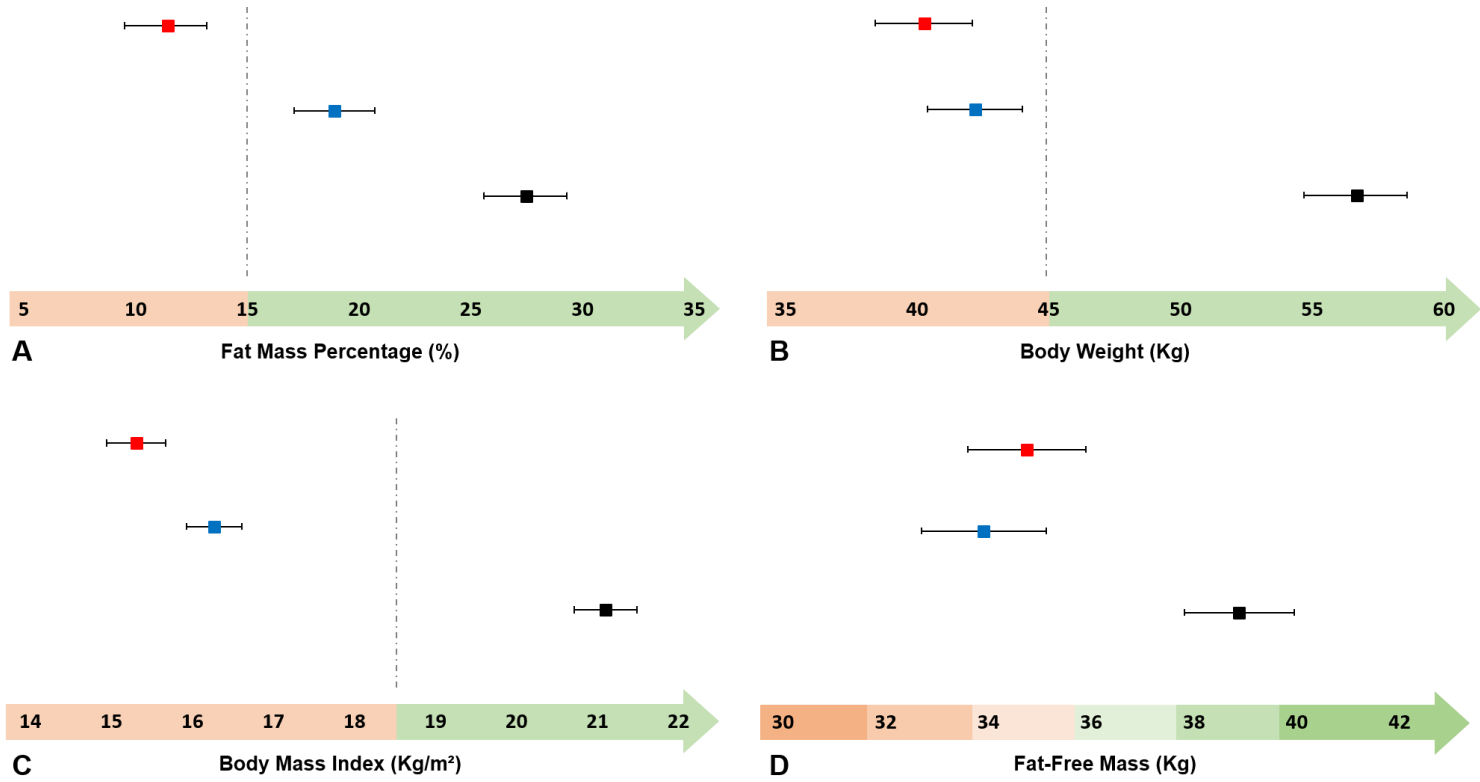
Marra *et al.*, 2009 [17]	Cross-sectional analysis	CT: n=10; 19.4 ± 2.4 yr; 16.8 ± 1 $kg.m^{-2}$ C: n=30; 20.0 ± 2.1 yr; 22.5 ± 2.8 $kg.m^{-2}$ AN: n=30; 19.0 ± 2.0 yr; 16.7 ± 0.5 $kg.m^{-2}$ (AN type: NR)	BMI, FM, FFM (Skinfold thickness – measured in triplicate to the nearest 0.2mm with a calibrated Harpenden caliper at 4 sites: biceps, triceps, subscapular and suprailiac – estimation with the sum of these 4 skinfold values)	BMI, and biceps, triceps, subscapular, suprailiac skinfolds [b]: AN=CT<C %FM: AN=CT<C FFM: CT=AN<C
Galusca *et al.*, 2012 [28]	Cross-sectional trial	CT: n=14; 23.7 ± 6 [a] yr; 16.0 ± 0.4 [a] $kg.m^{-2}$ C: n=10; 23.1 ± 5 [a] yr; 20.8 ± 0.6 [a] $kg.m^{-2}$ AN-R: n=19; 23.2 ± 8 [a] yr; 15.3 ± 0.4 [a] $kg.m^{-2}$	BMI, FM (DXA – LUNAR)	BMI, FM: AN<CT<C %FM: AN<CT<C
Pasanisi *et al.*, 2013 [18]	Cross-sectional analysis (same C group as Marra *et al.* 2007 [16])	CT: n=7; 21.7 ± 3.6 yr; 16.2 ± 0.9 $kg.m^{-2}$ C: n=20; 22.0 ± 3.7 yr; 21.7 ± 2.4 $kg.m^{-2}$ AN-R: n=7; 23.4 ± 4.5 yr; 15.3 ± 0.8 $kg.m^{-2}$	BW, BMI, FM, FFM (single frequency BIA – Akern)	BW, BMI, %FM, FFM: AN=CT [c]
Galusca *et al.*, 2015 [29]	Cross-sectional trial	CT: n=22; 23.2 ± 2.3 yr; 15.9 ± 0.5 $kg.m^{-2}$ C: n=14; 22.6 ± 6.0 yr; 21.6 ± 1.1 $kg.m^{-2}$ AN-R: n=23; 22.5 ± 6.2 yr; 14.6 ± 2.4 $kg.m^{-2}$	BMI, FM (DXA – LUNAR)	BMI: Missing significance symbol between CT and C, the result would therefore be: AN<CT<C %FM, FM: AN<CT<C FFM: NR
Germain *et al.*, 2016 [30]	Cross-sectional trial	CT: n=10; 20.6 ± 6.6 yr; 15.9 ± 0.9 $kg.m^{-2}$ C: n=10; 22.7 ± 1.6 yr; 21.4 ± 1.6 $kg.m^{-2}$ AN-R: n=10; 21.6 ± 4.7 yr; 15.1 ± 2.5 $kg.m^{-2}$	BW, BMI, FM (DXA – LUNAR)	BW, BMI: CT<C; AN=CT %FM: AN<C; p=0.05 for AN<CT; CT=C
Estour *et al.*, 2017 [5]	Cross-sectional trial	CT: n=56; 26.9 ± 7.6 yr; 16.5 ± 0.9 $kg.m^{-2}$ C: n=54; 23.4 ± 4.1 yr; 20.9 ± 2.2 $kg.m^{-2}$ AN-R: n=40; 25.0 ± 6.5 yr; 16.0 ± 0.8 $kg.m^{-2}$	BMI, FM, FFM (DXA – LUNAR)	BMI: AN=CT<C %FM, FM: AN<CT<C FFM: CT=AN; AN=C and trend for CT<C (with p-value significance at 0.001)

[a] Type of values dispersion (SD or SEM) not clearly reported; [b] Synthetized result from different results; [c] Result not clearly reported

AN: females with anorexia nervosa; AN-R: females with anorexia nervosa of restrictive type; BIA: bioelectrical impedance analysis; BMI: body mass index; BW: bodyweight; C: normal-weight control females; CT: constitutionally thin females; DXA: dual-energy X-ray absorptiometry; FFM: fat-free mass; FM: fat mass; NR: not reported

Statistical analyses. Data are presented as Mean [Min – Max] (Figure 2). The color code of the x-axis indicates in orange the pathological values and green the physiological healthy values. Statistical analysis was performed with Stata software (version 15, StataCorp, College Station, TX) and Comprehensive Meta-Analysis. The meta-analysis took account of between- and within-study variability. To address the non-independence of data due to the study effect, random-effects models as developed by DerSimonian were preferred to the usual statistical tests in order to estimate standardized mean differences (SMD) and their 95% confidence intervals.



Data are presented as Mean [Min – Max]. The color code of the x-axis indicates in orange the pathological values and green the physiological healthy values.

Figure 2. Fat Mass percentage (A), Body Weight (B), Body Mass index (C) and Fat-Free Mass (D) comparisons between patients with Anorexia Nervosa (■), Constitutional Thinness (■) and Normal-weight (■)

Results and Discussion

Constitutionally thin females cannot be considered as underfat. As illustrated by Figures 2B and 2C, CT females present significantly lower body weight (SMD [95% CI]: 2.55 [2.07 – 3.04]) and BMI (SMD [95% CI]: 3.94 [3.12 – 4.76]) compared with NW, without being different from patients with AN. This confirms and reinforces the underweight phenotype of CT. However, contrary to what is usually considered, Figure 1A clearly indicates that CT females cannot be considered as underfat. Although CT females effectively show a significantly lower percentage of FM compared with NW (18.9% *vs.* 27.4%; SMD [95% CI]: 1.92 [1.45 – 2.38]); this percentage remains significantly and largely higher (+7.5%FM) than AN patients (18.9% *vs.* 11.4% respectively; SMD [95% CI]: 1.62 [1.16 – 2.08]). Despite being as underweight as AN patients, CT females present %FM that seems to be within healthy FM ranges [31–34]. This distinction in %FM between AN and CT is highly important to consider since body fat has different implications on both sides of energy balance [26]. First, FM has a small but existing contribution to the variance in resting energy expenditure (REE) [35] and this contribution depends on the grade of adiposity [36]: %FM ≤10%: low contribution; %FM>10% and ≤30%: intermediate; %FM>30% and ≤40% moderately elevated [36]. Given the different grades of adiposity observed between AN and CT (Figure 2A), a different impact on REE might be expected between these two populations. The %FM difference between AN and CT may also have influences on energy intake regulation. For instance, leptin is well known to be positively associated with %FM [37] and to have some effects on the inhibition of food intake acting as a hunger suppressant signal [38,39]. As observed in the present study regarding %FM, the literature also shows intermediate levels of leptin in CT compared with AN and NW [5,7,40]. Contrary to AN, CT however seems to be characterized by normal energy intake [10,11], which suggests different regulations of energy balance. Finally, this %FM difference between AN and CT has probably different

implications on both energy intake and expenditure, which reinforces again the need to properly differentiate these two populations.

Can constitutional thinness really be considered within the normal range of percentage body fat?

The non-blunted %FM observed in CT females also raised the controversial issue of the healthy ranges of %FM. As introduced by Gallagher and her team, the literature shows no consensus about this question [41]. Regarding the growing interest for adiposity-based classifications, Gallagher *et al.* linked healthy BMI guidelines with %FM based on BMI thresholds established by the World Health Organization [41]. Their predictive model showed that a BMI of 18.5 kg/m² (which is also known for its own limitations) would be associated with a %FM of 21% in women [41]. Yet, they also recognized that this range should be cautiously applied given the low fraction of subjects included in their low BMI group [41] and some other publications mentioned lower thresholds, such as of 14% [31,32] or 16% [33] of FM as the minimal limit of a normal %FM. Despite the equivocal definition of being underfat, our present results give evidence of a significant difference in %FM between AN (11.4%) and CT (18.9%); therefore placing CT into, or at least very close to, healthy ranges.

New interests for lean-fat partitioning in constitutional thinness. From the relations between weight evolution and body composition usually presented in the literature [42], underweight would have been expected with a FM rather more affected than FFM by the low weight, as it is generally observed in AN with %FM around 10% [8,10,16]. One of the different hypotheses that might explain this unexpected non-blunted %FM in CT might be the 'collateral fattening' concept [42,43]. We are aware that Dulloo and his team developed this concept of body composition autoregulation regarding weight recovery patterns [42,43] and not for low but stable body weight as in CT [2,10]. Nevertheless, we formulate the hypothesis that a 'chronic' unsuccessful attempt of weight recovery, and more specifically of FFM

recovery in CT, might also lead to a specific lean-fat partitioning inducing collateral fattening. However, no longitudinal studies were conducted to date and the present analysis does not allow to test this hypothesis. Although the causes and consequences of the observed body fat in CT need to be further explored, it remains that females diagnosed with CT cannot be considered as 'underfat', which has huge implications when it comes to weight gain strategies. For this reason, it questions for instance the use of over-nutrition strategies based on high-fat diet, as recently proposed with short-term limited success [2].

Is Fat-Free mass the real key factor in constitutional thinness? Regarding these elements, it might be more relevant to try to increase FFM tissue in CT rather than body fat. Indeed, the Figure 2D provides a non-equivocal result showing that FFM of CT participants is lower compared to NW participants (33.9 kg *vs.* 38.8 kg; SMD [95% CI]: 1.26 [0.78 – 1.74]) and similar compared to AN patients, even showing a lower mean value (33.9 kg in CT *vs.* 34.7 kg in AN; SMD [95% CI]: -0.22 [-0.60 – 0.17]). In contrast to body fat which does not seem to be impaired in CT, FFM would on the contrary be greatly diminished. These contrasting FM and FFM results in CT might illustrate a specific lean-fat partitioning [42,43] in this population. While this assumption has yet to be investigated, present results clearly show a FFM at least as low in CT as it is in AN. This observation highlights a specific interest in developing FFM-targeted overfeeding strategies in CT. For this purpose, strength training eventually combined with a high-protein diet overfeeding might be an interesting strategy to help CT people gain weight through potential muscle hypertrophy.

Limitations. Several limitations have to be considered when interpreting the present analysis. First, the present results were obtained from studies conducted among young women which only represent a specific and limited part of the population. The analysis was performed on females to avoid sex bias,

given that only one study including males was eligible, and the inapplicability of these findings to males with CT must then be considered as a limitation here. Secondly, none of the clinical trials reported FM or FFM indexes, respectively calculated as FM/height² and FFM/height² [44]. As detailed by Dulloo and collaborators in 2010, the partitioning of BMI into FM and FFM indexes is however informative on body composition of certain conditions and might be very appropriate in characterizing CT condition. We therefore encourage the use of these indexes in future studies. Finally, different methods were used between the different publications to assess body composition. Among the 13 selected studies, 8 [5,10,11,23,27–30] used Dual-energy X-ray absorptiometry (DXA), recognized as the gold standard technique particularly reliable, while 4 [7,8,16,18] other studies used bioelectrical impedance (BIA). In healthy subjects, the accuracy of BIA measurements is discussed in the light of the different risks of bias related to hydration status, ambient temperature, or fasting status [45]. Moreover, in specific populations as obese or elderly people, the literature gave evidence of lower reliability of BIA compared with DXA [46–48]. Further studies should question the accuracy of BIA measurements among CT individuals. Skinfold thickness, a more accessible method, is known to be less trustworthy than DXA or BIA [49] but was however used in one of the included study [17]. Thus, even if more than half of the studies used the highly reliable DXA method to assess body composition, the present results have to be treated with caution given the different methods used by the other studies.

Conclusion

The present analysis provides more robust conclusions since the statistics were performed on large sample sizes: n=205 females with CT, n=228 NW control females, and n=258 females with AN. For the same state of thinness, %FM seems to be found in the normal ranges in CT and largely higher than in AN, whereas FFM was found as low in CT as in AN. From this observation, it would seem relevant to

investigate new FFM-targeted overfeeding and/or training strategies in order, hopefully, to help people with CT gain weight healthily.

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ÉTUDE 3 : PHYSIOLOGIE DE LA MAIGREUR CONSTITUTIONNELLE : REVUE SYSTÉMATIQUE ET MÉTA-ANALYTIQUE

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Is constitutional thinness really different from anorexia nervosa? A systematic review and meta-analysis

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Keywords: Constitutional thinness; weight gain resistance; energy metabolism; biochemical markers; appetite-regulating hormones; body composition

Abstract

A growing interest in constitutional thinness has been observed in the last decades, but the publications however cover various fields of study and report equivocal results. The present work systematically reviewed any clinical trials enrolling participants with constitutional thinness and bibliographic researches were performed between December 2018 and June 2020. From a total of 1 212 records initially identified, 402 records were removed as duplicates, 381 articles were excluded based on titles or abstracts and 390 references were excluded against eligibility criteria. Thirty-nine articles were finally included in the systematic review. The results showed that constitutionally thin people seem to be underweight but not underfat and present a fat-free mass as blunted as anorexic patients, despite being a little less underweight. The meta-analysis confirmed that constitutionally thin people present normal energy intake and revealed a trend toward a higher resting metabolic rate to fat-free mass ratio which suggests a highly metabolic fat-free mass. Contrary to patients with anorexia nervosa, constitutionally thin people present normal levels of insulin-like growth factor 1, estradiol, growth hormone, follicle-stimulating hormone, and luteinizing hormone. An intermediate level of leptin between anorexic and control participants was however observed in constitutional thinness. While all the studies reported normal free triiodothyronine and cortisol levels in constitutionally thin individuals, a higher fasting free triiodothyronine level ($p=0.033$) and a lower 24-hours mean cortisol level ($p=0.005$) were observed for the first time. Present results give robust evidence that constitutionally thin people present an atypical phenotype highly different from anorexia nervosa.

Abbreviations

3D-pQCT	three-dimensional peripheral quantitative computed tomography	**CIV**	complex IV of the mitochondrial respiratory chain
18F-FDG	18-fluorodeoxyglucose	**C/F**	capillary to muscle fibre ratio
AAT	amino acid transferase	**CAFA**	capillary contact per fibre area
ACTH	adrenocorticotropic hormone	**calo**	calorimetry
AEE	activity energy expenditure	**CC**	capillary contacts per muscle fibre
ALK	anaplastic lymphoma kinase	**CD**	capillary density
ALP	alkaline phosphatase	**CFPE**	capillary to fibre-perimeter exchange
ALT	alanine aminotransferase	**CI**	confidence interval
α-MSH	α-melanocyte-stimulating hormone	**CK**	creatine kinase
AN	subjects with anorexia nervosa	**COx**	cytochrome C oxidase
AN-BP	subjects with anorexia nervosa of bingeing/purging type	**CS**	citrate synthase
AN-R	subjects with anorexia nervosa of restrictive type	**CSA**	cross-sectional area
AST	aspartate aminotransferase	**CT**	constitutionally thin subjects
AUC	area under the curve	**DEBQ**	dutch eating behaviour questionnaire
BAT	brown adipose tissue	**DEPTOR**	DEP domain-containing MTOR interacting protein
BD	underweight ballet dancers	**DHEAS**	dehydroepiandrosterone sulfate
β-HAD	β-hydroxyacyl-CoA dehydrogenase	**DOMS**	delayed onset muscle soreness
BI	bioimpedance index	**DLW**	doubly labeled water
BIA	bioelectrical impedance analysis	**DXA**	dual-energy X-ray absorptiometry
BMC	bone mineral content	**E2**	estradiol
BMD	bone mineral density	**EDE**	eating disorder examination
BMI	body mass index	**EDI**	eating disorder inventory
BN	subjects with bulimia nervosa	**EDNOS**	subjects with eating disorders not otherwise specified
BW	bodyweight	**EGCUT**	estonian genome center of the university of tartu
C	normal-weight control participants	**FA**	fatty acids
CI	complex I of the mitochondrial respiratory chain	**FFM**	fat-free mass
CII	complex II of the mitochondrial respiratory chain	**FITM**	fat storage-inducing transmembrane

FM	fat mass	**MOSPA**	MONICA optional study of physical activity
FSH	follicle-stimulating hormone	**MPS**	multidimensional perfectionism scale
FT3	free triiodothyronine	**MRI**	magnetic resonance imaging
FT4	free thyroxine	**NA**	not applicable
FTI	free testosterone index	**NAA**	N-acetyl aspartate
GGT	gamma-glutamyl transpeptidase	**NEAT**	nonexercise activity thermogenesis
GH	growth hormone	**NN**	normal RR interval
GHRH	growth hormone-releasing hormone	**NPY**	neuropeptide Y
GLP-1	glucagon-like peptide-1	**NR**	not reported
Glx	glutamine/glutamate ratio	**NS**	not significant
GnRH	gonadotropin-releasing hormone	**OB**	participants with obesity
GWAS	genome-wide association study	**OF**	overfeeding
HF	high frequency	**OPG**	osteoprotegerin
HOMA	homeostasis model assessment	**PAL**	physical activity level
HR-pQCT	high-resolution peripheral quantitative computed tomography	**PET/CT**	positron emission tomography with computed tomography
iAUC	incremental area under the curve	**PFK**	phosphofructokinase
IBW	ideal bodyweight	**pNN50**	number of adjacent NN intervals differing by more than 50 ms in the entire recording divided by the total number of all NN intervals
IGF-1	insulin-like growth factor 1	**PPT**	postprandial thermogenesis
IL-7	interleukin-7	**pQCT**	peripheral quantitative computed tomography
IMTG	intramuscular triglycerides	**PTH**	parathyroid hormone
LC/PF	ratio between the length of contact of the capillaries with the muscle fibre to the perimeter of the muscle fibre	**PYY**	peptide YY
LDH	lactate dehydrogenase	**QUICKI**	quantitative insulin sensitivity check index
LF	low frequency	**RANKL**	receptor activator of nuclear factor-κB ligand
LH	luteinizing hormone	**RCT**	randomized Controlled Trial
Ln	logarithmic units	**rec**	after weight recovery or partial recovery

RMR	resting metabolic rate	**SHBG**	sex hormone-binding globulin
RMRc	calculated resting metabolic rate	**SMD**	standardized mean difference
RMRe	estimated resting metabolic rate	**SUVmax**	maximum standardized uptake values
RMRm	measured resting metabolic rate	**T3**	T3: triiodothyronine
RMR/FFM	resting metabolic rate to fat-free mass ratio	**T4**	tetraiodothyronine
rMSSD	root mean square successive difference	**TEE**	total energy expenditure
ROC	receiver operator characteristics	**TEI**	total energy intake
ROM	pain-free range motion	**TFEQ**	three-factor eating questionnaire
RQ	respiratory quotient	**TRACP 5b**	tartrate-resistant acid phosphatase type 5b
SCL-90-R	symptom check list – 90-revised	**TSH**	thyroid stimulating hormone
sCTX	serum C-telopeptide cross-link of type 1 collagen	**ULF**	ultra-low frequency
SD	standard deviation	**UPLC**	ultra-performance liquid chromatography
SDANN	index standard deviation of the average NN intervals for all 5-min segments	**VAS**	visual analog scale
SDNN	standard deviation of all NN intervals	**VLF**	very low frequency
SDNN index	mean of the standard deviation of all NN intervals for all 5-min segments	**W0**	week 0
SEM	standard errors of the means	**W4**	week 4
SERCA	sarco/endoplasmic reticulum Ca^{2+} –ATPase	**W8**	week 8
SF	sharing factor		

Introduction

Constitutional thinness (CT) is a natural state of underweight (BMI < 17.5 kg/m²) [1–4] characterized by the absence of any apparent imbalance between energy intake and expenditure [1, 4–6]. Despite the absence of undernourishment, eating disorders, associated diseases, or over-exercising, people with CT present a very low and stable bodyweight (BW) and have been shown resistant to weight gain [1, 2, 5, 7–12]. While obesity and its physiology have been widely explored for the last decades, only few studies have been interested in CT [12]. CT remains then today poorly known, understood, and recognized; which leads to both misdiagnosis and inefficiency of medical supports [3, 13, 14]. The actual literature effectively provides very limited insights on short-term issues related to CT, and its lifelong implications remain unknown. People with CT almost systematically report social stigmatization in addition to their own concerns about thinness [15–17]. Indeed, CT is frequently confounded with anorexia nervosa (AN) by both practitioners and non-practitioners. Based on these confusions and in order to enhance the diagnosis of individuals with CT, our research group recently systematically reviewed the criteria used so far in the literature to identify CT and proposed a new decision tree aiming at improving and facilitating their clinical diagnosis [12]. Although this systematic approach considered the inclusion criteria usually used, it also pointed out the need to better identify and clarify the physiological, metabolic, functional, and overall clinical characteristics of this underexplored population compared with AN and normal-weight control (C) individuals [12]. Although different specificities concerning CT have already been raised in the literature, whether in terms of body composition [3–5, 9, 10, 18, 19], energy balance [1, 5, 6, 18, 20, 21], appetite-regulating hormone levels [1, 6, 8, 11, 22–25] or biochemical parameters [1, 6, 8, 22, 24–27]; results remain inconsistent, mainly because of the important methodological heterogeneity observed between studies. A much more complete and comprehensive screening of these specificities of CT is necessary to better understand its physiology, which might help find more effective weight gain

strategies. There is, to date, a real need to systematically assess the findings of studies related to CT and to conduct evidence-based quantitative analyses to provide more robust conclusions. Hence, the present study proposed a systematic and meta-analytic (when appropriate) approach of the available clinical trials enrolling participants with CT.

Materials and methods

Study design and search strategy. The search process followed the recommendations from the PRISMA statements [28]. The study was preregistered in the International Prospective Register of Systematic Reviews (PROSPERO) with the following registration number: CRD42020196889. The literature search was performed using PubMed-Medline, Embase, the Cochrane Library, Google Scholar, and Clinical Trials from December 2018 and the last run was performed in June 2020. The eligibility criteria for the bibliographic research were discussed and established by the authors according to the aim of the systematic review and meta-analysis. The main keywords selected for a combined purpose of completeness and accuracy were: 'constitution/al/ly', 'thin/ness', 'lean/ness', 'weight gain resistance'. These keywords were structured under the following search equation: ((constitution[TI] OR constitutional[TI] OR constitutionally[TI]) AND (thinness[TI] OR leanness[TI] OR thin[TI] OR lean[TI])) OR "constitutional thinness"[TW] OR "constitutional leanness"[TW] OR (((resistance[TI] OR resistant[TI]) AND "weight gain"[TI]) NOT "insulin resistance"[TI]) OR ("thinness/physiology"[Mesh] OR ((physiological[TI] OR physiologically[TI] OR physiology[TI]) AND (thinness[TI] OR leanness[TI] OR thin[TI] OR lean[TI])) NOT "obesity"[Mesh]) AND ("humans"[Mesh] OR "humans"[TW] OR "human"[TW]).

Selection process. The inclusion criteria were: (1) clinical trials enrolling participants with CT; (2) adult participants; (3) diagnosis of CT with recognized criteria; (4) women or men (no gender restriction); (5)

publication in English or French language; (6) publication from 1950 to 2020; (7) any fields of study (no restriction) as long as the data provide information on the characterization of CT; (8) any other groups additional to that of the CT group; (9) any types of study designs (no restriction). The exclusion criteria were: (1) thinness not due to a well-identified 'constitutional' origin such as cases of thinness due to an associated pathology, undernourishment, eating disorders, or over-exercising; (2) studies without enough data as letters to the editor, abstracts without full-text, narrative reviews or case studies; (3) studies that use the 'lean' word to refer to a normal-weight participant but not to a constitutionally 'lean' underweight participant. All the records identified through database searching were directly extracted to Zotero Software (5.0.21, CHNM, GMU, USA) which allowed a rapid identification and removal of the duplicates. A first selection was performed based on titles only and then based on abstracts only. The full-text remaining articles were then screened to assess eligibility against inclusion and exclusion criteria. Two authors performed independently all the selection process and any disagreements regarding eligibility for inclusion were discussed among co-authors until a consensus was reached.

Data extraction and strategy for data synthesis. From the selected references, data extraction was performed using a standardized extraction file shared between the two reviewers who extracted data and made them available to the other co-authors. Any issue encountered by the authors during data extraction was discussed collectively. The perimeter of the variables to be considered in the selected articles extended to any variables allowing a characterization of CT, with no restriction about the fields of study. The extraction of the qualitative data was performed using a spreadsheet containing the following items: (1) reference; (2) population characteristics ('n' sample size, age, body mass index (BMI)); (3) quality of the CT diagnosis; (4) study design; (5) type of measurements or parameters assessed with details on the methods used; (6) main results regarding anthropometry/body composition; (7) main results regarding energy intake; (8) main results regarding energy expenditure; (9) main results regarding

biochemical parameters; (10) main results regarding hormonal regulation of appetite; (11) additional results. From this data extraction, the systematic analysis of the publication was divided into three tables (**Tab.1**, **Tab.2**, and **Tab.3**). The population characteristics, study design, and methodological aspects of the selected publications are displayed in **Tab.1**. **Tab.2** reports the main results of these different studies (anthropometry/body composition, energy intake, energy expenditure, biochemical parameters, and hormonal regulation of appetite), and the additional results are presented in **Tab.3**. As just aforementioned, an item was dedicated to the 'quality of CT diagnosis'. As already investigated in a previous publication [12], the definition and diagnosis of CT are found highly heterogeneous in the literature, even though CT diagnosis should be based on common and shared criteria. From this previous work [12], the following criteria were found to be the most important to consider in a CT diagnosis: the degree of thinness, absence of undernourishment, absence of eating disorders, absence of associated diseases, absence of over-exercising, physiological menstruations, weight gain resistance, and stable bodyweight. Based on these elements, we proposed an overall estimation of the CT diagnosis quality in the inclusion process of the included studies (**Tab.1**).

Risks of bias. The risks of bias were assessed with the Cochrane Collaboration's tool [29] (see **Tab.4**) by two authors using the following items: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias) and selective reporting (reporting bias). Regarding the inaccuracies or inconsistencies in the presentation of the results of some publications, an additional reporting bias item called 'inaccurate reporting' was added. For instance, some publications reported slightly different values between their results tables or graphs and their abstract/method part/result part, likely due to mistakes or inaccuracies in the reporting of results. Any disagreement regarding the risks of bias assessment was discussed among co-authors until a consensus

was reached. Although some risks were identified for some criteria in some studies, no overall risk in each study was found so 'high' as to exclude it.

Meta-analysis scope. Since the meta-analysis needs data consistency, more restrictive criteria were required for quantitative analyses listed as follows: (1) the meta-analysis was performed in women-only given the strong effect of gender and the little number of studies enrolling men with CT; (2) cohort-based data were not considered for statistical analyses due to the high risk of bias in the inclusion of such a large number of CT participants that would, in addition, gives a very huge weight in the analyses due to the large sample size; (3) data were included only once in the analyses when same data from same participants were presented in different studies; (4) data from the tables were favoured when mistakes in values reporting between the data tables and the text of the articles were made by the authors; (5) data were estimated from graphs, as accurately as possible, when the numerical values were not available elsewhere in the tables or text.

Meta-analysis procedure. For the extraction of quantitative data, a spreadsheet shared between the two authors was structured with 4 columns corresponding to the (1) 'n' sample size; (2) mean; (3) standard deviation (SD); (4) method used (when necessary), for each group (CT, C, and AN). To assess the dispersion of data, the standard errors of the means (SEM) provided by some publications were converted into SD. Quantitative analyses were performed when a sufficient number of studies reported the same outcomes. A meta-analysis was performed on the characteristics of population and anthropometry/body composition (age, BMI, bodyweight, height, % fat mass (%FM), fat-free mass (FFM), total bone mineral density (BMD) – **Tab.5**), energy intake, and hormonal regulation of appetite (total energy intake (TEI), % carbohydrates in TEI, % fat in TEI, % proteins in TEI, fasting leptin, 24h mean leptin, fasting total ghrelin, 24h mean total ghrelin, fasting acylated ghrelin – **Tab.6**), energy expenditure (total

energy expenditure (TEE), resting metabolic rate (RMR), resting metabolic rate to fat-free mass ratio (RMR/FFM), respiratory quotient (RQ), activity energy expenditure (AEE), physical activity level (PAL) – **Tab.7**), and biochemical parameters (fasting free triiodothyronine (FT3), fasting cortisol, 24h mean cortisol, fasting insulin-like growth factor 1 (IGF-1), fasting estradiol (E2), fasting growth hormone (GH), 24h mean GH, fasting dehydroepiandrosterone sulfate (DHEAS), Fasting follicle-stimulating hormone (FSH), fasting luteinizing hormone (LH), fasting sex hormone-binding globulin (SHBG), fasting testosterone, fasting free testosterone index (FTI), fasting glucose, fasting insulin, fasting homeostasis model assessment (HOMA), fasting triglycerides, fasting free thyroxine (FT4), fasting 25-hydroxy vitamin D3 – **Tab.8**).

Statistical analysis. Data are presented as Mean [Min – Max]. Statistical analysis was performed with Stata software (version 15, StataCorp, College Station, TX) and Comprehensive Meta-Analysis. The meta-analysis took account of between- and within-study variability. To address the non-independence of data due to study effect, random-effects models as developed by DerSimonian were preferred to the usual statistical tests to estimate standardized mean differences (SMD) and their 95% confidence intervals (CI). The heterogeneity in the study results was assessed by forest plots and the I^2 statistic, which is the most common metric for measuring the magnitude of between-study heterogeneity and is easily interpretable. I^2 values range between 0% and 100% and are typically considered low for 0% to 25%, modest for 25% to 50%, and high for values above 50%. Publication bias was assessed by funnel plots and confidence intervals. GraphPad Prism 5.0 software was used to create the graphs.

Results

Selection of studies. As detailed in the flow diagram (**Fig.1**), the search yielded a total of 1 043 publications identified from databases searching and 169 records identified through other sources, making a total of 1 212 records identified. After the removal of the duplicates, 810 records remained and were screened. Based on titles and abstract screening, 381 scientific papers were excluded. Four hundred and twenty-nine articles were fully read at this step of the selection process and 390 articles were removed in eligibility assessment against the inclusion and exclusion criteria. At the end of the process, 39 studies remained in the qualitative analysis of the systematic review, published from 1979 to 2020. These 39 selected studies were assessed regarding their risks of bias (**Tab.4**). While the different risks of bias remained relatively low for most of the different items, the performance bias related to the blinding of participants/personnel was found high in all but one study [30], and the reporting bias related to inaccurate reporting were found moderate or high in 18 [1, 3–6, 8, 9, 21–24, 26, 30–35] of the 39 studies (see **Tab.4**).

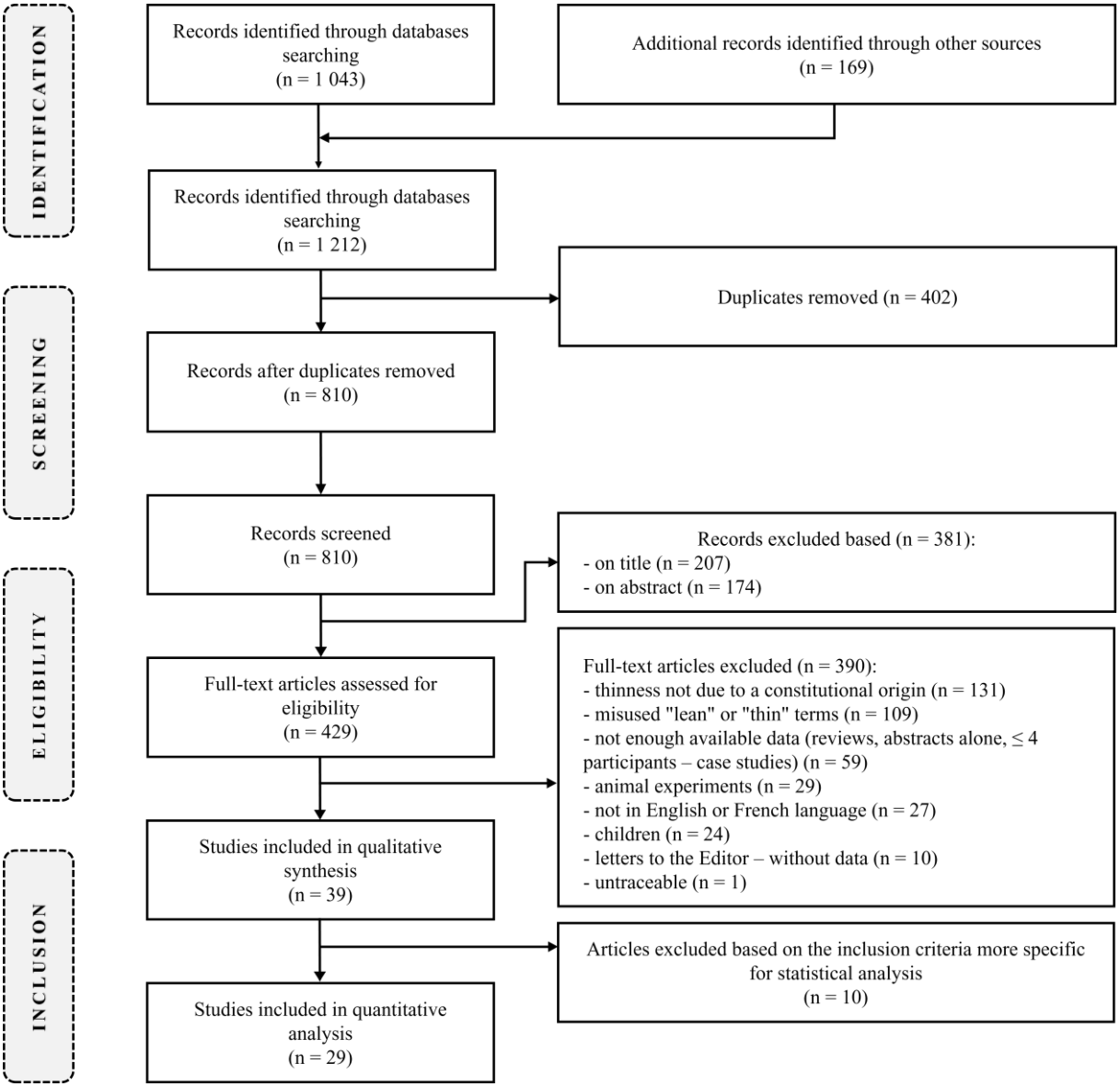

Fig.1 Flow diagram of the inclusion process

Characteristics of participants. As intended, all the studies included in the systematic review enrolled a CT group (**Tab.1**). In addition to the CT group, 35 studies [1, 3–11, 18–25, 27, 30–33, 35–46] included a C group, 24 studies [3, 5, 7–11, 18, 20–26, 31, 32, 34, 35, 38, 39, 42, 45, 47] included a group of patients with AN, 4 studies [24, 25, 38, 39] included a group of patients with bulimia nervosa (BN), one study [39]

included a group of patients with eating disorders not otherwise specified, 2 studies [10, 18] included a group of underweight ballet dancers, 10 studies [20, 25, 33, 38–40, 44, 46–48] included a group of people with obesity. Of the 39 studies, 21 [3, 5, 7–11, 18, 20–25, 31, 32, 35, 38, 39, 42, 45] included groups of CT, C, and AN participants at the same time. Overall, the selected studies included sample sizes ranging from n=6 [38] to n=1622 [45] for CT participants, n=6 [5] to n=96 [47] for AN patients, and n=7 [5, 8] to n=10 433 [45] for C participants. This represents a total of 3 396 CT participants, a total of 16 031 C participants, and a total of 515 AN patients. In the studies, the mean age of participants ranged from 19.4 [10] to 42.4 years [36] in CT individuals, from 19.3 [35] to 52.3 years [45] in C participants, and from 16.5 [47] to 26.4 years [42] in AN patients. Mean BMI ranged from 15.7 [8, 22] to 22.5 $kg.m^{-2}$ [37] in CT individuals, from 20.3 [38] to 27.6 $kg.m^{-2}$ [37] in C participants, and from 12.0 [26, 34] to 17.1 $kg.m^{-2}$ [18] in AN patients. Among the 39 selected studies, 27 [1, 3–5, 7–11, 20–27, 30–32, 34–36, 38, 40, 42, 44] exclusively enrolled female participants, 3 [18, 37, 48] enrolled only male subjects, and 9 [6, 19, 33, 39, 41, 43, 45–47] included both female and male participants.

Designs of studies. Of the 39 studies included, one [30] was a retrospective study, 18 [5, 9, 10, 18, 20, 21, 27, 32, 33, 35–39, 41, 45–47] were cross-sectional analyses (no intervention – and 5 [36, 37, 45–47] cohort-based among them), 15 [3, 4, 7, 8, 11, 22–26, 31, 34, 40, 42, 44] were cross-sectional trials (such as a standardized meal or a standardized exercise), 5 [1, 6, 19, 43, 48] were interventional control trials (long-term intervention) and no randomized controlled trials (RCT) were found in the selected studies (**Tab.1**). Among the interventional trials, one [48] performed a 3-week overfeeding (150% of the mean intake), another [1] performed a 4-week overfeeding (+630 kcal/day – fat exclusively), and 3 [6, 19, 43] reported the results of a 2-week overfeeding (+600 kcal/day – 48.5% carbohydrates, 20% proteins, 31.5% fat).

As detailed in the 'Materials and methods' part, an assessment of the CT diagnosis quality was performed on the basis of a previous work [12]. The quality of the CT diagnosis was estimated as very complete in 15 studies [1, 3, 5, 6, 8–11, 19, 23–25, 39, 43, 45], quite complete in 15 studies [4, 7, 18, 20–22, 26, 27, 30, 34, 35, 38, 41, 42, 46], not very complete in 4 studies [31, 32, 47, 48], and incomplete in 5 studies [33, 36, 37, 40, 44] (**Tab.1**).

Parameters assessed and methodological approaches

Anthropometry and body composition. Anthropometry (bodyweight, height, and/or BMI) was assessed in 36 [1, 3–11, 18–27, 30–44, 48] of the 39 studies (**Tab.1**). Body composition issues were provided in 25 studies [1, 3–6, 8–11, 18–25, 27, 33, 35, 38, 40, 43, 44, 48] and all of them investigated at least fat mass (FM) and/or FFM. Fifteen studies [3–6, 8, 9, 11, 19, 23–25, 27, 33, 35, 43] assessed FM and/or FFM using dual-energy X-ray absorptiometry (DXA), 5 [18, 20–22, 38] using bioelectrical impedance analysis (BIA), and 2 [40, 44] using skinfold thickness (**Tab.1**). One study [10] combined BIA and skinfold thickness methods, one study [1] used three different methods to assess FM and/or FFM (DXA, BIA, and magnetic resonance imaging (MRI)) and one study [48] used doubly labeled water (DLW) to calculate total body water and subsequently fat-free mass. Bone evaluation (such as mineral content (BMC) and/or BMD) were measured in 7 [3, 4, 9, 27, 33, 35, 43] of the 39 studies. Muscle functions and/or physical capacity were explored in 8 studies [1, 4–6, 19, 40, 43, 44].

Energy intake, energy expenditure, physical activity. Energy intake was evaluated in 10 [1, 4–6, 8, 33, 42–44, 48] of the 39 studies. Two studies [42, 48] performed this evaluation through food weighing, 6 studies [1, 4, 5, 8, 33, 44] through self-reporting, and 2 studies [6, 43] used both methods. Energy expenditure was assessed in 15 [1, 3–7, 18, 20, 21, 27, 32, 33, 42, 43, 48] of the 39 studies. Five studies [1, 5, 6, 43, 48] provided results on TEE – one [5] of them using DLW, 3 [1, 6, 43] of them calculating it as the product

between the RMR and the PAL, and one [48] of them using both calorimetric chamber and DLW. RMR was measured using indirect calorimetry in 14 studies [1, 3–7, 18, 20, 21, 27, 32, 33, 43, 48]: two studies [6, 43] assessed RMR using both portable device and calorimetric chamber and 9 studies [1, 3–5, 7, 18, 20, 21, 32] assessed it only using the portable device (**Tab.1**). Three studies combined different methods to assess the RMR: one of them [48] using both DLW and calorimetric chamber, and the 2 others [27, 33] using indirect calorimetry (portable device) and estimations through equations. Physical activity was assessed in 6 studies [1, 5, 6, 19, 43] – using an accelerometer in 5 studies [1, 4, 6, 19, 43] and calculated as the division between TEE and RMR in one study [5] (**Tab.1**).

Blood parameters. Biochemical assays were carried out in 22 [1, 3–6, 8, 9, 11, 22–27, 31, 32, 34, 35, 38, 40, 43, 44] of the 39 studies. In particular, FT3 was assessed in 13 studies [1, 3–6, 8, 9, 11, 22–25, 32, 43], IGF-1 in 13 studies [1, 3–6, 8, 9, 11, 22–25, 43], and cortisol in 12 studies [1, 3, 4, 8, 9, 11, 22–26, 34]. Bone markers were assessed in 3 studies [3, 4, 9]. Assays of regulating-appetite hormones were performed in 17 [1, 3–6, 8, 9, 11, 22–26, 34, 35, 38, 43] of the 39 studies, and all of them assessed leptin (**Tab.1**).

Additional results. Psychological or health profiles were reported in 6 studies [1, 3, 5, 36, 37, 42], genetic-related results were presented in 8 studies [1, 4–6, 43, 45–47], heart rate variability was specifically evaluated in one study [32], stomach sensations and discomforts were reported in one study [39], neurological parameters were explored in one study [42] (**Tab.1**).

Main results from the clinical trials systematically reviewed and meta-analysis. Considering the breadth and diversity of the qualitative results of the systematic review, the presentation of the results was structured in tables (**Tab.2** and **Tab.3**) in order to facilitate reading and understanding. As detailed in the 'Materials and methods' part, additional criteria specific to the quantitative analysis were applied

to assess eligibility and 29 studies [1, 3–11, 19–27, 30–35, 38, 40, 42, 44] were selected for meta-analysis part. The complete and detailed results of the meta-analysis are displayed in **Tab.5**, **Tab.6**, **Tab.7**, and **Tab.8**. A summary of qualitative and quantitative results is highlighted in **Tab.9** which gives an overview of the main results from both the systematic review and meta-analysis.

Discussion

A growing interest in CT has been observed over the last 20 years with 14 studies published from 2000 to 2010 and 19 studies from 2010 to 2020. However, the total number of publications investigating CT remains very low with only 39 clinical trials published from 1950. In addition, very different fields of study have been investigated and data appear relatively equivocal. Yet, it seems of major interest to better characterize CT in terms of body composition, energy balance, or biochemical parameters in order to better diagnose this condition and distinguish it from AN, but also to better understand this extreme state of underweight which would *a priori* not be associated with health issues. The present study aimed to systematically review the results of clinical trials enrolling CT participants and to perform a meta-analysis to compare CT *vs.* C and AN in terms of anthropometry, body composition, energy balance, appetite-regulating hormones, and biochemical markers.

The present meta-analysis logically confirmed the state of underweight of CT individuals who presented a lower bodyweight ($p<0.001$, SMD: 2.57 CI: [2.26; 2.87]) and subsequently a lower BMI than C people ($p<0.001$; 4.16 [3.53; 4.79]) (**Tab.5, Fig.2a, Fig.2b**). As underweight was one of the main inclusion criteria for CT in the studies, this observation was expected and all the studies without exception indeed reported this state of underweight in CT (**Tab.9**). However, an unexpected result was found in BW and BMI comparison between CT and AN. While 75% and 68% of the studies respectively missed reporting

BW and BMI difference between CT and AN, the meta-analysis however gave evidence of a lower BW ($p=0.001$; 0.94 [0.40; 1.47]) and a lower BMI ($p<0.001$; 1.12 [0.75; 1.49]) in AN patients than in CT participants. While AN and CT are both characterized by a severe state of underweight, our meta-analysis results seemed to show a slight but still significant difference between these two populations; with an even more severe state of underweight in AN patients which might be related to the pathological condition of AN. Since two [19, 33] of the 17 studies that assessed height in CT and C people found a significantly lower height in CT than C people, it raises the question that CT individuals would not only be underweight but might also present small body size. However, the meta-analysis did not show any height difference between CT and C people. The present paper also pointed out an untypical body composition in CT. Although CT participants showed a significantly lower percentage of FM compared with C ($p<0.001$; 1.82 [1.48; 2.17]), their %FM was found in the normal healthy ranges of body fat [14, 17, 49, 50] and remained significantly higher ($p<0.001$; 1.59 [1.18; 2.01]) than AN patients **(Fig.2c)**. However, regarding FFM, the values in CT subjects were largely reduced, in a similar proportion as in AN ($p=0.12$; -0.24 [-0.54; 0.06]), and found lower compared to C people ($p<0.001$; 1.60 [1.10; 2.10]) **(Fig.2d)**. While many scientists and practitioners are still sceptical about the existence of CT, the present observation reinforces once more the distinction between CT and AN. Given the important role of body fat mass in the regulation of energy metabolism [51], the higher %FM observed in CT *vs.* AN seems to suggest specific metabolic adaptions in CT that might bring new insights in the understanding of their weight gain resistance. This state of underweight without underfat points out the need to further explore the metabolic mechanisms behind this uncommon body composition profile. In addition, this atypical body composition also questions the relevance of the different weight gain strategies that might be proposed by some practitioners in certain cases or that are already naturally adopted by CT people themselves. Indeed, fat overfeeding does not seem to be a relevant nutritional strategy for this population, especially as CT subjects seemed to resist weight gain with fat overfeeding [1]. Bone mineral

content and density investigations would have also brought important information to better characterize and understand CT. Yet, only few studies evaluated BMC and BMD, thus limiting the relevance of a meta-analytical approach. One study found a lower BMC in CT *vs.* C [33] and another reported a similar BMC in CT *vs.* AN [35], which would suggest a BMC as low in CT as in AN, but no further studies presented BMC results. In addition, most of the studies found lower total, lumbar spine, or femoral neck BMD in CT *vs.* C [4, 9, 27, 35], along with similar values in CT *vs.* AN [3, 9, 35]. Even if the actual literature remains quite limited to date regarding bone function assessment, these first observations would suggest a low BMC and BMD in CT. The investigation of Galusca *et al.*[9] which was focused on bone evaluations in CT, depicted a low bone quality [9]. Despite a normal bone turnover [9], CT people would present a decreased calculated breaking strength [9] that may lead to health implications. Considering the natural history of bone tissue with aging [52], the risk of osteoporosis may be greater in the CT population, but this has not been demonstrated. To date, the short- and long-term consequences on the fracture risk in the living conditions of CT people remain unknown.

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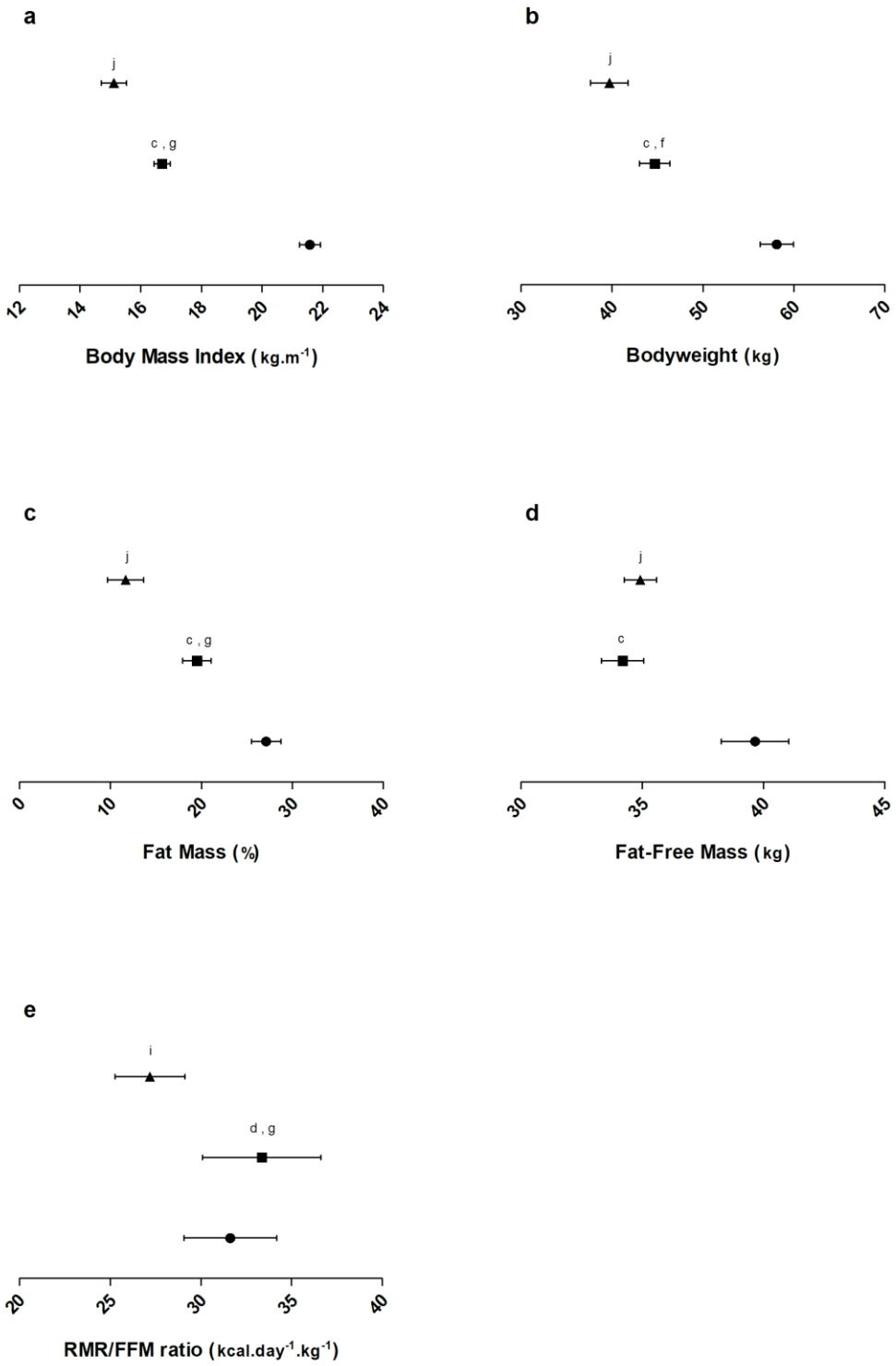
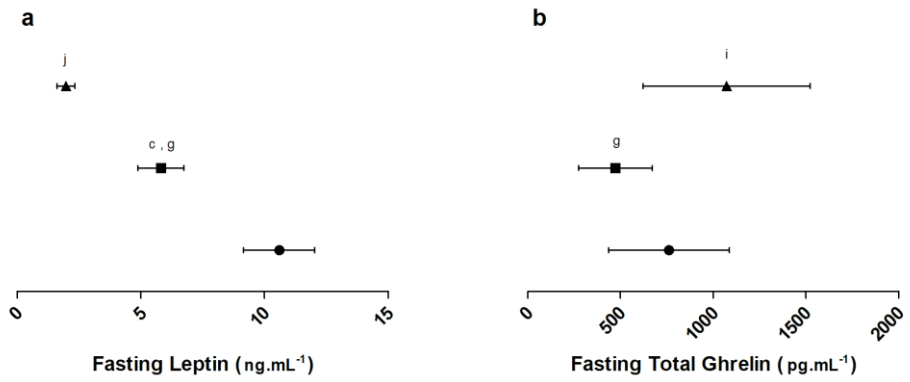


Data are presented as Mean [Min – Max]. [a]CT *vs.* C ($p<0.05$); [b]CT *vs.* C ($p<0.01$); [c]CT *vs.* C ($p<0.001$); [d]trend for difference between CT and C; [e]CT *vs.* AN ($p<0.05$); [f]CT *vs.* AN ($p<0.01$); [g]CT *vs.* AN ($p<0.001$); [h]AN *vs.* C ($p<0.05$); [i]AN *vs.* C ($p<0.01$); [j]AN *vs.* C ($p<0.001$).

Fig.2 Body Mass Index (**a**), Bodyweight (**b**), Fat Mass percentage (**c**), Fat-Free Mass (**d**) and RMR/FFM ratio (**e**) comparisons between patients with Anorexia Nervosa (▲), Constitutional Thinness (■) and Control (●)

The exploration of energy balance is a central issue in the characterization and understanding of the physiological mechanisms explaining the phenotype of CT. All the studies (n=7 [1, 4–6, 8, 33, 43]) investigating TEI in CT *vs.* C reported a similar TEI between CT and C participants which was also confirmed by our meta-analysis. This result supported, once more, the absence of eating disorders in CT, while the results obviously attested to a lower TEI in AN. In addition, almost all the studies (**Tab.9**) showed the same distribution in proteins, carbohydrates, and fat ingestion between CT and C participants, and this finding was also confirmed by the meta-analysis. The three studies [1, 6, 43] that investigated snacking however seemed to report more snacking in CT compared with C individuals, likely due to a satiety reached earlier during meal [53]. The results showed that 71% [1, 3, 4, 6, 9, 11, 23–25, 35] of the included studies reported lower levels of leptin in CT than in C individuals (when cumulating fasting and 24h mean results for all the studies that assessed leptin in both CT and C participants). For the 4 studies [5, 8, 38, 43] in which p-value did not reach significance, the absolute leptin values were nevertheless observed lower in CT compared with C. On the other hand, all the studies assessing leptin (n=12 [3, 5, 8, 9, 11, 23–26, 34, 35, 38]) (**Tab.9**) reported higher concentrations in CT compared with AN patients. The meta-analysis indeed showed different leptin levels in the two-by-two comparisons between each of the 3 groups (CT, C, AN) for both fasting and 24h mean leptin levels, with normal values in C people, blunted values in AN patients, and an intermediate profile in CT **(Fig.3a)**. Concerning ghrelin levels, results are difficult to interpret because of some methodological limitations. Several studies did not perform a statistical test for each assessment during the day, especially for the fasting point, which drastically reduced the number of studies that compared the fasting ghrelin concentration between groups. In addition, methodological differences led us to separate ghrelin variable into different variables (24h mean from fasting ghrelin values and a total from acylated active forms), again reducing the sample size and leading to samples often too small to give relevance to the meta-analysis. Taking these different

elements into account, ghrelin levels however seemed similar between CT and C participants, in contrast to increased levels in AN **(Fig.3b)**. Yet, due to the aforementioned limitations, this observation needs to be further confirmed. Finally, the present systematic and meta-analytic approaches seem to report different leptin and ghrelin hormonal-regulating profiles between CT and AN, which differentiates once more these two populations.



Data are presented as Mean [Min – Max]. [a]CT *vs.* C ($p<0.05$); [b]CT *vs.* C ($p<0.01$); [c]CT *vs.* C ($p<0.001$); [d]trend for difference between CT and C; [e]CT *vs.* AN ($p<0.05$); [f]CT *vs.* AN ($p<0.01$); [g]CT *vs.* AN ($p<0.001$); [h]AN *vs.* C ($p<0.05$); [i]AN *vs.* C ($p<0.01$); [j]AN *vs.* C ($p<0.001$).

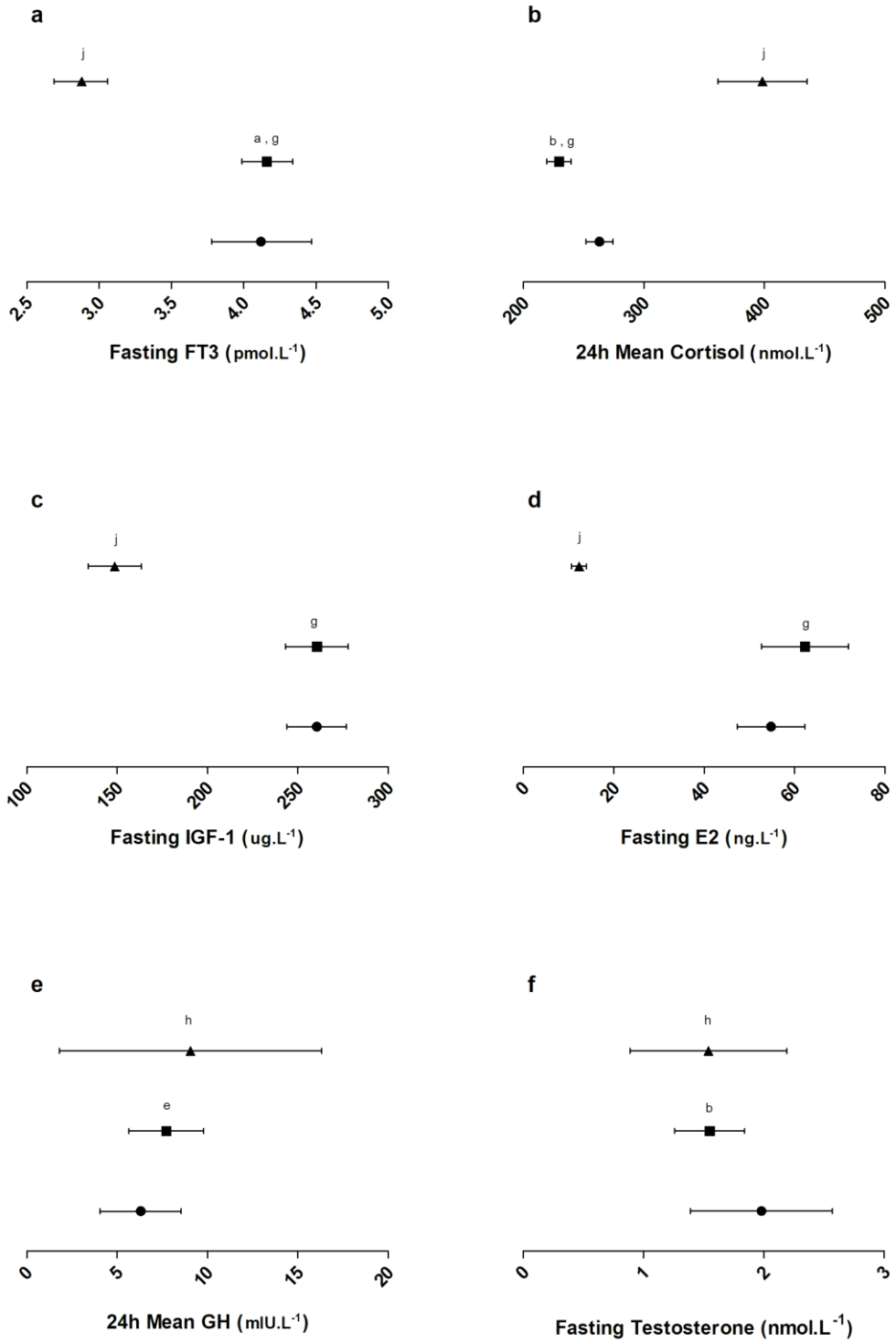
Fig.3 Fasting Leptin (**a**) and Fasting Total Ghrelin (**b**) comparisons between patients with Anorexia Nervosa (▲), Constitutional Thinness (■) and Control (●)

Only few studies investigated TEE [1, 5, 6, 43, 48], the other side of the energy balance, using moreover different methodological approaches. If results might rather go in the direction of a lower TEE in CT *vs.* C [6, 43] (in particular observed with the highly reliable method of calorimetric chamber), not all studies [1, 5] obtained such results, which makes it difficult to conclude. RMR, one of the major components of energy expenditure [54], was however reported in a large number of studies [1, 3, 5–7, 18, 20, 21, 27, 32, 33, 43]. RMR results appeared equivocal with 64% [1, 3, 5, 6, 27, 33, 43] of the studies showing a lower RMR in CT *vs.* C people while the remaining 36% [7, 18, 20, 32] did not report any difference.

Furthermore, RMR was found higher (or tended to be [3]) in CT *vs.* AN in 86% [5, 18, 20, 21, 32] of the studies. Based on the RMR mean values from the different studies, the meta-analysis concluded that the 3 groups displayed significantly different results from each other with a lower RMR in AN patients, a higher RMR in C participants, and an intermediate profile in CT individuals. Interestingly, different studies also normalised RMR with FFM by calculating RMR-to-FFM ratio (RMR/FFM). Results of both the systematic review and meta-analysis clearly indicated a higher RMR/FFM in CT than in AN (**Tab.9, Fig.2e**). Three studies [5, 6, 18] found a higher RMR/FFM in CT *vs.* C participants whereas the significance was not reached in 5 studies [1, 4, 20, 27, 43] (even if higher absolute RMR/FFM values were observed for these non-significant results). The meta-analysis concluded to a trend (p=0.083) toward a higher RMR/FFM ratio in CT *vs.* C **(Fig.2e)**. Since the meta-analysis was conducted on female subjects (to avoid gender bias and due to the limited number of studies conducted among males) and that 2 [6, 18] of the 3 studies which had found a higher RMR/FFM ratio reported results on males only, we propose that an even more pronounced RMR/FFM might characterize CT males compared with CT females. In spite of a low RMR logically observed in CT given their blunted FFM, RMR/FFM seemed to be higher in CT. This interesting finding clearly suggests that, despite their very low FFM, CT people might present an increased metabolic activity of their FFM. In addition, according to Germain *et al.* [1], a lipid overfeeding would increase the RMR/FFM ratio in CT contrary to control participants, which reinforces again the idea of a weight gain resistance in CT population. The other results relative to energy expenditure comparing CT and C participants seemed to show no difference in either the oxidation of substrates at rest (similar RQ) or AEE. Moreover, the meta-analysis missed showing any PAL difference between CT and C subjects but this finding has to be further explored given the low number of studies (n=4 [4, 5, 19, 43]) and the fact that 2 [19, 43] out of these 4 studies showed a lower PAL in CT, with no difference in the other 2 trials [4, 5].

Interestingly, biochemical assessments were often performed in the studies, therefore allowing more robust meta-analyses. The present meta-analysis confirmed that FT3 and cortisol differentiate CT from AN, but also showed, for the first time, that levels of FT3 and cortisol differ between CT and C people (in the opposite direction to that of AN) **(Fig.4a, Fig.4b)**. Such a result was definitely unexpected since 100% of the studies systematically analysed reported no difference between CT and C for fasting FT3 levels [1, 3–6, 8, 9, 11, 22–25, 32, 43], fasting cortisol [9], and 24h mean cortisol [1, 3, 4, 8, 11, 23–25]. In accordance with these studies, different reviews also communicated about the absence of biochemical disorders in FT3 and cortisol levels in CT contrary to AN, reporting similar FT3 and cortisol levels between CT and C people [13, 15, 16, 53, 55]. The meta-analysis however reported for the first time significantly different FT3 and cortisol levels between CT and C participants. Indeed, individuals with CT presented a slightly higher fasting FT3 level than controls (p=0.033; -0.42 [-0.81; -0.03]), a lower fasting cortisol level (p=0.003; 0.66 [0.22; 1.10]), and a lower 24h mean cortisol (p=0.005; 0.55 [0.16; 0.93]), but still within the norms. The higher FT3 values observed in CT compared to C may partly explain the trend towards a higher RMR/FFM ratio in CT. Indeed, FT3 was previously shown to be significantly associated with RMR/FFM [56]. One explanation could be that thyroid hormone signalling promotes a shift from slow type Ⅰ fibre phenotype to faster type Ⅱ fibres, with the associated increase in myosin and sarco/endoplasmic reticulum Ca^{2+}–ATPase (SERCA) expression leading to greater energy turnover and concomitant generation of heat during activity [57, 58]. However, although significant, the higher FT3 level observed in CT *vs.* C remains very modest. Its clinical relevance and consequences in CT individuals' physiology need to be further demonstrated. In 2017, Estour *et al.* performed Receiver Operator Characteristics (ROC) curves showing that FT3 constituted a strong significant tool for CT and AN distinction as displaying high sensitivity and specificity values [3] and the present meta-analysis even showed that FT3 and cortisol levels might differentiate CT and C people. As expected, both the systematic review and the results of the meta-analysis also confirmed the hypercortisolism and FT3

deficit characterizing AN. Almost all the studies systematically reviewed found similar levels of fasting IGF-1 [1, 3–6, 8, 9, 11, 22–25, 43], fasting GH [9], 24h mean GH [3, 8, 11, 22–24], and fasting E2 [1, 3, 4, 8, 9, 11, 23–25, 27] between CT and C people. These results are indeed confirmed by the meta-analysis. In accordance with most of the studies, the AN *vs.* CT and AN *vs.* C comparisons confirmed the low IGF-1 levels ($p<0.001$ and $p<0.001$ respectively), the low E2 levels ($p<0.001$ and $p<0.001$), the high fasting GH ($p=0.019$ and $p<0.001$) and the high 24h mean GH ($p=0.021$ and $p=0.027$) levels observed in AN, as opposed to CT **(Fig.4c, Fig.4d, Fig.4e)**. In agreement with most of the literature, FSH and LH hormonal levels were indeed found lower in AN and similar between CT and C participants by the meta-analysis (**Tab.9**). In a contrasting way with several results individually observed in the included studies, our meta-analysis showed a lower testosterone level in CT *vs.* C people ($p=0.004$; 0.48 [0.16; 0.81]) but similar to AN patients **(Fig.4f)**, a similar level of SHBG for the 3 groups, and a trend toward a lower DHEAS level in CT *vs.* C ($p=0.059$; 0.28 [-0.01; 0.57]) and AN ($p=0.086$; -0.28 [-0.60; 0.04]) subjects. The low testosterone levels observed in CT individuals might be consistent with their blunted FFM [59]. Yet, these observations have to be interpreted with caution given the small number of studies that have assessed these sex hormones. Only few studies investigated fasting glucose, triglycerides, insulin, vitamin D, and HOMA index, thus making it difficult to interpret these results. Yet, the first observations in CT would rather go in the direction of a normal fasting blood glucose, fasting vitamin D, and triglycerides levels, a normal-to-low insulin level, and a normal HOMA index. The meta-analysis showed similar results in CT *vs.* C for fasting glucose, triglycerides, and HOMA index, but lower levels of fasting insulin and vitamin D in CT *vs.* C were recorded. The lower fasting insulin values were in agreement with the lower levels of cortisol that we discussed above and with the literature [60]. Vitamin D deficiency can lead to complication found in CT people, such as poorer bone quality and muscle weakness [61]. However, further investigations are needed to provide more robust conclusions on these parameters.



Data are presented as Mean [Min – Max]. [a]CT *vs.* C ($p<0.05$); [b]CT *vs.* C ($p<0.01$); [c]CT *vs.* C ($p<0.001$); [d]trend for difference between CT and C; [e]CT *vs.* AN ($p<0.05$); [f]CT *vs.* AN ($p<0.01$); [g]CT *vs.* AN ($p<0.001$); [h]AN *vs.* C ($p<0.05$); [i]AN *vs.* C ($p<0.01$); [j]AN *vs.* C ($p<0.001$).

Fig.4 Fasting FT3 (**a**), 24h Mean Cortisol (**b**), Fasting IGF-1 (**c**), Fasting E2 (**d**), 24h Mean GH (**e**), and Fasting Testoterone (**f**) comparisons between patients with Anorexia Nervosa (▲), Constitutional Thinness (■) and Control (●)

Several limitations must be considered in the present analysis. First, our systematic approach revealed an important level of methodological heterogeneity between studies, which might impact our comparisons and analysis. This leads either to an increase in the variability which may eventually smooth out some statistical differences or to the need to split some variables according to the different methods used, thus drastically reducing the sample size. This limitation highlights the need to harmonize our methodological approaches in order to provide relevant comparisons between the different investigations. The relatively small sample size observed for many variables (due to the small number of investigations conducted in CT) should also be considered. This might reduce the power of some of the performed meta-analysis and sometimes made it impossible to perform such a meta-analytic approach for some variables. Moreover, most studies have been conducted among females while males also present CT, raising the need for more explorations in males with CT. Finally, we would like to draw attention to the inaccuracies, mistakes, or deficiencies observed in a high number of studies which can give rise to bias. Almost half of the studies presented some inaccuracies in their results, such as different values for the same result in the different parts of the article, mistakes in the units (in particular for biochemical assessment), outlandish values, absence of statistical tests between groups for each point for 24h analyses, errors in the sample size reporting, or lack of clarity in the description of the methods used.

Conclusion

The present work proposed the first systematic and meta-analytic analysis of the anthropometry, body composition, energy intake and expenditure, appetite-regulating hormones, and biochemical parameters in CT individuals. According to our results, CT would be characterized by a BW and BMI less decreased than in AN, a state of underweight without underfat, a FFM and testosterone as decreased as

in AN, a normal qualitative and quantitative energy intake, an intermediate level of leptin between AN and C participants, a diminished RMR but a trend toward an increased RMR/FFM that might suggest a metabolically highly active FFM. Levels of FT3, cortisol, IGF-1, E2, GH, FSH, and LH differentiates CT from AN, whereas these biochemical parameters were similar between CT and C participants, except for FT3 and cortisol. Indeed, higher FT3 and lower cortisol levels were observed in CT *vs.* C participants. In perspective, it might be of particular interest to explore if and how these FT3 and cortisol differences in CT might be related to their weight gain resistance. The hypothesis of a FFM potentially highly metabolically active in CT calls for further explorations, especially on muscle metabolism. At last, participants with CT displayed an untypical body composition with a non-blunted body fat mass despite a serious state of underweight, which may stand for important implications when it comes to weight gain strategies.

Table 1: Characteristics of population, CT diagnosis, study design, and methodology of the clinical trials selected in the systematic review

Reference	Population characteristics (sample size, age; BMI) (means ± SD)	CT diagnosis†	Study design	Measurements – Parameter (method)
Schneider *et al.*, 1979 [30]	Females: CT: n=53; 25.3 ± 5.2§ yr; NR C: n=100; 25.8 ± 4.2§ yr; NR	2	Retrospective analysis	Anthropometry/body composition BW, height
van Binsbergen *et al.*, 1990 [31]	Females: CT: n=10; 26.4§ yr; 18.4§ kg.m^{-2} C: n=10; 25.1§ yr; 20.8§ kg.m^{-2} AN: n=20; 24.8§ yr; 14.3§ kg.m^{-2} (AN type: NR)	3	Cross-sectional trial Administration of GnRH (100 µg)	Anthropometry/body composition BW, height, BMI, IBW, %IBW (=BW/IBW) Biochemical parameters Fasting E2, estrone, progesterone, testosterone, androstenedione, DHEAS, SHBG, LH, FSH, prolactin (t=-10min) LH, FSH (t=0min) and gived 100ug GnRH: determinated LH, FSH at 20, 60, 90 and 120min
Diaz *et al.*, 1992 [48]	Males: CT: n=7; 26.3 ± 4.5 yr; 21.7 ± 1.3 kg.m^{-2} OB: n=3; 37.3 ± 9.0 yr; 26.8 ± 1.0 kg.m^{-2}	3	Interventional trial (overfeeding) Baseline period: 3 weeks (standard kcal diet + any extra food the subject wanted to eat) Overfeeding period: 6 weeks (calculated by DLW as 150% of the mean intake and expenditure observed during baseline) Post-overfeeding period: 6 weeks (uncontrolled) Overfeeding protocol 6 weeks diet supplying 50% more than the baseline energy requirements – composition of the diet reflected that of a typical British diet (12% protein, 42% fat, 46% carbohydrates) and no artificial food	Anthropometry/body composition BW, height, BMI, FM, FFM (total body water determined by DLW calculated, FFM estimated as total body water/0.73, FM calculated as BW – FFM) Energy intake Accurately weighed and homogenised samples of each meal were freeze-dried for analysis of gross energy content by bomb calorimetry and of nitrogen by Kjeldahl analysis Nitrogen balance and metabolizable energy of the diet calculated by total urine and faecal collections throughout the entire overfeeding period, then analysed by bomb calorimetry for gross energy and by Kjeldahl analysis for nitrogen Energy expenditure TEE, RMR (measured twice: 1. indirect calorimetry – 11m^3 whole-body calorimeters, between 12.5 and 13.5h postabsorption, measured for 1h immediately on waking, 2. DLW) [activity + thermogenesis] calculated as TEE – RMR
Scalfi *et al.*, 1992 [7]	Females: CT: n=7; 28.6 ± 5.6 yr; 16.8 ± 0.8 kg.m^{-2} C: n=8; 28.5 ± 3.4 yr; 22.5 ± 2.5 kg.m^{-2} AN: n=7; 21.3 ± 3.7 yr; 15.3 ± 2.1 kg.m^{-2} (AN: restrictive-type)	2	Cross-sectional trial Mixed test meal at 9:00am (3,56MJ, 850kcal: 16% proteins, 50% carbohydrates, 34% fat)	Anthropometry/body composition BW, height, BMI Energy expenditure RMR (40min before meal: absolute and weight as covariate ; 240min postmeal: incremental above baseline) (indirect calorimetry, Weir's formula), RQ (40min before meal; 240min postmeal: incremental above baseline), PPT (240min postmeal: absolute and incremental above baseline) (trapezoid method)
Hinney *et al.*, 1997 [47]	Females: CT: n=48; 24.7 ± 3.9 yr; 17.6 ± 0.8 kg.m^{-2} AN: n=92; 16.6 ± 3.4 yr; 14.5 ± 1.5 kg.m^{-2} (AN: restrictive and binge eating/purging type) OB: n=51; 49.6 ± 13.6 yr; 38.4 ± 4.6 kg.m^{-2} Males:	3	Cohort-based cross-sectional analysis	Genetics Allele and genotype frequencies (blood samples – DNA-Isolation, PCR) Parental transmission of the long allele (Transmission Disequilibrium Test)

	CT: n=64; 26.1 ± 4.1 yr; 19.0 ± 1.0 kg.m^{-2} AN: n=4; 15.3 ± 0.9 yr; 13.9 ± 2.0 kg.m^{-2} (AN: restrictive and binge eating/purging type) OB: n=37; 50.0 ± 9.6 yr; 38.7 ± 4.9 kg.m^{-2}			
Petretta *et al.*, 1997 [32]	Females: CT: n=10; 22 ± 3 yr; 16.6 ± 1,1 kg.m^{-2} C: n=10; 21 ± 3 yr; 23.4 ± 2,4 kg.m^{-2} AN: n=13; 20 ± 2 yr; 15.7 ± 2,4 kg.m^{-2} (AN: restrictive-type)	3	Cross-sectional analysis	Anthropometry/body composition BW, height, BMI Energy expenditure RMR (indirect calorimetry – Beckamn Metabolic Measurement Cart, after an overnight fast and 30min of acclimatization – period mean of 10 measurements, each lasting 3min) Biochemical parameters Fasting FT3, FT4, T3, T4, TSH, plasma renin activity, glucose, Na^+, K^+ Heart rate variability *Time domain measures* Average NN interval, SDNN, SDANN index, SDNN index, rMSSD, pNN50 (24h Holter recordings analysed at the National Research Council – custom analyser built around a Motorola 68030-50MHz microprocessor) *Frequency domain measure* Power spectrum computed by means of the fast Fourier transform algorithm, averaging at least 51 spectra (total power: 0.00066 to 0.40Hz, ULF from 0.00066 to 0.0033Hz, VLF from 0.0033 to 0.04Hz, LF from 0.04 to 0.15Hz, HF from 0.15 to 0.40Hz)
Slof *et al.*, 2003 [36]	Females: CT: n=80; 42.4 ± 7.2 yr; 20.3 ± 1.5 kg.m^{-2} C: n=881; 43.0 ± 7.7 yr; 26.8 ± 6.2 kg.m^{-2}	4	Cohort-based cross-sectional analysis (population-based Virginia Twin Registry)	Anthropometry/body composition BMI Psychological and health profile Health behaviours (caffeine use, cigarette smoking - Faserstrom tolerance questionnaire, number of sick days) Food-related behaviour (EDI - body dissatisfaction, drive to thinness, TFEQ - eating restraint, disinhibition, susceptibility to hunger) Perfectionism (MPS) Personality (symptom checklist-90-R, rosenberg self-esteem scale, locus of control scale, neuroticism and extroversion scales from the Eysenck personality questionnaire), learned resourcefulness subscale from the attributional style questionnaire Anxiety, phobias (interviews)
Tolle *et al.*, 2003 [22]	Females: CT: n=8; 23.3 ± 8.8[§] yr; 15.7 ± 1.1[§] kg.m^{-2} C: n=10; 23.2 ± 3.5[§] yr; 21.5 ± 2.2[§] kg.m^{-2} AN: n=9; 17.2 ± 2.7[§] yr; 14.6 ± 1.2[§] kg.m^{-2} (AN: restrictive-type) AN-rec: n=9; NR but probably AN age + 2 to 15 months; 17.9 ± 2.1[§] kg.m^{-2}	2	Cross-sectional trial Meals at 8:00am and 12:00am immediately after samples collect, and 7:00pm. GHRH administration	Anthropometry/body composition BW, BMI, FM (BIA – Analicor 2) Biochemical parameters Fasting FT3, IGF-1, E2 Samples collected every 4h for 24h (8:00am, 12:00am, 4:00pm, 8:00pm, 12:00pm, 4:00am) for cortisol and GH GHRH injection: samples collected (-20, 0, 15, 30, 60, 90, 120min) Hormonal regulation of appetite Samples collected every 4h for 24h (8:00am, 12:00am, 4:00pm, 8:00pm, 12:00pm, 4:00am) for total ghrelin and leptin GHRH injection: samples collected (-20, 0, 15, 30, 60, 90, 120min)
Bosy-Westphal *et al.*, 2004 [33]	CT (12 females): n=12; 26.4 ± 6.8 yr; 16.9 ± 0.9 kg.m^{-2} C (12 females and 13 males): n=25; 25.4 ± 2.4 yr; 22.3 ± 2.0 kg.m^{-2}	4	Cross-sectional analyses Fasting, measured at 7:00am to 9:00am	Anthropometry/body composition BW, height, BMI, FM, FFM, muscle mass, lean body mass trunk (DXA – HOLOGIC) Bone evaluation

	OB (9 females and 9 males): n=18; 26.7 ± 4.5 yr; 33.4 ± 4.5 $kg.m^{-2}$			BMC (DXA - HOLOGIC) Volume of internal organs Brain, heart, liver, kidneys, organ mass, spleen (MRI) Residual (=BW – muscle mass – organ mass (FM×0,8)–(BMC×1,85) <u>Energy intake</u> TEI, proteins, carbohydrates, lipids (7-day dietary record, analysed with Prodis-version 4.5) <u>Energy expenditure</u> RMRm (indirect calorimetry: during 1h, data obtained during the first 15min were omitted), RMRc (calculated with 8 compartments: $brain_{MRI}$, heart $liver_{MRI}$, $kidneys_{MRI}$, MM_{DXA}, skeletal $bone_{DXA}$, adipose $tissue_{DXA}$ and residual mass), RMR adjusted for FFM according to an equation
Mazzeo *et al.*, 2004 [37]	Males: CT: n=158; NR but probably 29-69 yr; 22.5 ± 2.1 $kg.m^{-2}$ C: n=915; NR but probably 29-69 yr; 27.6 ± 4.2 $kg.m^{-2}$	4	Cohort-based cross-sectional analysis (population-based Virginia Twin Registry)	<u>Anthropometry/body composition</u> BMI <u>Psychological and health profile</u> Cigarette smoking - Faserstrom tolerance questionnaire Food-related behaviour (EDI - body dissatisfaction, drive to thinness, TFEQ - eating restraint, disinhibition, susceptibility to hunger) Perfectionism (MPS) Personality (rosenberg self-esteem scale, locus of control scale, neuroticism and extroversion scales from the Eysenck personality questionnaire), novelty seeking with Cloninger's tridimensional personality questionnaire, interpersonal dependency with Hirschfeld's measure Anxiety, phobias (interviews)
Tagami *et al.*, 2004 [38]	Females: CT: n=6; 27.5 ± 4.2 yr; 17.7 ± 0.5 $kg.m^{-2}$ C: n=16; 25.7 ± 2.9 yr; 20.3 ± 1.5 $kg.m^{-2}$ AN: n=31; 25.5 ± 8.1 yr; 14.0 ± 2.5 $kg.m^{-2}$ (AN: probably restrictive-type) BN: n=11; 23.5 ± 3.9 yr; 20.5 ± 1.8 $kg.m^{-2}$ OB: n=9; 27.0 ± 6.8 yr; 30.3 ± 5.6 $kg.m^{-2}$	2	Cross-sectional analysis	<u>Anthropometry/body composition</u> BMI, FM (BIA – Tanita, analyser TBF-110) <u>Biochemical parameters</u> Fasting adiponectin, glucose, insulin <u>Hormonal regulation of appetite</u> Fasting leptin
Miljic *et al.*, 2006 [26]	Females: CT: n=10; 22.5 ± 4.4 yr; 17.6 ± 1.3 $kg.m^{-2}$ AN: n=9; 25.1 ± 5.1 yr; 12.0 ± 1.2 $kg.m^{-2}$ (AN: restrictive and binge eating/purging type) AN rec: n=6; 24.8 ± 4.7 yr; 17.2 ± 3.2 $kg.m^{-2}$	2	Cross-sectional trial Ghrelin infusion during 5 hours (5pmol/kg/min), start at 9:00am	<u>Anthropometry/body composition</u> BW, BMI <u>Biochemical parameters</u> Fasting cortisol, GH, prolactin, insulin, c-peptide, adiponectin, glucose Samples collected at baseline (t=0), every 15min during first 2h, and every 30min for last hours for cortisol, GH, prolactin <u>Hormonal regulation of appetite</u> Fasting leptin, total ghrelin Samples collected at baseline (t=0), every 15min during first 2h, and every 30min for last hours for total ghrelin *Appetite* Hunger, satiety, palatability, sickness (VAS: 0-100mm): before and hourly infusion *Sleepiness* Sleepiness (VAS: 0-100mm): before and hourly infusion

Bossu *et al.*, 2007 [5]	Females: CT: n=7; NR but 18-26 yr; 16.1 ± 0.6 kg.m^{-2} C: n=7; NR but 18-26 yr; 21.2 ± 0.8 kg.m^{-2} AN: n=6; NR but 18-26 yr; 15.8 ± 0.8 kg.m^{-2} (AN: restrictive-type)	1	Cross-sectional analysis	Anthropometry/body composition BW, BMI, FM, FFM (DXA – LUNAR) Energy intake TEI, proteins, carbohydrates, lipids (during a period of 4 days including 2 weekdays and a weekend – 24h dietary records, using photographs previously validated for the SU.VI.MAX study) FQ (using Black's formula) Energy expenditure TEE (DLW) RMR, RQ (indirect calorimetry – Deltatrac device, after a 12-h fast and 1h of resting) Physical activity PAL (=TEE/RMR), AEE (=TEE – RMR), physical activity (MOSPA questionnaire) Biochemical parameters Fasting FT3, IGF-1 Hormonal regulation of appetite Fasting leptin Psychological profile Food-related behavioural problems ("silhouette" questionnaire, DEBQ, EDI, EDE)
Germain *et al.*, 2007 [8]	Females: CT: n=10; 20.2 ± 3.8 yr; 15.7 ± 0.6 kg.m^{-2} C: n=7; 23 ± 2.1 yr; 20.4 ± 0.8 kg.m^{-2} AN: n=12; 20.7 ± 4.2 yr; 15.2 ± 1.4 kg.m^{-2} (AN: probably restrictive-type)	1	Cross-sectional trial Standardized meals were served at 8.15am, 12.15pm, 7.00pm (breakfast: 382kcal, lunch: 789kcal, dinner 789kcal)	Anthropometry/body composition BMI, FM, FFM (DXA – LUNAR) Energy intake TEI (during a period of 4 days including 2 weekdays and a weekend, using photographs previously validated for the SU.VI.MAX study) Biochemical parameters Fasting FT3, IGF-1, E2, FSH, LH, total testosterone, SHBG, FTI (testosterone/SHBG), DHEAS Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for cortisol, GH Hormonal regulation of appetite Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for leptin, total (1-36 and 3-36) PYY, GLP-1, total (acylated and deacylated) ghrelin
Marra *et al.*, 2007 [20]	Females: CT: n=20; 22.5 ± 5.8 yr; 17.2 ± 1.0 kg.m^{-2} C: n=20; 22.0 ± 3.7 yr; 21.7 ± 2.4 kg.m^{-2} AN: n=20; 18.8 ± 3.4 yr; 15.1 ± 1.6 kg.m^{-2} (AN: restrictive-type) OB: n=20; 21.9 ± 0.9 yr; 43.8 ± 10.0 kg.m^{-2}	2	Cross-sectional analysis	Anthropometry/body composition BW, height, BMI, FM, FFM (single frequency BIA – Akern) Energy expenditure RMR, RQ (indirect calorimetry – Canopy system, after a 12-14h fast, after a 30min adaptation period) Fidgeting (representing NEAT – based of the assumption that in the steady state of measurements with indirect calorimetry, increased fidgeting will increase RMR variability)
Miljic *et al.*, 2007 [34]	Females: CT: n=10; 22.5 ± 4.4 yr; 17.6 ± 1.3 kg.m^{-2} AN: n=9; 25.1 ± 5.1 yr; 12.0 ± 1.2 kg.m^{-2} (AN: restrictive and binge eating/purging type) AN rec: n=6; 24.8 ± 4.7 yr; 17.2 ± 3.2 kg.m^{-2}	2	Cross-sectional trial (same participants as Miljic *et al.* 2006 [26]) Ghrelin infusion during 5 hours (5pmol/kg/min), start at 9:00am	Anthropometry/body composition BW, BMI Biochemical parameters Fasting cortisol, GH, prolactin, insulin, c-peptide, adiponectin, glucose Samples collected at baseline (t=0), every 15min during first 2h, and every 30min for last hours for cortisol, GH, prolactin, glucose, insulin, c-peptide

				Samples collected at baseline (t=0) for adiponectin HOMA = (Insulin x glucose)/22,5 Hormonal regulation of appetite Fasting leptin, total ghrelin Samples collected at baseline (t=0), every 15min during first 2h, and every 30min for last hours for total ghrelin
Galusca *et al.*, 2008 [9]	Females: CT: n=25; 23.1 ± 6.0 yr; 15.8 ± 0.5 $kg.m^{-2}$ C: n=28; 23.9 ± 7.4 yr; 20.7 ± 2.1 $kg.m^{-2}$ AN: n=44; 23.4 ± 8.0 yr; AN: 15.5 ± 0.7 $kg.m^{-2}$ (AN: restrictive-type)	1	Cross-sectional analysis	Anthropometry/body composition Height, BMI, FM, FFM (DXA – HOLOGIC) Bone evaluation BMD (DXA – HOLOGIC – femoral neck, lumbar spine) Breaking strength, trabecular density and structure, cortical density and structure (multislice 3D-pQCT – XtremeCT – distal radius, distal tibia) Biochemical parameters Fasting FT3, IGF-1, GH, E2, cortisol, testosterone, SHBG, FTI (testosterone/SHBG), DHEAS, Bone markers Samples collected every 4h for 24h for osteocalcin, PTH, ALP, sCTX, TRACP 5b, OPG, RANKL, vitamin D (25-hydroxy vitamin D3) Hormonal regulation of appetite Fasting leptin
Fernández-García *et al.*, 2009 [35]	Females: CT: n=22; 19.7 ± 5.3 yr; 16.7 ± 1.0 $kg.m^{-2}$ C: n=20; 19.3 ± 1.6 yr; 22.3 ± 1.6 $kg.m^{-2}$ AN: n=48; 19.0 ± 5.1 yr; 16.1 ± 1.5 $kg.m^{-2}$ (AN: restrictive-type and binge eating/purging type)	2	Cross-sectional analysis	Anthropometry/body composition BW, height, BMI, FM, FFM (DXA – LUNAR) Bone evaluation BMD, BMC (DXA – LUNAR) Biochemical parameters Fasting GH Hormonal regulation of appetite Fasting leptin
Germain *et al.*, 2009 [23]	Females: CT: n=9; 24.1 ± 3.6 yr; 16.1 ± 0.3 $kg.m^{-2}$ C: n=10; 23.1 ± 4.4 yr; 20.5 ± 1.3 $kg.m^{-2}$ AN: n=15; 20.4 ± 5.0 yr; 14.8 ± 0.4 $kg.m^{-2}$ (AN: restrictive-type)	1	Cross-sectional trial Standardized meals were proposed at 8.15am, 12.15pm, 7.15pm (breakfast: 400kcal, lunch: 800kcal, dinner 800kcal) Certainly eaten only in C and CT	Anthropometry/body composition Height, BMI, FM, FFM (DXA – LUNAR) Biochemical parameters Fasting FT3, IGF-1, E2, SHBG Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for cortisol, GH Hormonal regulation of appetite Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for leptin, obestatin, total ghrelin, acylated ghrelin
Marra *et al.*, 2009 [10]	Females: CT: n=10; 19.4 ± 2.4 yr; 16.8 ± 1 $kg.m^{-2}$ C: n=30; 20.0 ± 2.1 yr; 22.5 ± 2.8 $kg.m^{-2}$ AN: n=30; 19.0 ± 2.0 yr; 16.7 ± 0.5 $kg.m^{-2}$ (AN type: NR) BD: n=15; 18.9 ± 1.7 yr; 17.4 ± 0.6 $kg.m^{-2}$	1	Cross-sectional analysis	Anthropometry/body composition BMI, FM, FFM (Skinfold thickness – measured in triplicate to the nearest 0.2mm with a calibrated Harpenden caliper at 4 sites: biceps, triceps, subscapular and suprailiac – estimation with the sum of these 4 skinfold values) Impedance and phase angle (BIA – 101 analyser Akern, for whole body, arms and legs)

Hasegawa *et al.*, 2011 [27]	Females: CT: n=20; 23.2 ± 2.3 yr; 17.6 ± 0.8 kg.m^{-2} C: n=20; 23.1 ± 2.1 yr; 21.9 ± 1.2 kg.m^{-2}	2	Cross-sectional analysis	Anthropometry/body composition BW, height, BMI, FM, FFM, bone mass, adipose tissue, skeletal muscle mass, residual mass (DXA – HOLOGIC) Bone evaluation BMD, BMC (DXA – HOLOGIC) Energy expenditure RMRm (indirect calorimetry – Minato AE300, after a 10-12h overnight fast and at least 30min of rest) RMRe (calculated from a prediction model) Biochemical parameters Fasting T3, E2, leukocytes, erythrocytes, platelets, hemoglobin, hematocrit, glucose, total cholesterol, HDL-cholesterol, triglycerides, AST, ALT, GGT
Galusca *et al.*, 2012 [24]	Females: CT: n=14; 23.7 ± 6§ yr; 16.0 ± 0.4§ kg.m^{-2} C: n=10; 23.1 ± 5§ yr; 20.8 ± 0.6§ kg.m^{-2} AN-R: n=19; 23.2 ± 8§ yr; 15.3 ± 0.4§ kg.m^{-2} AN-BP: n=10; 23.4 ± 6§ yr; 15.4 ± 4.4§ kg.m^{-2} BN: n=10; 22.9 ± 5§ yr; 22.0 ± 2.2§ kg.m^{-2}	1	Cross-sectional trial Standardized meals were proposed at 8.15am, 12.15pm, 7.15pm (breakfast: 400kcal, lunch: 800kcal, dinner 800kcal) Certainly eaten in C, CT, BN Snacks were not allowed	Anthropometry/body composition BMI, FM (DXA – LUNAR) Biochemical parameters Fasting FT3, IGF-1, E2, SHBG Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for cortisol, GH Hormonal regulation of appetite Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for leptin Samples collected 12 times over a period of 24h (4.00am, 7.00am, 8.00am, 9.00am, 10.00am, 12.00pm, 1.00pm, 2.00pm, 4.00pm, 7.00pm, 8.00pm, 12.00am) for 26RFa
Santonicola *et al.*, 2012 [39]	Females and males (not clearly reported): CT: n=9; 24.9 ± 6.6 yr; NR C: n=22; 23.7 ± 3.3 yr; NR AN: n=20; 22.5 ± 4.2 yr; NR (AN: probably restrictive-type) BN: n=6; 24.8 ± 6.8 yr; NR EDNOS: n=10; 24.5 ± 5.8 yr; NR OB: n=32; 23.8 ± 4.2 yr; NR	1	Cross-sectional analysis	Anthropometry/body composition BW Stomach sensations and discomforts Standardized questionnaire of food disorders (Rome III symptom questionnaire - 18 questions allowing the diagnosis of food disorders and its subgroups (postprandial distress syndrome and epigastric pain syndrome) The frequency of early satiety, epigastric fullness, epigastric pain and burning (the 4 cardinal symptoms pragmatically described by the Rome III Committee) and other dyspeptic symptoms such as epigastric pressure, belching, nausea and vomiting was scored from 0 to 3 (0: absent, 1: 2 days/week, 2: 3-5 days/week, 3: 6-7 days/week) and the intensity of these symptoms was scored from 0 to 3 (0: absent, 1: not very bothersome, not interfering with daily activities, 2: bothersome, but not interfering with daily activities, 3: interfering with daily activities) A intensity-frequency score from 0 to 6 was obtained for each symptom
Pasanisi *et al.*, 2013 [21]	Females: CT: n=7; 21.7 ± 3.6 yr; 16.2 ± 0.9 kg.m^{-2} C: n=20; 22.0 ± 3.7 yr; 21.7 ± 2.4 kg.m^{-2} AN: n=7; 23.4 ± 4.5 yr; 15.3 ± 0.8 kg.m^{-2} (AN: restrictive-type) AN rec: n=3; 21.3 ± 1.5 yr; 18.8 ± 1.1 kg.m^{-2}	2	Cross-sectional analysis (same C group as Marra *et al.* 2007 [20])	Anthropometry/body composition BW, height, BMI, FM, FFM (single frequency BIA – Akern) Energy expenditure RMR, RQ (indirect calorimetry – Canopy system) BAT activity: 18F-FDG PET/CT scans (PET/CT Discovery LS8 Device) between October and March to minimize seasonal influences, acquired after a 8h fast and 15min wait – FDG uptake considered present if greater than background soft-tissue activity – the bodyweight-corrected SUVmax was recorded (Volumetrix for PET-CT) and BAT activity was evaluated by the number of typical anatomical areas of BAT showing FDG uptake and SUVmax for each area – finally the sum of SUVmax was used for analyses

				Fidgeting (representing NEAT – SD from the average of 30 RMR measurements (every min for 30min), based on the assumption that in the steady state, increased fidgeting will increase RMR variability)
Paschalis *et al.*, 2013 [40]	Females: CT: n=8; 21.4 ± 1.1 yr; 17.3 ± 0.6 $kg.m^{-2}$ C: n=12; 20.2 ± 1.4 yr; 22.0 ± 1.0 $kg.m^{-2}$ OB: n=12; 20.8 ± 1.0 yr; 29.4 ± 1.7 $kg.m^{-2}$	4	Cross-sectional trial Exercise session consisted of 5 sets of 15 isokinetic eccentric maximal voluntary contractions (MVCs) in the seated position Measurement were performed between 9:00 - 12:00am	Anthropometry/body composition BMI, FM (Siri skinfold equation with 7 skinfold measures - using Harpenden caliper) Biochemical parameters CK (pre, post, 24h, 48h, 72h) Muscle function and damage Isometric and concentric peak torque, ROM (CYBEX - pre, post, 24h, 48h, 72h), DOMS at squat, DOMS walking (VAS - pre, post, 24h, 48h, 72h) Proprioception Position sense in absolute values of angle, position sense in signed values, force mismatch, knee joint reaction angle to release (CYBEX - pre, post, 24h, 48h, 72h) Physical state VO_{2max}
Germain *et al.*, 2014 [1]	Females: CT: n=8; 21.6 ± 5.4 yr; 17.1 ± 0.8 $kg.m^{-2}$ C: n=8; 22.1 ± 2.3 yr; 22.1 ± 0.8 $kg.m^{-2}$	1	Interventional control trial (overfeeding) W0: Week 0 – baseline analyses W4: Week 4 – analyses at the end of the overfeeding period W8: Week 8 – analyses 4 weeks after the end of the overfeeding period Overfeeding protocol: 4-week overfeeding for both CT and C of 630kcal/day excess daily (fat exclusively – fixed daily quantity of olive oil, peanuts, gruyere cheese, butter – 31 ± 7.4% saturated FA, 52 ± 5.4% monounsaturated FA, 17 ± 2.7% polyunsaturated FA) CT and C asked not to maintain their normal lifestyle (baseline diet and physical activity) Appointments for delivering food, evaluating bodyweight evolution, checking compliance Standardized test meals performed after a 12h overnight fast (328kcal: 80% carbohydrates, 13% lipids, 7% proteins) and blood samples collected at T0, 15, 30, 60, 90min – test meal fully eaten between T0 and T15 min in the presence of a study investigator	Anthropometry/body composition BW, height, BMI, FM, FFM (DXA + BIA – Bodystat + MRI) Abdominal fat area (Magnetom Symphonie 1.5T) Energy intake Daily self-reporting during 5 days (over- and under-reporting tracked) Snacking (food eaten between the 3 official meals: breakfast, lunch, dinner – expressed in % of total daily energy intake) Energy expenditure TEE (calculated as RMR×PAL) RMR, RQ (indirect calorimetry – Deltatrac device, after a 12h overnight fast) Energy gap: difference between TEI and TEE Physical activity PAL, AEE (ActiHeart accelerometer – 5 days) Biochemical parameters Fasting FT3, IGF-1, E2, insulin, blood glucose, triglycerides, β-hydroxybutyrate, glycerol, free FA, AAT Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for cortisol Insulin, blood glucose at T0, 15, 30, 60, 90min after the standardized test meal Hormonal regulation of appetite Fasting leptin Total and acylated ghrelin, obestatin, PYY_{3-36}, total GLP-1 at T0, 15, 30, 60, 90min after the standardized test meal Psychological profile Food-related behavioural problems (DEBQ, EDE) Metabolomics analysis 24h urine samples (15ml) (reversed phase UPLC chromatography system coupled to a time-of-flight mass spectrometer)
Galusca *et al.*, 2015 [11]	Females: CT: n=22; 23.2 ± 2.3 yr; 15.9 ± 0.5 $kg.m^{-2}$	1	Cross-sectional trial	Anthropometry/body composition BMI, FM (DXA – LUNAR)

	C: n=14; 22.6 ± 6.0 yr; 21.6 ± 1.1 kg.m^{-2} AN: n=23; 22.5 ± 6.2 yr; 14.6 ± 2.4 kg.m^{-2} (AN: restrictive-type)		Standardized meals were proposed at 8.15am, 12.15pm, 7.15pm (breakfast: 400kcal, lunch: 800kcal, dinner 800kcal) Certainly eaten in C and CT Food intake was not imposed or verified Snacks were not allowed	<u>Biochemical parameters</u> Fasting FT3, IGF-1, E2, SHBG Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for cortisol, GH <u>Hormonal regulation of appetite</u> Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for leptin Samples collected 12 times over a period of 24h (4.00am, 7.00am, 8.00am, 9.00am, 10.00am, 12.00pm, 1.00pm, 2.00pm, 4.00pm, 7.00pm, 8.00pm, 12.00am) for plasma NPY, plasma α-MSH
Germain *et al.*, 2016 [25]	Females: CT: n=10; 20.6 ± 6.6 yr; 15.9 ± 0.9 kg.m^{-2} C: n=10; 22.7 ± 1.6 yr; 21.4 ± 1.6 kg.m^{-2} AN-R: n=10; 21.6 ± 4.7 yr; 15.1 ± 2.5 kg.m^{-2} AN-R rec: n=5; 21.8 ± 4.5 yr; 20.9 ± 3.8 kg.m^{-2} AN-BP: n=5; 21.8 ± 5.4 yr; 15.4 ± 2.7 kg.m^{-2} BN: n=4; 24.2 ± 8.0 yr; 19.8 ± 1.2 kg.m^{-2} OB: n=7; 27 ± 5.6 yr; 46.2 ± 9.0 kg.m^{-2}	1	Cross-sectional trial Standardized meals were proposed at 8.15am, 12.15pm, 7.15pm (breakfast: 400kcal, lunch: 800kcal, dinner 800kcal) Certainly eaten in C, CT, BN, OB Snacks were not allowed	<u>Anthropometry/body composition</u> BW, height, BMI, FM (DXA – LUNAR) <u>Biochemical parameters</u> Fasting FT3, IGF-1, E2, FSH, LH, testosterone Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for cortisol Samples collected 12 times over a period of 24h (4.00am, 7.00am, 8.00am, 9.00am, 10.00am, 12.00pm, 1.00pm, 2.00pm, 4.00pm, 7.00pm, 8.00pm, 12.00am) for plasma IL-7 <u>Hormonal regulation of appetite</u> Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for leptin
Gunes *et al.*, 2016 [41]	CT (16 females, 8 males): n=24; 22.1 ± 3.7 yr; 17.4 ± 1.2 kg.m^{-2} C (9 females, 15 males): n=24; 23.5 ± 4.0 yr; 22.1 ± 2.4 kg.m^{-2}	2	Cross-sectional analysis	<u>Anthropometry/body composition</u> BMI <u>Eye corneal parameters</u> Spherical equivalent, best-corrected visual acuity, intraocular pressure with Goldmann applanation tonometry, axial length (Scheimpflug imaging – detailed slit-lamp ophthalmic examination – Sonomed PacSan 300AP + Biometric/pachymeter) Corneal power measurements, pachymetric measurements, corneal volume measurements – Scheimpflug imaging system (HR Pentacam, Oculus) – cornea thickness at the center of the pupil, apex point, thinness point: automatically evaluated
Estour *et al.*, 2017 [3]	Females: CT: n=56; 26.9 ± 7.6 yr; 16.5 ± 0.9 kg.m^{-2} C: n=54; 23.4 ± 4.1 yr; 20.9 ± 2.2 kg.m^{-2} AN: n=40; 25.0 ± 6.5 yr; 16.0 ± 0.8 kg.m^{-2} (AN: restrictive-type)	1	Cross-sectional trial Standardized meals were offered at 8.15am, 12.15pm, 7.15pm (breakfast: 400kcal, lunch: 800kcal, dinner 800kcal) Certainly eaten in C, CT	<u>Anthropometry/body composition</u> BMI, FM, FFM (DXA – LUNAR) <u>Bone evaluation</u> BMD (DXA – HOLOGIC – femoral neck, lumbar spine) <u>Energy expenditure</u> RMR (indirect calorimetry – Deltatrac device, after a 12-h overnight fast) <u>Biochemical parameters</u> Fasting FT3, IGF-1, FT4, E2, TSH, FSH, LH, total testosterone, DHEAS, albumin Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for cortisol, GH, ACTH, prolactin <u>Bone markers</u> Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for osteocalcin, sCTX, vitamin D (25-hydroxy vitamin D3) <u>Hormonal regulation of appetite</u> Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for leptin

				Psychological profile Food-related behavioural problems (DEBQ, EDI, EDE, SCL-90-R questionnaires)
Galusca *et al.*, 2018 [4]	Females: CT: n=10; 22.1 ± 5.1 yr; 17.0 ± 0.9 kg.m^{-2} C: n=10; 22.2 ± 2.5 yr; 21.7 ± 1.3 kg.m^{-2}	2	Cross-sectional trial Standardized meals were eaten at 8.15am, 12.15pm, 7.15pm (breakfast: 400kcal, lunch: 800kcal, dinner 800kcal)	Anthropometry/body composition BW, height, BMI, FM, FFM, proportion of fat mass at lower limb level (legs fat mass×100/total fat mass) (DXA – LUNAR) Bone evaluation BMD (DXA – HOLOGIC – femoral neck, lumbar spine) Energy intake TEI (dietary self-reporting record on 7 days using photographs previously validated for the SU.VI.MAX study) Energy expenditure RMR, RQ, fat oxidation index (indirect calorimetry – Deltatrac device, after a 12h overnight fast) AEE (ActiHeart accelerometer – ambulatory position during 5 days), PAL, inactivity time (MOSPA questionnaire) Biochemical parameters Fasting FT3, IGF-1, E2, blood glucose, insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, QUICKI, HOMA, creatinine, AST, ALT, ALP, GGT Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for cortisol Bone markers Fasting vitamin D, calcium, PTH Hormonal regulation of appetite Samples collected every 4h for 24h (8.00am, 12.00pm, 4.00pm, 8.00pm, 12.00am, 4.00am) for leptin Histological muscle fibres evaluation Analyses from the *vastus lateralis* skeletal muscle biopsies (right leg) Fibre size (CSA of fibres), fibre-typing (immunohistochemical staining – % of fibre types), capillary network (CD31 antibody – CD, C/F for global indexes and CC, CC with SF, LC/PF for local indexes), COx activity (3,3'-diaminobenzidine staining) Enzymological muscle evaluation PFK, CS, β-HAD, CK (SFM25 fluorometer – Kontron instruments) Muscle transcriptomics RNA profiling of skeletal muscle biopsies (Affymetrix)
Florent *et al.*, 2019 [42]	Females: CT: n=10; 22.4 ± 2.5 yr; 17.1 ± 0.9 kg.m^{-2} C: n=10; 21.8 ± 2.2 yr; 21.9 ± 1.3 kg.m^{-2} AN: n=10; 26.4 ± 6.0 yr; 15.3 ± 1.9 kg.m^{-2} (AN: restrictive-type)	2	Cross-sectional trial Calibrated breakfast (nutritional supplement as breakfast – breakfast calibrated to 25% of the predicted RMR for each participant)	Anthropometry/body composition BW, BMI Energy intake Energy intake at breakfast (around 8:45am – assessed by a medical team trained for the purpose in order to limit the anxiety of patients with AN) Energy expenditure Predicted RMR for calibration breakfast (Harris and Benedict equation) Neural regulation of appetite Glx, creatine, NAA, myoinositol, choline (MRI - 3T Philips Achieva Scanner - fasting at 8:00am, and 1h postprandial at 10:00am) Psychological profile TFEQ

Ling *et al.*, 2019 [43]	Females and males: CT: n=29; 25.0 ± 4.7 yr; 17.0 ± 0.7 $kg.m^{-2}$ C: n=29; 22.6 ± 2.9 yr; 23.0 ± 1.0 $kg.m^{-2}$	1	Interventional control trial (overfeeding) Overfeeding: 2 weeks – bottle of Renutryl Booster (Nestlé) +600kcal/day in 300mL (48,5% carbohydrates, 20% proteins, 31,5% fat) consumed between dinner and bedtime	Anthropometry/body composition BMI, FM, FFM (DXA – LUNAR) Bone evaluation HR-pQCT Energy intake TEI (in free living conditions by self-reported dietary records during 7 days using photographs previously validated for the SU.VI.MAX study and in calorimetric chamber with controlled meals of 2300kcal/day but with measurements of the real food intake by dieticians), quantity of proteins, carbohydrates and lipids intake, snacking (defined as food intake out of the 3 main meals), compliance to the overfeeding protocol (urea excretion) Energy expenditure TEE (calculated as RMR×PAL), RMR by indirect calorimetry (canopy system Quark RMR COSMED – after a 12h-fast) and in calorimetric chamber (monitored during 24h – open-circuit whole-body – RMR calculated as the 30min average after waking up in the fasting state) RQ (indirect calorimetry – canopy system Quark RMR COSMED – after a 12h-fast) Fat stools – near-infrared deflectance analysis (and when stools were liquid: titrimetry) Physical activity PAL (ActiHeart accelerometer – 5 days) Biochemical parameters Fasting FT3, IGF-1, FT4, TSH, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, fasting glucose, glycerol, nonesterified fatty acid, metadrenaline, normetadrenaline Urinary creatinine, urea, uric acid Hormonal regulation of appetite Fasting leptin General parameters Body core temperature – noninvasive Jonah capsule temperature sensor (Respironic) for at least 3h postingestion Adipose tissue evaluation Adipocytes area (hematoxylin and eosin staining) Mitochondrial respiration – on fresh tissue within 3h after the biopsy using high-resolution respirometry (Oxygraph-2k, OROBOROS) Muscle tissue evaluation Mitochondrial respiration – on fresh tissue within 3h after the biopsy using high-resolution respirometry (Oxygraph-2k, OROBOROS) Genetic analyses Plasma proteomics: a total of 999 proteins were measured Mitochondrial DNA content – real time PCR Gene expression analysis by RNA sequencing (extraction of total RNA, PCR, validation of gene expression using Nanostring technology)
Margaritelis *et al.*, 2019 [44]	Females: CT: n=12; 21.2 ± 1.4 yr; 17.8 ± 0.8 $kg.m^{-2}$	4	Cross-sectional trial Exercise session consisted of 5 sets of 15 isokinetic	Anthropometry/body composition BW, BMI, FM (Siri skinfold equation with 7 skinfold measures - using Harpenden caliper) Energy intake

	C: n=14; 20.4 ± 1.8 yr; 22.4 ± 1.1 kg.m^{-2} OB: n=13; 20.8 ± 1.3 yr; 36.6 ± 1.5 kg.m^{-2}		eccentric maximal voluntary contractions in the seated position Measurements were performed between 08:00 - 09:00am after overnight fasting in order to avoid any effect of diet on resting redox status	Carbohydrates, proteins, lipids, Vitamin C, Vitamin E, Selenium (self-reporting during a period of 4 days including 3 days of the familiarization process and 1 day of the eccentric exercise test) Biochemical parameters Plasma protein carbonyls, erythrocytes glutathione, CK (pre, post, 24h, 48h, 72h, 96h), urinary F2-isoprostanes Muscle function and damage ROM, isometric peak torque (CYBEX - pre, post, 24h, 48h, 72h, 96h), DOMS (VAS - pre, post, 24h, 48h, 72h, 96h)
Marra *et al.*, 2019 [18]	Males: CT: n=15; 23.3 ± 5.2 yr; 17.9 ± 0.6 kg.m^{-2} C: n=18; 22.3 ± 3.7 yr; 22.3 ± 1.7 kg.m^{-2} AN: n=17; 22.3 ± 5.3 yr; 17.1 ± 1.2 kg.m^{-2} (AN: probably restrictive-type) BD: n=12; 19.7 ± 1.6 yr; 20.0 ± 1.3 kg.m^{-2}	2	Cross-sectional analysis	Anthropometry/body composition BW, height, BMI, FM, FFM (BIA – Human Im Plus II) Energy expenditure RMR, RQ (indirect calorimetry – Canopy system, after a 12-14h overnight fast and after a 15min adaptation period)
Riveros-McKay *et al.*, 2019 [45]	Females: CT: n=1 325; 36.6 ± 14.3 yr; 17.6 ± 0.9 kg.m^{-2} (STILTS cohort) C: n=5 837; 52.0 ± 16.7 yr; 27.0 ± 7.9 kg.m^{-2} (UKHLS cohort) AN type: NR Males: CT: n=297; 35.2 ± 14.5 yr; 17.6 ± 1.1 kg.m^{-2} (STILTS cohort) C: n=4 596; 52.7 ± 17.3 yr; 26.9 ± 7.8 kg.m^{-2} (UKHLS cohort) AN type: NR	1	Cohort-based cross-sectional analysis	Genetic parameters DNA salivary or blood samples
Bailly *et al.*, 2020 [19]	Females: CT: n=15; 27.4 ± 4.6 yr; 16.5 ± 0.8 kg.m^{-2} C: n=16; 22.4 ± 2.8 yr; 23.0 ± 1.1 kg.m^{-2} Males: CT: n=15; 23.6 ± 3.8 yr; 17.4 ± 0.8 kg.m^{-2} C: n=15; 23.9 ± 2.9 yr; 23.0 ± 1.2 kg.m^{-2}	1	Interventional control trial (overfeeding) (same participants as Ling *et al.*, 2019 [43]) Overfeeding: 2 weeks – bottle of Renutryl Booster (Nestlé) +600kcal/day in 300mL (48,5% carbohydrates, 20% proteins, 31,5% fat) consumed between dinner and bedtime	Anthropometry/body composition BW, height, BMI, FM, FFM (DXA – LUNAR) Physical activity PAL (ActiHeart accelerometer – 5 days) Histological muscle fibres evaluation Analyses from the *vastus lateralis* skeletal muscle biopsies (right leg) Fibre size (CSA of fibres), fibre-typing (immunohistochemical staining – % of fibre types), capillary network (CD31 antibody – CD, C/F for global indexes and CC, CC with SF, CFPE, CAFA for local indexes), IMTG content (Oil Red O staining), glycogen content (periodic acid schiff staining), COx activity (3,3'-diaminobenzidine staining) Enzymological muscle evaluation Enolase, β-HAD, CS, CII, CIV, LDH, myokinase, CK (spectrophotometry – microplate reader CLARIOstar)
Ling *et al.*, 2020 [6]	Females: CT: n=12; 26.9 ± 4.7 yr; 16.6 ± 0.7 kg.m^{-2} C: n=12; 21.9 ± 3.0 yr; 22.9 ± 1.2 kg.m^{-2} Males: CT: n=11; 23.0 ± 3.9 yr; 17.3 ± 0.7 kg.m^{-2} C: n=10; 23.3 ± 2.8 yr; 22.9 ± 1.0 kg.m^{-2}	1	Interventional control trial (overfeeding) (same participants as Ling *et al.*, 2019 [43]) Overfeeding: 2 weeks – bottle of Renutryl Booster (Nestlé) +600kcal/day in 300mL (48,5% carbohydrates, 20% proteins, 31,5% fat) consumed between dinner and bedtime Standardized meal: bottle of Renutryl Booser (Nestlé)	Anthropometry/body composition BW, BMI, FM, FFM (DXA – LUNAR) Energy intake TEI, nitrogen intake (in free living conditions by self-reported dietary records during 7 days using photographs previously validated for the SU.VI.MAX study and in calorimetric chamber with controlled meals of 2300kcal/day but with measurements of the real food intake by dieticians), quantity of proteins, carbohydrates and lipids intake, snacking (defined as food intake out of the 3 main meals), compliance to the overfeeding protocol (urea excretion) Nitrogen loss (24h urine samples) Energy expenditure

			consumed slowly during 15min under surveillance – blood collection at 7 times points: 0min (after a 12h fast), 15min (immediately after the Renutryl Booster consumption), 30min, 60min, 90min, 120min, 150min	TEE (calculated as RMR×PAL), RMR by indirect calorimetry (canopy system Quark RMR COSMED – after a 12h fast) and in calorimetric chamber (monitored during 24h – open-circuit whole-body – RMR calculated as the 30min average after waking up in the fasting state) Energy gap: difference between TEI and TEE <u>Physical activity</u> PAL (ActiHeart accelerometer – 5 days) <u>Biochemical parameters</u> Fasting FT3, IGF-1, insulin, blood glucose, triglycerides, albumin, nonesterified fatty acids, glycerol <u>Hormonal regulation of appetite</u> Fasting leptin, total ghrelin, acylated ghrelin, PYY, GLP-1 Samples collected during the standardized meal (at 0min (after a 12h fast), 15min (immediately after the Renutryl Booster consumption), 30min, 60min, 90min, 120min, 150min for total ghrelin, acylated ghrelin, PYY, GLP-1 <u>Genetic analyses</u> 24h urine samples were collected for metabolome analysis Metabolomics analysis (^{1}H NMR spectroscopy) – a total of 79 signals out of 155 were assigned to biochemical molecular species, corresponding to 51 unique metabolites
Orthofer *et al.* 2020 [46]	Females and males: CT: n=881; NR but 20-44 yr; NR but 6th lowest %BMI C: n=3173; NR but 20-44 yr; NR but 30-50th %BMI (cohort from EGCUT biobank) OB: NR; NR; NR	2	Cohort-based cross-sectional analysis	<u>Genetic parameters</u> GWAS performed – genotype data generated using HumanCorePsy array (Illumina, San Diego USA)

[†] CT diagnosis estimated 1: very complete, 2: quite complete, 3: not very complete, 4: incomplete – based on a previous publication [12]; [§] Type of values dispersion (SD or SEM) not clearly reported

18F-FDG: 18-fluorodeoxyglucose; AAT: amino acid transferase; ACTH: adrenocorticotropic hormone; AEE: activity energy expenditure; ALP: alkaline phosphatase; ALT: alanine aminotransferase; α-MSH: α-melanocyte-stimulating hormone; AN: subjects with anorexia nervosa; AN-BP: subjects with anorexia nervosa of bingeing/purging type; AN-R: subjects with anorexia nervosa of restrictive type; AST: aspartate aminotransferase; BAT: brown adipose tissue; BD: underweight ballet dancers; β-HAD: β-hydroxyacyl-CoA dehydrogenase; BIA: bioelectrical impedance analysis; BMC: bone mineral content; BMD: bone mineral density; BMI: body mass index; BN: subjects with bulimia nervosa; BW: bodyweight; C: normal-weight control participants; C/F: capillary to muscle fibre ratio; CAFA: capillary contact per fibre area; CC: capillary contacts per muscle fibre; CD: capillary density; CFPE: capillary to fibre-perimeter exchange; CII: complex II of the mitochondrial respiratory chain; CIV: complex IV of the mitochondrial respiratory chain; CK: creatine kinase; COx: cytochrome C oxidase; CS: citrate synthase; CSA: cross-sectional area; CT: constitutionally thin subjects; DEBQ: Dutch eating behaviour questionnaire [62]; DHEAS: dehydroepiandrosterone sulfate; DOMS: delayed onset muscle soreness; DWL: doubly labeled water; DXA: dual-energy X-ray absorptiometry; E2: estradiol; EDE: eating disorder examination [63]; EDI: eating disorder inventory [64]; EDNOS: subjects with eating disorders not otherwise specified; EGCUT: Estonian genome center of the university of tartu; FA: fatty acids; FFM: fat-free mass; FITM: fat storage-inducing transmembrane; FM: fat mass; FSH: follicle-stimulating hormone; FT3: free triiodothyronine; FT4: free thyroxine; FTI: free testosterone index; GGT: gamma-glutamyl transpeptidase; GH: growth hormone; GHRH: growth hormone-releasing hormone; GLP-1: glucagon-like peptide-1; Glx: glutamine/glutamate ratio; GnRH: gonadotropin-releasing hormone; GWAS: genome-wide association study; HF: high frequency; HOMA: homeostasis model assessment; HR-pQCT: high-resolution peripheral quantitative computed tomography; IBW: ideal bodyweight; IGF-1: insulin-like growth factor 1; IL-7: interleukin-7; IMTG: intramuscular triglycerides; LC/PF: ratio between the length of contact of the capillaries with the muscle fibre to the perimeter of the muscle fibre; LDH: lactate dehydrogenase; LF: low frequency; LH: luteinizing hormone; MOSPA: MONICA optional study of physical activity [65]; MPS: multidimensional perfectionism scale; MRI: magnetic resonance imaging; NAA: N-acetyl aspartate; NEAT: nonexercise activity thermogenesis; NN: normal RR interval; NPY: neuropeptide Y; NR: not reported; OB: participants with obesity; OPG: osteoprotegerin; PAL: physical activity level; PET/CT: positron emission tomography with computed tomography; PFK: phosphofructokinase; pNN50: number of adjacent NN intervals differing by more than 50 ms in the entire recording divided by the total number of all NN intervals; PPT: postprandial thermogenesis; 3D-pQCT: three-dimensional peripheral quantitative computed tomography; PTH: parathyroid hormone; PYY: peptide YY; QUICKI: quantitative insulin sensitivity check index; RANKL: receptor activator of nuclear factor-κB ligand; rec: after weight recovery or partial recovery; RMR: resting metabolic rate; RMRc: calculated resting metabolic rate; RMRe: estimated resting metabolic rate; RMRm: measured resting metabolic rate; rMSSD: root mean square successive difference; ROM: pain-free range motion; RQ: respiratory quotient; SCL-90-R: symptom check list – 90-revised; sCTX: serum C-telopeptide cross-link of type 1 collagen; SDANN index standard deviation of the average NN intervals for all 5-min segments; SDNN: standard deviation of all NN intervals; SDNN index: mean of the standard deviation of all NN intervals for all 5-min segments; SF: sharing factor; SHBG: sex hormone-binding globulin; SUVmax: maximum standardized uptake values; T3: triiodothyronine; T4: tetraiodothyronine; TEE: total energy expenditure; TEI: total energy intake; TFEQ: three-factor eating questionnaire [66], TRACP 5b: tartrate-resistant acid phosphatase type 5b; TSH: thyroid stimulating hormone; ULF: ultra-low frequency; UPLC: ultra-performance liquid chromatography; VAS: visual analog scale; VLF: very low frequency

Table 2: Main results of the clinical trials selected in the systematic review, classified as four sub-groups: anthropometry/body composition, energy intake, energy expenditure, and biochemical parameters

Reference	Anthropometry/body composition	Energy intake	Energy expenditure	Biochemical parameters	Hormonal regulation of appetite
Schneider *et al.*, 1979 [30]	BW: CT<C Height: CT=C $BWgain_{28/32/36/total}$: C=CT	NR	NR	NR	NR
van Binsbergen *et al.*, 1990 [31]	BW, BMI, %IBW: lower values in AN IBW: AN=C=CT Height: AN=CT=C	NR	NR	Baseline Fasting E2, testosterone, basal LH, prolactin, estrone, progesterone, androstenedione: significant difference among the 3 groups (C, CT, AN) – lower values in AN Fasting DHEAS: AN=CT=C Fasting SHBG: C=CT; trend (p=0.052) for higher values in AN Response to GnRH: LH mean increment: C ↗; CT ↗; AN ↗ FSH mean increment: C →; CT →; AN ↗	NR
Diaz *et al.*, 1992 [48]	Baseline BW, BMI, %FM: CT<OB Height: OB=CT Overfeeding BW gain: OB=CT Individual analysis: all subjects gained weight almost linearly except 1 CT subject (range in CT: +5.0kg to +10.5kg) Post-overfeeding Individual analysis: all subjects lost BW	Overfeeding Good compliance despite the rigorous demands of the study protocol Good agreement between metabolizable energy calculated from food tables and the measurements obtained from the gross, fecal, and urinary energy The mean percent of energy between baseline and overfeeding were respectively 11.7% and 12.7% from proteins, 39.0% 41.5% from fat, 48.8% and 45.4% from carbohydrates	Overfeeding Individual analysis: almost all subjects increased TEE_{DLW} and TEE_{calo}, RMR_{calo} increased in all subjects, TEE_{calo} seems to increase more in OB than CT Individual analysis: $[activity + thermogenesis]_{DLW}$ increased but not significantly, $[activity + thermogenesis]_{calo}$ increased significantly Post-overfeeding Individual analysis: TEE_{calo}, RMR_{calo}, $[activity + thermogenesis]_{calo}$ values: returned to values similar to those at baseline Overall Fat oxidation seem to be: OB<CT, especially with overfeeding Carbohydrates oxidation seem to be CT<OB especially with overfeeding	NR	NR
Scalfi *et al.*, 1992 [7]	BW, BMI: AN<CT<C Height: C=AN=CT	NR	Before meal: RMR: AN<C, AN=CT, CT=C $RMR_{weight-adjusted}$: AN=CT=C RQ: C=AN=CT Post meal: $RQ_{0-120min}$, $RQ_{120-240min}$, $RQ_{0-240min}$: AN=CT=C	NR	NR

			$PPT_{30min\ absolute}$: AN=CT<C $PPT_{90\ and\ 120min\ absolute}$: CT<C, CT=AN, AN=C $PPT_{0-120min}$: CT<C, AN=C, CT=AN $PPT_{120-240min}$: CT=AN=C $PPT_{0-240min}$: CT<AN=C		
Hinney *et al.*, 1997 [47]	NR	NR	NR	NR	NR
Petretta *et al.*, 1997 [32]	BW, BMI: AN=CT<C Height: AN=CT=C	NR	RMR: AN<CT=C	Fasting FT3, FT4, T3, plasma renin activity: AN<CT=C Fasting glucose, T4: AN<C=CT Na^+: C=AN=CT K^+: AN=C=CT TSH: AN=CT=C	NR
Slof *et al.*, 2003 [36]	BMI: CT<C	NR	NR	NR	NR
Tolle *et al.*, 2003 [22]	BW: AN=CT<AN rec<C BMI: AN=CT<AN rec<C %FM: AN<AN rec<C AN<CT=C	NR	NR	<u>Baseline:</u> Fasting FT3: AN<AN rec=C=CT Fasting IGF-1: AN<C=CT=AN rec Fasting E2: AN=AN rec<C<CT 24h mean GH: C<AN, C=CT, C=AN 24h mean cortisol: C, AN rec, CT<AN <u>24h circadian profile</u>: Cortisol: CT=AN rec=C<AN GH: C=AN rec=CT<AN <u>After GHRH injection</u>: GH: C<AN rec=CT<AN AUC GH/120min: C=AN rec=CT<AN	<u>Baseline:</u> Fasting total ghrelin: C=AN rec=CT<AN <u>24h circadian profile:</u> Total ghrelin: C=AN rec<CT<AN Leptin: AN<CT<AN rec<C
Bosy-Westphal *et al.*, 2004 [33]	BW, BMI, FFM: CT<C<OB Height, lean body mass trunk, muscle mass: CT<C=OB %FM: C=CT<OB Total BMC: CT<OB=C <u>Volume of internal organs</u> Brain: CT<OB<C Heart: CT<C<OB Liver, spleen: CT<C=OB Kidneys, OM: CT<OB=C Residual: CT<C<OB	TEI: CT=OB=C %proteins: CT=C=OB %carbohydrates: C=OB<CT %fat: CT=OB, CT<C, OB=C	RMRm, RMRc: CT<C=OB RMR adjusted for FFM: CT<C, OB=C, CT=OB RMRm– RMRc: OB=CT=C	NR	NR
Mazzeo *et al.*, 2004 [37]	BMI: CT<C	NR	NR	NR	NR

Tagami *et al.*, 2004 [38]	BMI: AN, CT<C; C=BN; C<OB %FM: AN, CT, BN<C; C<OB (statistical tests *vs.* C only)	NR	NR	Fasting adiponectin: OB, AN, BN<C; C=CT Fasting glucose: AN<C; BN, CT=C; C=OB Fasting insulin: AN<C; CT=C; C=BN; C<OB Fasting HOMA: AN<C; C=CT, BN; C<OB (statistical tests *vs.* C only)	Fasting leptin: AN<C; CT, BN=C; C<OB (statistical tests *vs.* C only)
Miljic *et al.*, 2006 [26]	BW, BMI: AN<AN rec=CT	NR	NR	<u>Baseline:</u> Fasting GH, cortisol: CT=AN rec<AN Fasting adiponectin: AN=CT=AN rec Fasting prolactin, insulin: CT=AN rec=AN Fasting glucose: AN=AN rec=CT Fasting C-peptide: AN rec=AN=CT[£] <u>During infusion:</u> Cortisol response: AN rec=AN=CT GH response: AN=AN rec<CT Prolactin response: AN=CT=AN rec	<u>Baseline:</u> Fasting leptin: AN<AN rec=CT Fasting total ghrelin: CT<AN rec<AN <u>During infusion:</u> Total ghrelin response: CT=AN=AN rec <u>Appetite:</u> Hunger 1h: (AN+AN rec)<CT
Bossu *et al.*, 2007 [5]	BW, BMI: AN=CT<C CT: BMI was very low (3rd percentile) throughout the growth period until the age of 18 %FM: AN<CT<C FFM: CT=AN<C	TEI: AN<CT=C %proteins: CT=C<AN %carbohydrates: C=AN=CT %fat: AN<C; C=CT; AN=CT	TEE: CT=C=AN TEE/FFM: C<CT; C=AN; AN =CT RMR: AN<CT<C RMR/FFM: AN=C<CT RQ: CT=C <u>Physical activity</u> PAL, AEE: C=CT and trends for higher values in AN	Fasting FT3: AN<CT=C Fasting IGF-1: AN<C; AN=CT; CT=C	Fasting leptin: AN<CT=C
Germain *et al.*, 2007 [8]	BMI: AN=CT<C %FM: AN<CT<C FFM: NR	TEI: AN<CT=C	NR	Fasting FT3, IGF-1, E2, FSH, LH: AN<C=CT Fasting FTI, SHBG: CT=AN=C 24h mean cortisol: CT=C<AN 24h mean GH: C=CT<AN	24h mean leptin: AN<CT=C Total PYY: AN=C<CT GLP-1: CT<C=AN 24h mean total ghrelin: CT<C<AN <u>Circadian profiles, results at nadir and peak points:</u> Leptin: AN<CT=C Total PYY: AN=C<CT
Marra *et al.*, 2007 [20]	BW, BMI: AN=CT<C<OB Height: AN=CT=OB=C %FM: AN<CT<C<OB FFM: AN=CT; CT=C; CT<OB; AN<C<OB	NR	RMR: AN<CT=C<OB RMR/FFM: AN<C=CT<OB Fidgeting: AN=C<OB<CT Fidgeting/RMR: C=AN=OB<CT RQ: CT=C=AN=OB <u>Correlation between RQ and fidgeting:</u> CT: negative C: no correlation AN, OB: positive	NR	NR

Miljic *et al.*, 2007 [34]	BW, BMI: AN<AN rec=CT	NR	NR	<u>Baseline:</u> Fasting adiponectin: AN=CT=AN rec Fasting GH, cortisol: CT=AN rec<AN Fasting glucose, HOMA: AN=AN rec=CT Fasting insulin: CT=AN rec=AN Fasting C-peptide: CT=AN rec=AN[£] <u>During infusion:</u> $Glucose_{45\text{-}240min}$: AN rec<CT $Glucose_{15\text{-}300min}$: AN<CT $Glucose_{45\text{-}120min}$: AN<AN rec	<u>Baseline:</u> Fasting leptin: AN<AN rec=CT Fasting total ghrelin: CT<AN rec<AN <u>During infusion:</u> Total ghrelin: CT=AN=AN rec
Galusca *et al.*, 2008 [9]	BMI: AN=CT<C Height: AN=C=CT %FM: AN<CT<C FFM: NR Femoral neck BMD, lumbar spine BMD: AN=CT<C Up to 44% of CT and 50% of AN have low bone mass (Z score < -2.0)	NR	NR	Fasting FT3, IGF-1, E2: AN<C=CT Fasting cortisol, DHEAS: CT=C<AN Fasting GH: C=CT<AN Fasting SHBG: AN=CT=C Fasting FTI: CT=AN=C <u>Bone markers</u> 24h mean osteocalcin: AN<C=CT Fasting vitamin D: CT=AN=C Fasting PTH: AN=C=CT 24h mean ALP: CT=AN=C 24h mean sCTX: C=CT<AN 24h mean TRACP 5b, OPG: CT=C<AN 24h mean RANKL: CT<AN=C 24h mean OPG/RANKL: AN<CT; C<CT	Fasting leptin: AN<CT<C
Fernández-García *et al.*, 2009 [35]	BW: CT=AN<C Height: AN=CT=C BMI: AN=CT<C %FM: AN<CT (*vs.* C: NR) FFM: CT=AN (*vs.* C: NR) Total BMC: CT=AN (*vs.* C: NR) Total BMD, lumbar spine BMD: CT=AN<C	NR	NR	Fasting GH: CT=AN (*vs.* C: NR)	Fasting leptin: AN<CT<C
Germain *et al.*, 2009 [23]	Height: CT=C=AN BMI: AN=CT<C %FM: AN<CT<C FFM: NR	NR	NR	Fasting FT3, IGF-1, E2: AN<C=CT Fasting SHBG: CT=C<AN 24h mean cortisol: CT=C<AN 24h mean GH: C=CT<AN	24h mean leptin: AN<CT<C 24h mean total ghrelin: C=CT<AN 24h mean acylated ghrelin, mean obestatin: CT=C; C<AN; CT=AN Acylated ghrelin/obestatin: AN<CT=C Total ghrelin/obestatin: AN<C=CT Acylated ghrelin/total ghrelin: CT<AN=C

Marra *et al.*, 2009 [10]	BMI, and biceps, triceps, subscapular, suprailiac skinfolds‡: AN=CT=BD<C %FM: AN=BD=CT<C FFM: CT=BD=AN<C BI – whole body: CT=AN=BD<C BI arms: BD=AN=CT<C BI legs: CT=AN=C=BD Phase angle whole body: AN<C=CT<BD Phase angle arms: AN<CT<BD; C=CT<BD; AN=C Phase angle legs: AN=CT<C=BD	NR	NR	NR	NR
Hasegawa *et al.*, 2011 [27]	BW, BMI, %FM, FFM, total BMD: CT<C Height: C=CT Head & trunk, arms, legs BMD: CT=C Bone mass, adiopose tissue, skeletal muscle mass: CT<C Residual mass: CT=C	NR	RMRm, RMRe: CT<C RMRm/BW: C<CT RMRm/FFM: C=CT Bone mass RMRe, adipose tissue RMRe, skeletal muscle mass RMRe: CT<C Residual mass RMRe: CT=C	Leukocytes: CT<C Fasting glucose, triglycerides, platelets, T3, E2: CT=C Fasting erythrocytes, hemoglobin, hematocrit, total cholesterol, ALT, GGT: C=CT Fasting HDL-cholesterol, AST: C<CT	NR
Galusca *et al.*, 2012 [24]	BMI, FM: AN-R=AN-BP<CT<C=BN %FM: AN-R=AN-BP<CT<BN=C	NR	NR	Fasting FT3, E2: AN-BP=AN-R<C=CT; AN-BP=AN-R<BN<CT; BN=C 24h mean cortisol: CT=C<AN-R=AN-BP; CT<BN<AN-R=AN-BP; C=BN Fasting IGF-1: AN-BP=AN-R<BN=CT=C 24h mean GH: BN=C=CT<AN-BP=AN-R	24h mean leptin: AN-BP=AN-R<CT<C=BN 24h mean 26RFa: BN=C=CT<AN-BP=AN-R – but a trend (p=0.06) for C<CT <u>Circadian profile of 26RFa, broadly:</u> C≤CT≤AN-R, but CT do not show significant increase at lunchtime contrary to C When converting into relative values: no differences of the 26RFa AUC between the 5 groups (CT, C, AN-R, AN-BP, BN)
Santonicola *et al.*, 2012 [39]	BW£: overall difference between the 6 groups (CT, C, AN, BN, EDNOS, OB) but unspecified between which groups the differences are	NR	NR	NR	NR
Pasanisi *et al.*, 2013 [21]	BW, BMI, %FM, FFM: AN=CT=AN rec£ Height: no difference between the 4 groups (CT, C, AN, AN rec) Phase angle: AN=AN rec<CT£ (comparison tests between C and other groups not performed for the above parameters)	NR	RMR, %fidgeting/RMR: AN=AN rec<CT RMR/FFM: AN rec=AN<CT RQ: AN rec=CT=AN (comparison tests between C and other groups not performed for the above parameters) <u>BAT activity:</u> 18F-FDG uptake: present in all CT (neck and supraclavicular area in 7/7 CT, thoracic and paravertebral in 6/7, mediastinal in 5/7, prevertebral and intercostal in 3/7, paracardiac and sovrarenal in	NR	NR

			2/7); absent in all AN and AN rec; present in 3/24 C limited to the neck and supraclavicular regions SUVmax, mean SUVmax: C<CT (from the 7/7 CT and 3/24 C in whom BAT activity was detected) <u>Correlations of the sum of SUVmax in CT with:</u> RMR: trend (p<0.07) for positive correlation RMR/FFM: positive correlation Fasting RQ: negative correlation		
Paschalis *et al.*, 2013 [40]	BMI: C<OB, CT<C %FM: C<OB, CT<C (comparison tests between OB and CT probably not performed/reported for the above parameters)	NR	NR	<u>CK</u> Post: NR 24h: C ↗; CT ↗; OB ↗ (CT=C, C=OB) 48h: C ↗; CT ↗; OB ↗ (C<OB, C=CT) 72h: C ↗; CT ↗; OB ↗ (CT=C, C=OB) (comparison tests between OB and CT probably not performed/reported for the above parameters)	NR
Germain *et al.*, 2014 [1]	<u>W0</u> BW, BMI, %FM (DXA), %FM (BIA), total abdominal fat (MRI): CT<C Height: CT=C <u>W4 *vs.* W0</u> BMI, %FM (DXA), total abdominal fat (MRI): C: →; CT: → (W4: CT<C) BW, %FM (BIA): C: ↗; CT: → (W4: CT<C) <u>W8 *vs.* W4</u> BW: C →; CT ↘ <u>W8 *vs.* W0</u> BMI, total abdominal fat (MRI): C: →; CT: → (W8: CT<C) BW, %FM (DXA), %FM (BIA): C: ↗; CT: → (W8: CT<C)	<u>W0</u> Total daily energy intake, %fat, fat, %mono unsaturated FA, %poly unsaturated FA: C=CT %proteins, %carbohydrates, %saturated FA: CT=C Snacking: C<CT <u>W4 *vs.* W0</u> Total daily energy intake, fat: C: ↗; CT: ↗ (W4: C=CT) %fat, %poly unsaturated: C: ↗; CT: ↗ (W4: CT=C) %carbohydrates: C: ↘; CT: ↘ (W4: C=CT) Snacking: C: →; CT: → (W4: C<CT) %proteins, %saturated FA: C: →; CT: → (W4: CT=C) %mono saturated FA: C: →; CT: ↗ (W4: C=CT) <u>W8 *vs.* W0</u> NR	<u>W0</u> RMR: CT<C RMR/FFM, RQ: C=CT TEE, carbohydrates oxidation index, fat oxidation index: CT=C W0 in C: energy intake=TEE, W0 in CT: TEE=energy intake Energy gap: NS in CT, NS in C <u>W4 *vs.* W0</u> RMR: C: →; CT: ↗ (W4: CT<C) RMR/FFM: C: →; CT: ↗ (W4: C<CT) TEE, RQ, carbohydrates oxidation index, fat oxidation index: C: →; CT: → (W4: CT=C) W4 in C: TEE=energy intake, W4 in CT: TEE<energy intake Energy gap: C: →; CT: ↗ (W4: significant energy gap in CT but NS in C) <u>W8 *vs.* W0</u> RMR: C: →; CT: → (W8: CT<C) RMR/FFM, TEE, RQ, carbohydrates oxidation index, fat oxidation index: C: →; CT: → (W8: CT=C) Energy gap: NR <u>Physical activity</u> <u>W0</u> PAL: NR	<u>W0</u> Fasting FT3, IGF-1, E2, triglycerides, β-hydroxybutyrate, glycerol, free FA: C=CT 24h mean cortisol, AAT: CT=C <u>W4 *vs.* W0</u> Fasting triglycerides free FA: C: ↗(trend); CT: ↗(trend) (W4: C=CT) Fasting β-hydroxybutyrate: C: →; CT: → (W4: C=CT) Fasting glycerol: C: →; CT: → (W4: CT=C) <u>W8 *vs.* W0</u> Fasting triglycerides, β-hydroxybutyrate, glycerol, free FA: C: →; CT: → (W8: C=CT)	<u>W0</u> Fasting leptin: CT<C Total ghrelin iAUC, acylated ghrelin iAUC, obestatin iAUC, total GLP-1 iAUC: CT=C PYY_{3-36} iAUC: C=CT <u>W4 *vs.* W0</u> Fasting total ghrelin, fasting acylated ghrelin: C: ↗, CT: → (W4: CT<C) Total ghrelin iAUC, acylated ghrelin iAUC: C: ↘; CT: ↗[£] (W4: C<CT) Obestatin iAUC: C: →; CT: → (W4: CT=C) PYY_{3-36} iAUC: C: ↘ (p=0.05); CT: →[£] (W4: C<CT) Total GLP-1 iAUC: C: ↘ (trend); CT: → (W4: C<CT p=0.05) <u>W8 *vs.* W0</u> Total ghrelin iAUC: C: →; CT: → (W8: CT=C) Acylated ghrelin iAUC, obestatin iAUC: C: →; CT: ↘ (W8: CT<C) PYY_{3-36} iAUC, total GLP-1 iAUC: C: →; CT: → (W8: C=CT) (Kinetic change analyses of each hormones at T0, 15, 30, 60, 90min after the standardized test meal for each visit are not reported here but are available in the original article)

			AEE: C=CT W4 *vs.* W0 PAL: NR AEE: C: →; CT: → (W4: CT=C) W8 *vs.* W0 PAL: NR AEE: C: →; CT: → (W8: CT=C)		
Galusca *et al.*, 2015 [11]	BMI: Missing significance symbol between CT and C, the result would therefore be: AN<CT<C %FM, FM: AN<CT<C FFM: NR	NR	NR	Fasting FT3, E2: AN<CT=C Fasting IGF-1: AN<C=CT 24h mean cortisol, 24h mean GH: CT=C<AN	24h mean leptin: AN<CT<C 24h mean plasma NPY: C=AN=CT 24h mean plasma α-MSH: AN<CT=C Circadian profile of plasma NPY: Difference at 9.00am: CT=C<AN In C: progressive decrease in the evening and night whereas in CT: peak at 4.00am (C=AN<CT) Circadian profile of plasma α-MSH: Broadly: AN≤CT≤C CT: in the morning: CT<C, peak at 2.00pm (C<CT) and 12.00am (AN=C<CT)
Germain *et al.*, 2016 [25]	BW, BMI: AN-R, AN-BP, CT, BN, AN-R rec, C<OB[£]; CT<BN=AN-R rec=C; AN-R=CT; AN-BP=CT[£] Height: CT=AN-R rec=AN-R=C=BN=AN-BP=OB %FM: AN-BP=AN-R<C=AN-R rec; p=0.05 for AN-R<CT; CT=BN=C=AN-R rec (given the complexity of 2-group comparisons with 7 groups, main results only were reported)	NR	NR	Fasting FT3: AN-BP<AN-R<AN-R rec=C=CT<OB; BN=CT<OB Fasting IGF-1: AN-R<AN-BP=AN-R rec=CT; AN-BP=OB<C; OB=BN=CT; C=CT Fasting E2: AN-R=AN-BP<CT=C; AN-R<AN-R rec; BN=AN-R rec=CT=C 24h mean cortisol: CT=C=AN-R rec=AN-BP<AN-R; CT=BN 24h mean level of plasma IL-7: AN-BP, OB, AN-R rec<AN-R<C; OB<CT; AN-R<BN, CT; C=BN, CT Fasting FSH: AN-R<AN-BP=AN-R rec=C=CT; AN-R=BN; BN=AN-BP=AN-R rec=C=CT Fasting LH: AN-R<AN-BP<CT=C; BN=CT; BN<C; CT<AN-R rec; C=AN-R rec Fasting testosterone: BN=AN-BP=AN-R rec=CT=AN-R=C Circadian profile of cortisol, broadly: CT≤BN, C, AN-R rec≤AN-BP≤AN-R Circadian rhythm conserved in all groups (CT, BN, C, AN-R-rec, AN-BP, AN-R) Circadian profile of plasma IL-7, broadly: AN-BP≤AN-R rec, OB≤BN, AN-R≤C≤CT No variation in all groups during meal schedules (AN-BP, AN-R rec, OB, BN, AN-R, C, CT)	24h mean leptin: AN-R<AN-BP, CT, AN-R rec, C<OB; AN-BP=CT=BN<OB; CT<AN-R rec=C Circadian profile of leptin, broadly: AN-R, AN-BP≤CT, BN≤AN-R rec, C Blunted pattern in AN-R Correlation between 24h mean leptin and 24h mean plasma IL-7: C: positive AN-R, AN-R rec, AN-BP, BN, CT, OB (given the complexity of 2-group comparisons with 7 groups, main results only were reported)

				Possibly biphasic in AN-R, CT, OB with an increase during the 2nd part of the nychthemeron compared to the morning (given the complexity of 2-group comparisons with 7 groups, main results only were reported)	
Gunes *et al.*, 2016 [41]	BMI: CT<C	NR	NR	NR	NR
Estour *et al.*, 2017 [3]	BMI: AN=CT<C CT: BMI remains around the 5th percentile throughout the growth period until at least the age of 18 AN: BMI remains around 50th percentile before starting weight loss %FM, FM: AN<CT<C FFM: CT=AN; AN=C and trend for CT<C Femoral BMD: AN=CT and trend for AN<C and CT<C£ Lumbar spine BMD: CT=C and trends for AN<CT, C (with p-value significance at 0.001)	NR	RMR: AN<C, CT<C and trend for AN<CT (with p-value significance at 0.001)	Fasting FT3, IGF-1, E2, LH: AN<C=CT Fasting FSH: AN=C=CT Fasting total testosterone, albumin, DHEAS: CT=AN=C 24h mean ACTH: C=CT and trend for C, CT<AN 24h mean prolactin: AN=CT=C Fasting FT4: CT=C and trends for AN<CT, C Fasting TSH: CT=C=AN 24h mean cortisol: CT=C<AN 24h mean GH: CT=C=AN <u>Bone markers</u> 24h mean osteocalcin: C=CT and trends for AN<C, CT£ 24h mean sCTX: C=CT and trends for C, CT<AN£ 24h mean vitamin D: CT=AN=C (with p-value significance at 0.001)	24h mean leptin: AN<CT<C (with p-value significance at 0.001)
Galusca *et al.*, 2018 [4]	BW, BMI, %FM, FFM, right leg FFM (site of the biopsy): CT<C Height, waist/hip ratio, proportion of fat mass at lower limb level: CT=C Total BMD, right leg (site of the biopsy) BMD: CT<C	TEI, %fat, %monounsaturated FA, % polyunsaturated FA, cholesterol daily intake, fibres daily intake, calcium daily intake, sodium daily intake: C=CT %proteins, %carbohydrates, saturated FA: CT=C	RMR/FFM, fasting fat oxidation index/FFM: CT=C RQ: C=CT <u>Physical activity</u> PAL, daily AEE, time per 24h at metabolic equivalent <1, time per 24h at metabolic equivalent 3-6, weekly inactivity time: CT=C Time per 24h at metabolic equivalent 1-3, time per 24h at metabolic equivalent >6: C=CT	Fasting FT3, IGF-1, E2, total cholesterol, HDL-cholesterol, LDL-cholesterol, ALP: C=CT 24h mean cortisol: CT=C Fasting blood glucose, insulin, insulin/blood glucose, triglycerides, QUICKI, HOMA, creatinine, AST, ALT, GGT: CT=C <u>Bone markers</u> Fasting vitamin D, calcium: CT=C Fasting PTH: C=CT	24h mean leptin: CT<C
Florent *et al.*, 2019 [42]	BW: AN=CT<C BMI: AN<CT<C	Energy intake at breakfast (calibrated breakfast): AN<C – trend (p=0.087) for AN<CT and trend (p=0.07) for CT<C	RMR (predicted for calibration breakfast with Harris and Benedict equation): AN<C – trend (p=0.089) for AN<CT and trend (p=0.07) for CT<C	NR	NR
Ling *et al.*, 2019 [43]	<u>Baseline</u> BMI: CT<C£ %FM, FFM: CT<C Waist-hip ratio: CT=C£ Many bone quality parameters: CT<C (observed in both sexes)	<u>Baseline</u> TEI (free living conditions) C=CT TEI (calorimetric chamber): CT=C£ Carbohydrates, fat: C=CT Proteins: CT=C Snacking energy intake, snacking	<u>Baseline</u> TEE (indirect calorimetry), TEE (calorimetric chamber), RMR (indirect calorimetry), RMR (calorimetric chamber): CT<C RMR corrected for FFM (calorimetric chamber), RMR corrected for FFM (indirect calorimetry): C=CT	<u>Baseline</u> Fasting FT3, TSH, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, glycerol, nonesterified fatty acids, metadrenaline, normetadrenaline: C=CT Fasting IGF-1: CT=C£	<u>Baseline</u> Fasting leptin: CT=C

	Overfeeding BW: C ↗; CT ↗ (no significant difference in weight gain between C and CT)	frequency: C<CT Overfeeding Compliance confirmed (urea excretion: C ↗; CT ↗)	Fat stools: CT=C RQ: C=CT Fat oxidation index: C<CT Carbohydrates oxidation index: C=CT[£] Physical activity Baseline PAL: CT<C	Fasting FT4, glucose: CT=C Fasting insulin, creatinine, urea, uric acid: CT<C	
Margaritelis *et al.*, 2019 [44]	BW, BMI, %FM: CT<C<OB[£] Height: C=OB=CT	%carbohydrates, vitamin E, selenium: C=CT=OB %proteins: C=CT=OB %fat: OB=CT=C Vitamin C: CT=OB=C	NR	*Plasma protein carbonyls* Pre, 72h: C<OB=CT Post, 24h: C=OB=CT 48h, 96h: C<CT=OB *Erythrocytes glutathione* Pre, 96h: OB=CT=C Post, 24h: CT=OB=C 48h, 72h: OB=C=CT *CK* Pre: CT=C=OB Post: NR 24h, 96h: C=CT=OB 48h: C=CT, C<OB, CT=OB 72h: C<CT=OB	NR
Marra *et al.*, 2019 [18]	BW, BMI: AN=CT<BD<C Height: AN=BD=C=CT %FM: BD<AN=CT<C FFM: AN=CT<C=BD Phase angle: AN<C<BD; AN=CT; C=CT<BD	NR	RMR: AN<BD<CT; BD=C; AN<C=CT RMR/FFM: AN=BD<CT; AN<C<CT RMR adjusted for FFM: AN<BD<C=CT RQ: AN=C=CT=BD	NR	NR
Riveros-McKay *et al.*, 2019 [45]	NR	NR	NR	NR	NR
Bailly *et al.*, 2020 [19]	Baseline BW, height, BMI, %FM, FFM, right leg FFM (site of the biopsy): CT<C Overfeeding BW, BMI: C ↗; CT ↗	NR	Physical activity PAL: CT<C	NR	NR
Ling *et al.*, 2020 [6]	Baseline All (females+males) FM, FFM: CT<C Females %FM, FM, FFM, BW, BMI: CT<C	Baseline All (females+males) TEI (free living conditions), mean carbohydrates intake (free living), nitrogen balance (calorimetric chamber):	Baseline All (females+males) TEE (indirect calorimetry), TEE (calorimetric chamber), RMR (indirect calorimetry): CT<C	Baseline Females Fasting FT3, triglycerides, glycerol: C=CT Fasting IGF-1: trend (p=0.089) for CT<C Fasting blood glucose, albumin, nonesterified fatty acids: CT=C	Baseline All (females+males) Fasting total ghrelin, fasting acylated ghrelin, fasting PYY, fasting GLP-1, test meal mean total ghrelin$_{0\text{-}150min}$, test meal mean acylated ghrelin$_{0\text{-}150min}$: C=CT

	Males %FM, FM, FFM, BW, BMI: CT<C Overfeeding All (females+males) FM: C ↗; CT ↗ (OF: CT<C) FFM: C ↗; CT trend (p=0.07) for ↗ (OF: CT<C) Females BW: C ↗; CT ↗ Males BW: C ↗; CT ↗	C=CT TEI (calorimetric chamber), mean protein intake (free living): CT=C Nitrogen intake (calorimetric chamber), nitrogen losses (calorimetric chamber): CT<C Mean fat intake (free living): trend (p=0.0997) for C<CT Snacking energy intake: C<CT Overfeeding All (females+males) TEI (free living conditions), mean fat intake (free living), mean carbohydrates intake (free living): C ↗; CT ↗ (OF: CT=C) TEI (calorimetric chamber), mean protein intake (free living), nitrogen intake (calorimetric chamber), nitrogen losses (calorimetric chamber): C ↗; CT ↗ (OF: CT<C) Nitrogen balance (calorimetric chamber): C ↗; CT ↗ (OF: C=CT)	Fat oxidation index: C=CT Carbohydrates oxidation index, energy gap: C<CT Energy gap: seems to be negative in C and positive in CT Females RMR, TEE (indirect calorimetry), TEE (calorimetric chamber): CT<C RMR/FFM: NS[£] Males RMR, TEE (calorimetric chamber): CT<C TEE (indirect calorimetry): trend (p=0.094) for CT<C[£] RMR/FFM: C<CT Overfeeding All (females+males) TEE (indirect calorimetry): C →; CT → (OF: CT<C) TEE (calorimetric chamber): C ↗; CT → (OF: CT<C) RMR (indirect calorimetry): C ↗; CT → (OF: CT<C) Fat oxidation index: C ↘; CT trend (p=0.079) for ↘ (OF: C=CT) Carbohydrates oxidation index: C ↗; CT → (OF: CT=C) (further results depending on gender available in the article) Energy gap OF: C<CT (further results depending on gender available in the article) Physical activity PAL: CT no excessive physical activity	HOMA: CT=C Fasting insulin: trend (p=0.06) for CT<C Males Fasting FT3, nonesterified fatty acid, glycerol: C=CT Fasting IGF-1, glucose, insulin, triglycerides, albumin: CT=C HOMA: CT=C	Test meal mean $PYY_{0\text{-}150min}$, test meal mean $GLP\text{-}1_{0\text{-}150min}$: C<CT Females Fasting leptin: CT<C Males Fasting leptin: CT<C Overfeeding All (females+males) Fasting total ghrelin, fasting acylated ghrelin, fasting GLP-1: C →; CT → (OF: C=CT) Fasting PYY: C ↗; CT → (OF: C=CT) Test meal mean total $ghrelin_{0\text{-}150min}$: C ↘; CT ↘ (OF: trend (p=0.09) for C<CT) Test meal mean acylated $ghrelin_{0\text{-}150min}$: C →; CT ↘ (OF: C=CT) Test meal mean $PYY_{0\text{-}150min}$: C →; CT → (OF: C<CT) – OF: trend for earlier secretory response of PYY to the test meal in CT Test meal mean $GLP\text{-}1_{0\text{-}150min}$: C ↘; CT → (OF: C<CT) (further results depending on gender available in the article)
Orthofer *et al.* 2020 [46]	NR	NR	NR	NR	NR

Even when there is no significance between the groups (reported as '='), the groups are listed in ascending order of values in the columns of results; ‡ Synthetized result from different results; £ Result not clearly reported

18F-FDG: 18-fluorodeoxyglucose; AAT: amino acid transferase; ACTH: adrenocorticotropic hormone; AEE: activity energy expenditure; ALP: alkaline phosphatase; ALT: alanine aminotransferase; α-MSH: α-melanocyte-stimulating hormone; AN: subjects with anorexia nervosa; AN-BP: subjects with anorexia nervosa of bingeing/purging type; AN-R: subjects with anorexia nervosa of restrictive type; AST: aspartate aminotransferase; AUC: area under the curve; BAT: brown adipose tissue; BD: underweight ballet dancers; BI: bioimpedance index; BIA: bioelectrical impedance analysis; BMC: bone mineral content; BMD: bone mineral density; BMI: body mass index; BN: subjects with bulimia nervosa; BW: bodyweight; C: normal-weight control participants; calo: calorimetry; CK: creatine kinase; CT: constitutionally thin subjects; DHEAS: dehydroepiandrosterone sulfate; DWL: doubly labeled water; DXA: dual-energy X-ray absorptiometry; E2: estradiol; EDNOS: subjects with eating disorders not otherwise specified; FA: fatty acids; FFM: fat-free mass; FM: fat mass; FSH: follicle-stimulating hormone; FT3: free triiodothyronine; FT4: free thyroxine; FTI: free testosterone index; GGT: gamma-glutamyl transpeptidase; GH: growth hormone; GHRH: growth hormone-releasing hormone; GLP-1: glucagon-like peptide-1; GnRH: gonadotropin-releasing hormone; HOMA: homeostasis model assessment; iAUC: incremental area under the curve; IBW: ideal bodyweight; IGF-1: insulin-like growth factor 1; IL-7: interleukin-7; LH: luteinizing hormone; MRI: magnetic resonance imaging; NPY: neuropeptide Y; NR: not reported; NS: not significant; OB: participants with obesity; OF: overfeeding; OPG: osteoprotegerin; PAL: physical activity level; PPT: postprandial thermogenesis; PTH: parathyroid hormone; PYY: peptide YY; QUICKI: quantitative insulin sensitivity check index; RANKL: receptor activator of nuclear factor-κB ligand; rec: after weight recovery or partial recovery; RMR: resting metabolic rate; RMRc: calculated resting metabolic rate; RMRe: estimated resting metabolic rate; RMRm: measured resting metabolic rate; RQ: respiratory quotient; SCL-90-R: symptom check list – 90-revised; sCTX: serum C-telopeptide cross-link of type 1 collagen; SHBG: sex hormone-binding globulin; SUVmax: maximum standardized uptake values; T3: triiodothyronine; T4: tetraiodothyronine; TEE: total energy expenditure; TEI: total energy intake; TRACP 5b: tartrate-resistant acid phosphatase type 5b; TSH: thyroid stimulating hormone; W0: week 0; W4: week 4; W8: week 8

Table 3: Additional results of the clinical trials selected in the systematic review

Reference	Additional results
Schneider *et al.*, 1979 [30]	Pregnancy
	Association CT and pregnancy: 0.32%.
	Premature deliveries: trend (p=0.08) for C<CT
	Low birth canal, toxaemia, child weight$_{37}$, child height, neurological condition: CT=C
	Instrumental extraction, child weight$_{38}$: C=CT
	Child weight$_{39/40/41}$: CT<C
	Correlation between mother weight gain and child weight: NS
van Binsbergen *et al.*, 1990 [31]	NA
Diaz *et al.*, 1992 [48]	NA
Scalfi *et al.*, 1992 [7]	Correlation between RMR and BW
	All subjects/C: positive
	AN/CT: no correlation
Hinney *et al.*, 1997 [47]	Genetics
	Allele frequencies (%)
	Short allele: CT=AN=OB
	Long allele: OB=AN=CT
	Genotype frequencies (%)
	Homozygotes short allele: CT=AN=OB
	Heterozygotes: CT=OB=AN
	Homozygotes long allele: OB=AN=CT
	Parental transmission of the long allele
	OB: NS
	AN: NS
Petretta *et al.*, 1997 [32]	General parameters
	Systolic blood pressure, diastolic blood pressure: AN<CT=C
	Heart rate variability (LF and HF)
	24h recordings: LF power: CT<C=AN, HF power: C=CT<AN
	Daytime recordings: LF power: AN=C=CT, HF power: CT=C<AN
	Night-time recordings: LF power: C=CT=AN, HF power: C=CT<AN
	Heart rate variability (24h Holter monitoring)
	Time domain measures
	Average NN interval, SDNN, SDNN index, rMSSD, pNN50 CT=C<AN
	SDANN index: C=CT<AN
	Frequency domain measure
	Ln total power, Ln ULF power, Ln VLF power, Ln LF power: CT<C=AN
	Ln HF power: CT=C<AN
	Heart rate variability (only daytime)
	Time domain measures
	Average NN interval, SDNN index, rMSSD, pNN50 CT=C<AN
	SDNN, SDANN index: CT<C<AN
	Frequency domain measure
	Ln total power, Ln VLF power, Ln HF power: CT=C<AN
	Ln LF power: CT=C=AN
Slof *et al.*, 2003 [36]	Psychological and health profile
	Dieting (lifetime), binge eating (lifetime), number of sick days, EDI body dissatisfaction, EDI drive for thinness, TFEQ disinhibition, TFEQ hunger: CT<C
	Dependence (personality): trend (p=0.07) for CT<C
	Age at menarche, TFEQ restraint, self-esteem (personality): C<CT
	Locus of control, mastery, optimism: trends for C<CT
	Fagerstrom tolerance questionnaire (cigarettes, smoking), personal standards (personality), general anxiety disorder, major depressive disorder, panic disorder broad, phobia: CT=C
	Ever married, number of pregnancy, education, parent education, caffeine (servings per day), doubts about actions, concern about mistakes, extraversion, neuroticism, alcohol abuse/dependence: C=CT
Tolle *et al.*, 2003 [22]	Correlations between ghrelin and BMI, FT3
	C, CT, AN (3 groups as one): negative
	C, CT, AN rec (3 groups as one): negative
	Correlation between ghrelin and leptin

	C, CT, AN (3 groups as one): negative C, CT, AN rec (3 groups as one): no correlation Correlations between ghrelin and cortisol, GH C, CT, AN (3 groups as one): no correlation C, CT, AN rec (3 groups as one): no correlation Correlation between AUC GH_{120min} and ghrelin C, CT, AN (3 groups as one): positive C, CT, AN rec (3 groups as one): no correlation
Bosy-Westphal *et al.*, 2004 [33]	General parameters Arterial pressure: CT=C<OB Correlations Between RMRc and RMRm: C, CT, AN (3 groups as one): positive Between RMRm and FFM: C, CT, AN (3 groups as one): positive Correlations between the following variables in pairs – lean body mass trunk, brain mass, heart mass, liver mass, spleen mass, kidneys mass, residual mass, muscle mass, BMC: C, CT, AN (3 groups as one): positive
Mazzeo *et al.*, 2004 [37]	Psychological and health profile CT not associated with education or with smoking CT associated with lower scores of: EDI body dissatisfaction, EDI drive for thinness, TFEQ restraint, TFEQ, disinhibition, TFEQ hunger, and dieting CT associated with more generalized anxiety disorder No association between CT and: bingeing, perfectionism, neuroticism, extraversion, dependency, novelty seeking, self-esteem, alcohol dependence, major depressive disorder, panic disorder, and phobias
Tagami *et al.*, 2004 [38]	NA
Miljic *et al.*, 2006 [26]	During infusion Sleepiness 4 and 5h CT<(AN+AN rec)
Bossu *et al.*, 2007 [5]	Psychological profile Typical psychological profile of food limitation, emotionality, and external excitement was evidence in AN but not in CT, CT wanted to gain weight Heredity/genetic component 2.5 thin people in CT family, for 0.5 for AN
Germain *et al.*, 2007 [8]	NA
Marra *et al.*, 2007 [20]	NA
Miljic *et al.*, 2007 [34]	NA
Galusca *et al.*, 2008 [9]	Bone evaluation Bone resistance parameters: CT<C; AN<C Distal radius and tibial CSA: CT<C; $AN_{recently\ diagnosed}$=C; C=$AN_{long\text{-}standing}$ Distal radius and tibial breaking strength: $AN_{long\text{-}standing}$<C; CT<C; $AN_{recently\ diagnosed}$=C Trabecular bone: lower density, lower trabecular number, larger trabecular separation in CT *vs.* C (in distal tibia but not in distal radius) Cortical bone: reduced thickness but normal density in CT *vs.* C (in distal tibia) Only AN with long history of disease display impaired trabecular and cortical bone density and microarchitecture Correlation between osteocalcin and sCTX CT, C: positive AN: no correlation
Fernández-García *et al.*, 2009 [35]	Correlation between BMD and BMI: positive for CT and AN BMD and FFM: positive for AN, no correlation for CT BMC and BMI: positive for AN and CT BMC and FFM: positive for AN and CT BMC and GH: positive for CT, no correlation for AN Multivariate analysis CT: BMI explains 57% of the variance in BMD and 72% of the variance in lumbar spine BMD
Germain *et al.*, 2009 [23]	NA
Marra *et al.*, 2009 [10]	NA
Hasegawa *et al.*, 2011 [27]	NA
Galusca *et al.*, 2012 [24]	NA
Santonicola *et al.*, 2012 [39]	Stomach sensations and discomforts Postprandial distress syndrome frequency: C: 18%, CT: 56%, BN: 83%, EDNOS: 90%, AN: 90% Vomiting: C: 6%, AN: 15%, EDNOS: 20%, CT: 22%, BN: 100% Only 1 BN met the epigastric pain syndrome criteria Postprandial distress syndrome distribution: C: 10%, CT:12%, BN: 12%, EDNOS: 22%, AN: 44%

	Intensity-frequency scores Postprandial fullness: OB, C, CT<EDNOS, AN, BN Early satiety: OB, C, BN<AN Nausea: OB, CT, C, AN<EDNOS, BN Epigastric pressure: C, OB, CT<EDNOS, BN CT: no nausea, no epigastric burning
Pasanisi *et al.*, 2013 [21]	NA
Paschalis *et al.*, 2013 [40]	Physical capacity VO_{2max}: C=OB=CT Muscle function and damage OB: suffered more from exercise-induced muscle damage than CT and C *Isometric peak torque* Post *vs.* pre: C ↘; CT ↘; OB ↘ (post: CT<C, C=OB) 24h *vs.* pre, 48h *vs.* pre: C ↘; CT ↘; OB ↘ (24h, 48h OB<C, CT=C) 72h *vs.* pre: C →; CT ↘; OB ↘ (72h: OB<C, CT=C) *DOMS walking* Post *vs.* pre: C ↗; CT ↗; OB ↗ (post: OB=C, C=CT) 24h *vs.* pre, 72h *vs.* pre: C ↗; CT ↗; OB ↗ (24h, 72h: C=CT, C=OB) 48h *vs.* pre: C ↗; CT ↗; OB ↗ (48h: C<OB, C=CT) (comparison tests between OB and CT probably not performed/reported for the above parameters) Proprioception *Position sense in absolute values of angle at 45°* Pre: C<OB, C<CT Post *vs.* pre: C →; CT →; OB ↗ (post: C<OB, C=CT) 24h *vs.* pre: C ↗; CT ↗; OB ↗ (24h: C<CT, C<OB) 48h *vs.* pre: C →; CT →; OB ↗ (48h: C<OB, C=CT) 72h *vs.* pre: C →; CT →; OB → (72h: C=CT, C=OB) *Force mismatch at 30%* Pre: OB=C, C=CT Post *vs.* pre: C ↘; CT ↘; OB ↘ (post: CT<C, OB<C) 24h *vs.* pre, 48h *vs.* pre: C ↘; CT ↘; OB ↘ (24h, 48h: OB<C, C=CT) 72h *vs.* pre: C →; CT →; OB ↘ (72h: OB<C, C=CT) *Knee joint reaction angle to release at 45°* Pre: OB<C, C=CT Post *vs.* pre, 24h *vs.* pre: C ↗; CT ↗; OB ↗ (post, 24h: OB=C, C=CT) 48h *vs.* pre: C →; CT ↗; OB ↗ (48h: C<OB, C<CT) 72h *vs.* pre: C →; CT →; OB → (72h: C<CT, C=OB) (comparison tests between OB and CT probably not performed/reported for the above parameters)
Germain *et al.*, 2014 [1]	Psychological profile W0 Restrained eating score (DEBQ), restrained eating score (EDE): CT<C CT: no eating-disorder-related traits such as perfectionism, drive to thinness, excessive bodyweight/shape concern compared with C Metabolomics analysis 2 clusters with opposite profiles: 1 cluster with C at W0, C at W8, CT at W4, 1 cluster with C at W4, CT at W0, CT at W8 – suggesting different/inverse metabolic pathways in response to the same dietary stress W0 *vs.* W4 Each metabolic phenotype switched to the other group (including metabolites from mitochondria) Discrimination at W4 for C and CT with a larger amplitude for CT W8 *vs.* W0 C: →; CT: →
Galusca *et al.*, 2015 [11]	NA
Germain *et al.*, 2016 [25]	Correlation between 24h mean plasma IL-7 and: %FM, BMI, 24h mean cortisol, age: No correlation for any groups (C, AN-R, AN-R rec, AN-BP, BN, CT, OB)
Gunes *et al.*, 2016 [41]	Eye corneal parameters Best-corrected visual acuity (Snellen), astigmatism (Diopter), spherical equivalent, axial length: CT=C Intraocular pressure, pachymetric measurements (central, apex, thinnest, corneal volume): CT<C Corneal power (front) of flat axis, steep axis, and mean: C=CT Correlations Positive correlations between intraocular pressure and: BMI, pachymetric measurements (central, apex, thinnest, corneal volume) Absence of correlation between intraocular pressure and: age, astigmatism, spherical equivalent, axial length, corneal power

	(front)of flat axis, steep axis, and mean Positive correlations between BMI and: pachymetric measurements (central, apex, thinnest) – p=0.05 for the positive correlation between BMI and corneal volume (pachymetric measurement) Absence of correlation between BMI and: age, astigmatism (Diopter), spherical equivalent, axial length, corneal power (front) of flat axis, steep axis, and mean
Estour *et al.*, 2017 [3]	<u>Psychological profile</u> <u>EDE questionnaire</u> Restraint: CT<C<AN, shape concern: C=CT=AN, weight concern: CT=C<AN, eating concern: C=CT<AN <u>EDI questionnaire</u> Drive for thinness: CT=C<AN, bulimia: CT=C=AN, body dissatisfaction, interpersonal distrust: C=CT=AN, ineffectiveness, perfectionism, interoceptive awareness, maturity fears: C<AN; C=CT; CT=AN <u>DEBQ questionnaire</u> Restrained eating: CT<C=AN, emotional eating: CT=C=AN, external eating: AN=C=CT <u>SCL-90 questionnaire</u> Global severity index: C<AN; C=CT; CT=AN (with p-value significance at 0.001)
Galusca *et al.*, 2018 [4]	<u>General parameters</u> Systolic blood pressure, diastolic blood pressure: CT<C Pulse: C=CT <u>Histological muscle fibres evaluation</u> Mean CSA, type I fibres CSA, type IIA fibres CSA, type IIX fibres CSA, %type I fibres, global C/F, type I fibres CC, type IIA fibres CC, type IIX fibres CC, type I fibres CC with SF, type IIA fibres CC with SF, type IIX fibres CC with SF, type I fibres LC/PF, type IIA fibres LC/PF, type IIX fibres LC/PF, type I fibres COx, type I fibres COx combined with %area occupied by type I fibres: CT<C Global CD, type IIA fibres COx, type IIX fibres COx, %area occupied by type I fibres: CT=C %type IIA fibres, %type IIX fibres: C=CT Mean CSA of every kind of fibre adjusted to right leg (site of the biopsy) FFM: no significant difference between CT and C <u>Enzymological muscle evaluation</u> PFK, CS: C=CT β-HAD, CK: CT=C <u>Muscle transcriptomics</u> 386 genes with significantly different level of expression between CT and C, in which 293 genes less expressed in CT than in C, such as genes related to mitochondria and respiratory chain CT: downregulation of transcripts involved in structural muscular proteins and downregulation of genes playing a role in triglyceride storage and metabolism (for instance FITM1 and FITM2)
Florent *et al.*, 2019 [42]	<u>Psychological profile</u> TFEQ: CT=C<AN <u>Neural regulation of appetite</u> Numbers of fibres passing by the arcuate nucleus of the hypothalamus: CT=AN<C Numbers of fibres passing by the lateral hypothalamic area: C=CT, C<AN, CT=AN In the hypothalamus: Fasting Glx/creatine: C<AN, C=CT, CT=AN 1h Postprandial Glx/creatine: AN<CT=C NAA/creatine: NS (order not precised) In the thalamus: Fasting + 1h Postprandial Glx/creatine: AN=CT=C NAA/creatine: NS (order not precised)
Ling *et al.*, 2019 [43]	<u>General parameters</u> <u>Baseline</u> Systolic and diastolic blood pressure: CT<C Fc: C=CT$^{£}$ Body core temperature: C<CT$^{£}$ <u>Adipose tissue evaluation</u> <u>Baseline</u> Adipocyte area: CT<C Mitochondrial DNA content in adipocytes: C<CT Leak (uncoupled) respiration: CT=C CI respiration: C=CT CI+CII respiration, CII respiration, maximal electron transport system capacity: C<CT <u>Overfeeding</u> Leak (uncoupled) respiration, CI respiration, CI+CII respiration: C: →; CT: →

	CII respiration, maximal electron transport system capacity: C: ↗; CT: ↗
	Muscle tissue evaluation
	Baseline
	Leak (uncoupled) respiration, CI respiration, CII respiration: CT=C
	CI+CII respiration, maximal electron transport system capacity: trend for CT<C
	Genetic analyses
	Baseline
	Adipose tissue: 88 genes differentially expressed between CT and C – some of them belong to mitochondria
	Pathway analyses of the entire data set revealed increased oxidative metabolism (upregulation of fatty acid oxidation but also of triglyceride biosynthesis)
	Downregulation of IL-8 signalling pathway, several antiangiogenic factors, several stress factors
	Muscle tissue: lack of robust transcriptomic alterations
	Overfeeding
	Differences upon the intervention but none demonstrated a differential response to the overfeeding between CT and C
Margaritelis *et al.*, 2019 [44]	Oxydative stress
	F2-isoprostanes
	Pre: C<OB=CT
	Post: C=OB=CT
	24h, 48h, 96h: C=CT=OB
	72h: CT=C=OB
	Muscle function
	DOMS
	Pre , 96h: CT=C=OB
	Post: C=OB=CT
	24h, 48h: C<CT=OB
	72h: C<OB, C=CT, CT=OB
	ROM
	Pre, 96h: OB=CT=C
	Post: CT=OB=C
	24h: OB=C=CT
	48h, 72h: OB=CT<C
	Isometric peak torque
	Pre: CT=C<OB
	Post: CT=C, CT<OB, C=OB
	24h, 48h, 72h: CT=OB=C
	96h: CT=C=OB
Marra *et al.*, 2019 [18]	NA
Riveros-McKay *et al.*, 2019 [45]	Genetic parameters
	Heritable trait: CT=OB
	Percentage of phenotypic variance (on the 97 loci associated with BMI): CT<OB
	Effect of increased or decreased BMI genetic risk score: CT<OB
	"74% of the STILTS cohort have a family history of persistent thinness throughout life, suggesting we have enriched for genetically driven thinness"
	Correlation between CT and severe childhood obesity: negative
	CT and severe childhood obesity share a number of genetic risk loci
	No correlation between AN and CT
	No correlation between AN and severe early onset obesity
Bailly *et al.*, 2020 [19]	Histological muscle fibres evaluation
	Baseline
	Overall IMTG, type I fibres IMTG, type IIA fibres IMTG, type I fibres glycogen, type IIA fibres glycogen, mean CSA, type I fibres CSA, type IIA fibres CSA, type IIX fibres CSA, %area occupied by type I fibres, global C/F, type I fibres CC, type IIA fibres CC, type IIX fibres CC, type I fibres CC with SF, type IIA fibres CC with SF, type IIX fibres CC with SF, type I fibres CFPE, type IIA fibres CFPE: CT<C
	Overall glycogen: trend (p=0.07) for CT<C
	%type IIX fibres: C<CT
	%area occupied by type IIX fibres: trend (p=0.051) for C<CT
	%type I fibres, %type IIA fibres, global CD, type IIX fibres CFPE: CT=C
	%area occupied by type IIA fibres, overall COx activity, type I COx activity, type IIA COx activity, type I fibres CAFA, type IIA fibres CAFA, type IIX fibres CAFA: C=CT
	Overfeeding
	Indexes of CSA, indexes glycogen content, indexes of COx activity: C: →; CT: →
	Almost all indexes of capillary supply: C: →; CT: →

	Overall IMTG, type I fibres IMTG, type IIA fibres IMTG: C: →; CT: ↗
	Enzymological muscle evaluation
	Baseline
	CS, myokinase: CT<C
	CK: trend (p=0.076) for CT<C
	β-HAD/CS ratio: C<CT
	CII: trend (p=0.078) for C<CT
	Enolase, β-HAD, CIV, LDH: CT=C
	Overfeeding
	Enolase, β-HAD, CS, LDH, myokinase, CK: C: →; CT: →
	CII, CIV: C: →; CT: trends (p=0.054 for CII and p=0.052 for CIV) for ↗
Ling *et al.*, 2020 [6]	Urinary metabolomics
	Baseline
	Specific metabolism in CT
	Orthogonal partial least squares discriminant analysis models on total urine content normalization: metabolic differences between CT and C
	Data normalized to creatinine: major/essential amino acids and central energy metabolism intermediates in 24h urine samples: C<CT
	Overfeeding
	Orthogonal partial least squares discriminant analysis models on total urine content normalization: differences in metabolome remained but were attenuated
	Urinary metabolic phenotypic differences not affected by the overfeeding
Orthofer *et al.* 2020 [46]	Genetic parameters
	ALK gene identified as a candidate thinness gene
	Association of the thinness phenotype with a variant in an uncharacterized long non-coding RNA AC013652.1
	Two variants located within genes rs79938778 within DEPTOR and rs568057364 within the first intron of ALK

Even when there is no significance between the groups (reported as '='), the groups are listed in ascending order of values in the columns of results

[£] Result not clearly reported

ALK: anaplastic lymphoma kinase; AN: subjects with anorexia nervosa; AUC: area under the curve; β-HAD: β-hydroxyacyl-CoA dehydrogenase; BMC: bone mineral content; BMD: bone mineral density; BMI: body mass index; BN: subjects with bulimia nervosa; BW: bodyweight; C: normal-weight control participants; CI: complex I of the mitochondrial respiratory chain; C/F: capillary to muscle fibre ratio; CAFA: capillary contact per fibre area; CC: capillary contacts per muscle fibre; CD: capillary density; CFPE: capillary to fibre-perimeter exchange; CII: complex II of the mitochondrial respiratory chain; CIV: complex IV of the mitochondrial respiratory chain; CK: creatine kinase; COx: cytochrome C oxidase; CS: citrate synthase; CSA: cross-sectional area; CT: constitutionally thin subjects; DEBQ: Dutch eating behaviour questionnaire [62]; DEPTOR: DEP domain-containing MTOR interacting protein; EDE: eating disorder examination [63]; EDI: eating disorder inventory [64]; EDNOS: subjects with eating disorders not otherwise specified; FFM: fat-free mass; FITM: fat storage-inducing transmembrane; FT3: free triiodothyronine; GH: growth hormone; Glx: glutamine/glutamate ratio; HF: high frequency; IMTG: intramuscular triglycerides; LC/PF: ratio between the length of contact of the capillaries with the muscle fibre to the perimeter of the muscle fibre; LDH: lactate dehydrogenase; LF: low frequency; Ln: logarithmic units; NA: not applicable; NAA: N-acetyl aspartate; NN: normal RR interval; NS: not significant; OB: participants with obesity; PFK: phosphofructokinase; pNN50: number of adjacent NN intervals differing by more than 50 ms in the entire recording divided by the total number of all NN intervals; rec: after weight recovery or partial recovery; RMR: resting metabolic rate; RMRc: calculated resting metabolic rate; rMSSD: root mean square successive difference; ROM: pain-free range motion; SCL-90-R: symptom check list – 90-revised; sCTX: serum C-telopeptide cross-link of type 1 collagen; SDANN index standard deviation of the average NN intervals for all 5-min segments; SDNN: standard deviation of all NN intervals; SDNN index: mean of the standard deviation of all NN intervals for all 5-min segments; SF: sharing factor; TFEQ: three-factor eating questionnaire [66], ULF: ultra-low frequency; VLF: very low frequency; W0: week 0; W4: week 4; W8: week 8

Table 4: Risks of bias analysis

Reference	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Inaccurate/inconsistent reporting (reporting bias)
Schneider *et al.*, 1979 [30]	Moderate risk	NR	Low risk	Moderate risk	Moderate risk	Moderate risk	Moderate risk
van Binsbergen *et al.*, 1990 [31]	Low risk	NR	High risk	Low risk	NR	Low risk	Moderate risk
Diaz *et al.*, 1992 [48]	Low risk	NR	High risk	High risk	High risk	Low risk	Low risk
Scalfi *et al.*, 1992 [7]	Low risk	NR	High risk	Moderate risk	NR	Low risk	Low risk
Hinney *et al.*, 1997 [47]	Low risk	NR	High risk	Low risk	Low risk	Low risk	Low risk
Petretta *et al.*, 1997 [32]	Low risk	NR	High risk	Low risk	NR	Low risk	Moderate risk
Slof *et al.*, 2003 [36]	Moderate risk	NR	High risk	High risk	Low risk	Low risk	Low risk
Tolle *et al.*, 2003 [22]	Moderate risk	NR	High risk	Low risk	Moderate risk	Low risk	Moderate risk
Bosy-Westphal *et al.*, 2004 [33]	Low risk	NR	High risk	Low risk	High risk	Low risk	High risk
Mazzeo *et al.*, 2004 [37]	Moderate risk	NR	High risk	High risk	Low risk	Low risk	Low risk
Tagami *et al.*, 2004 [38]	Low risk	NR	High risk	Low risk	Moderate risk	Low risk	Low risk
Miljic *et al.*, 2006 [26]	Moderate risk	NR	High risk	Low risk	Moderate risk	Low risk	Moderate risk
Bossu *et al.*, 2007 [5]	Low risk	NR	High risk	Moderate risk	NR	Low risk	Moderate risk
Germain *et al.*, 2007 [8]	Low risk	NR	High risk	Low risk	NR	Low risk	High risk
Marra *et al.*, 2007 [20]	Moderate risk	NR	High risk	Low risk	NR	Low risk	Low risk
Miljic *et al.*, 2007 [34]	Moderate risk	NR	High risk	Low risk	Moderate risk	Low risk	Moderate risk
Galusca *et al.*, 2008 [9]	Low risk	NR	High risk	Low risk	NR	Low risk	High risk
Fernández-García *et al.*, 2009 [35]	Low risk	NR	High risk	Low risk	Moderate risk	Low risk	Moderate risk
Germain *et al.*, 2009 [23]	Low risk	NR	High risk	Low risk	Moderate risk	Low risk	High risk
Marra *et al.*, 2009 [10]	Low risk	NR	High risk	Low risk	NR	Low risk	Low risk
Hasegawa *et al.*, 2011 [27]	Low risk	NR	High risk	Low risk	NR	Low risk	Low risk
Galusca *et al.*, 2012 [24]	Low risk	NR	High risk	Low risk	NR	Low risk	High risk
Santonicola *et al.*, 2012 [39]	Moderate risk	NR	High risk	High risk	NR	Low risk	Low risk
Pasanisi *et al.*, 2013 [21]	Moderate risk	NR	High risk	Low risk	Moderate risk	Low risk	High risk
Paschalis *et al.*, 2013 [40]	Moderate risk	NR	High risk	Moderate risk	NR	Low risk	Low risk
Germain *et al.*, 2014 [1]	Low risk	NR	High risk	Moderate risk	NR	Low risk	Moderate risk
Galusca *et al.*, 2015 [11]	Low risk	NR	High risk	Low risk	NR	Low risk	Low risk

(end of the table on the next page)

Reference	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Inaccurate/inconsistent reporting (reporting bias)
Germain *et al.*, 2016 [25]	Low risk	NR	High risk	Low risk	NR	Low risk	Low risk
Gunes *et al.*, 2016 [41]	Low risk	NR	High risk	Low risk	NR	Low risk	Low risk
Estour *et al.*, 2017 [3]	Low risk	NR	High risk	Low risk	NR	Low risk	Moderate risk
Galusca *et al.*, 2018 [4]	Low risk	NR	High risk	Low risk	NR	Low risk	High risk
Florent *et al.*, 2019 [42]	Low risk	NR	High risk	Moderate risk	Moderate risk	Low risk	Low risk
Ling *et al.*, 2019 [43]	Low risk	NR	High risk	Low risk	High risk	Low risk	Low risk
Margaritelis *et al.*, 2019 [44]	Low risk	NR	High risk	Moderate risk	NR	Low risk	Low risk
Marra *et al.*, 2019 [18]	Moderate risk	NR	High risk	Low risk	NR	Low risk	Low risk
Riveros-McKay *et al.*, 2019 [45]	Low risk	NR	High risk	Low risk	Low risk	Low risk	Low risk
Bailly *et al.*, 2020 [19]	Low risk	NR	High risk	Low risk	Moderate risk	Low risk	Low risk
Ling *et al.*, 2020 [6]	Low risk	NR	High risk	Moderate risk	Moderate risk	Low risk	Moderate risk
Orthofer *et al.* 2020 [46]	Low risk	NR	High risk	Low risk	Low risk	Low risk	Low risk

NR: not reported

Table 5: Results of the meta-analysis on characteristics of population and body composition in CT, AN, and C

	Mean [Min ; Max]			Comparison between groups					
	CT	AN	C		n	SMD [95% CI]	p-value	I^2 (%)	p-value
Age (yrs)	23.2 [22.3 ; 24.0]	21.8 [20.6 ; 23.1]	22.7 [21.8 ; 23.7]	CT *vs.* C	23	-0.16 [-0.33 ; 0.01]	0.069	23.3	0.15
				CT *vs.* AN	18	0.21 [-0.00 ; 0.42]	0.051	34.1	0.078
				AN *vs.* C	16	0.29 [0.01 ; 0.57]	0.043	60.9	0.001
BMI ($kg.m^{-2}$)	16.7 [16.4 ; 17.0]	15.1 [14.7 ; 15.5]	21.6 [21.2 ; 21.9]	CT *vs.* C	23	4.16 [3.53 ; 4.79]	<0.001	81.1	<0.001
				CT *vs.* AN	19	1.12 [0.75 ; 1.49]	<0.001	75.2	<0.001
				AN *vs.* C	17	3.82 [3.29 ; 4.35]	<0.001	70.6	<0.001
Weight (kg)	44.7 [43.0 ; 46.4]	39.7 [37.6 ; 41.8]	58.1 [56.3 ; 59.9]	CT *vs.* C	17	2.57 [2.26 ; 2.87]	<0.001	31.0	0.11
				CT *vs.* AN	12	0.94 [0.40 ; 1.47]	0.001	76.2	<0.001
				AN *vs.* C	10	3.10 [2.62 ; 3.58]	<0.001	40.4	0.089
Height (cm)	162.1 [160.8 ; 163.4]	161.5 [159.9 ; 163.1]	162.7 [161.5 ; 164.0]	CT *vs.* C	16	0.12 [-0.05 ; 0.29]	0.16	0.0	0.53
				CT *vs.* AN	9	0.07 [-0.16 ; 0.31]	0.54	0.0	0.48
				AN *vs.* C	9	0.20 [-0.02 ; 0.42]	0.073	0.0	0.81
FM (%)	19.5 [18.0 ; 21.1]	11.7 [9.7 ; 13.7]	27.1 [25.5 ; 28.8]	CT *vs.* C	18	1.82 [1.48 ; 2.17]	<0.001	60.8	<0.001
				CT *vs.* AN	14	1.59 [1.18 ; 2.01]	<0.001	72.3	<0.001
				AN *vs.* C	12	3.24 [2.58 ; 3.90]	<0.001	80.0	<0.001
FFM (kg)	34.2 [33.3 ; 35.1]	34.9 [34.2 ; 35.6]	39.7 [38.3 ; 41.0]	CT *vs.* C	7	1.60 [1.10 ; 2.10]	<0.001	66.0	0.007
				CT *vs.* AN	6	-0.24 [-0.54 ; 0.06]	0.12	18.5	0.29
				AN *vs.* C	4	1.14 [0.71 ; 1.57]	<0.001	43.4	0.15
Total BMD ($g.cm^{-2}$)	1.063 [1.028 ; 1.099]	1.076 [1.035 ; 1.117]	1.131 [1.108 ; 1.153]	CT *vs.* C	3	0.80 [0.25 ; 1.35]	0.005	42.2	0.18
				CT *vs.* AN	NA	NA	NA	NA	NA
				AN *vs.* C	NA	NA	NA	NA	NA

AN: subjects with anorexia nervosa; BMD: bone mineral density; BMI: body mass index; C: normal-weight control participants; CI: Confidence Interval; CT: constitutionally thin subjects; FFM: fat-free mass; FM: fat mass; SMD: standardized mean difference

Table 6: Results of the meta-analysis on energy intake and hormonal regulation of appetite in CT, AN and C

	Mean [Min ; Max]			Comparison between groups					
	CT	**AN**	**C**		**n**	**SMD [95% CI]**	**p-value**	**I^2 (%)**	**p-value**
TEI (kcal.day^{-1})	1931 [1837 ; 2025]	1145 [1023 ; 1267]	1832 [1733 ; 1932]	CT *vs.* C	5	-0.26 [-0.68 ; 0.16]	0.22	0.0	0.47
				CT *vs.* AN	2	2.20 [0.47 ; 3.93]	0.013	68.1	0.077
				AN *vs.* C	2	1.99 [1.10 ; 2.88]	<0.001	0.0	0.33
Carbohydrates (%)	55.9 [52.8 ; 59.1]	50.6 [45.3 ; 55.9]	55.7 [48.9 ; 62.4]	CT *vs.* C	4	0.09 [-0.58 ; 0.76]	0.79	51.4	0.10
				CT *vs.* AN	NA	NA	NA	NA	NA
				AN *vs.* C	NA	NA	NA	NA	NA
Fat (%)	28.0 [22.5 ; 33.6]	30.5 [24.0 ; 37.0]	27.3 [18.7 ; 35.9]	CT *vs.* C	4	-0.23 [-0.84 ; 0.39]	0.47	42.8	0.16
				CT *vs.* AN	NA	NA	NA	NA	NA
				AN *vs.* C	NA	NA	NA	NA	NA
Proteins (%)	15.9 [13.3 ; 18.4]	18.8 [14.5 ; 23.1]	16.4 [14.1 ; 18.7]	CT *vs.* C	4	0.04 [-0.42 ; 0.49]	0.88	0.0	0.55
				CT *vs.* AN	NA	NA	NA	NA	NA
				AN *vs.* C	NA	NA	NA	NA	NA
Fasting leptin (ng.mL^{-1})	5.8 [4.9 ; 6.7]	2.0 [1.6 ; 2.3]	10.6 [9.2 ; 12.0]	CT *vs.* C	9	1.14 [0.64 ; 1.65]	<0.001	62.6	0.006
				CT *vs.* AN	8	1.51 [1.21 ; 1.82]	<0.001	0.0	0.44
				AN *vs.* C	7	2.51 [1.61 ; 3.41]	<0.001	82.7	<0.001
24h mean leptin (ng.mL^{-1})	6.4 [5.9 ; 6.9]	2.0 [1.8 ; 2.2]	12.4 [10.1 ; 14.8]	CT *vs.* C	6	1.69 [0.90 ; 2.48]	<0.001	80.1	<0.001
				CT *vs.* AN	6	2.30 [1.35 ; 3.25]	<0.001	86.2	<0.001
				AN *vs.* C	6	3.97 [2.43 ; 5.52]	<0.001	90.8	<0.001
Fasting total ghrelin (pg.mL^{-1})	472 [272 ; 671]	1072 [621 ; 1522]	761 [436 ; 1086]	CT *vs.* C	5	-0.20 [-0.81 ; 0.41]	0.53	49.4	0.095
				CT *vs.* AN	4	-1.08 [-1.62 ; -0.53]	<0.001	19.4	0.29
				AN *vs.* C	3	-1.11 [-1.93 ; -0.29]	0.008	50.4	0.13
24h mean total ghrelin (pg.mL^{-1})	1876 [0 ; 5005]	2401 [0 ; 5810]	1739 [0 ; 4188]	CT *vs.* C	2	1.01 [-1.82 ; 3.83]	0.49	92.0	<0.001
				CT *vs.* AN	2	-1.50 [-3.83 ; 0.84]	0.21	90.5	0.001
				AN *vs.* C	2	-0.98 [-1.66 ; -0.30]	0.005	5.0	0.31
Fasting acylated ghrelin (pg.mL^{-1})	279 [97 ; 462]	1150 [601 ; 1699]	325 [95 ; 555]	CT *vs.* C	3	-0.23 [-0.74 ; 0.29]	0.39	0.0	0.60
				CT *vs.* AN	NA	NA	NA	NA	NA
				AN *vs.* C	NA	NA	NA	NA	NA

AN: subjects with anorexia nervosa; BMD: bone mineral density; BMI: body mass index; C: normal-weight control participants; CI: Confidence Interval; CT: constitutionally thin subjects; FFM: fat-free mass; FM: fat mass; SMD: standardized mean difference; TEI: total energy intake

Table 7: Results of the meta-analysis on energy expenditure in CT, AN and C

	Mean [Min ; Max]			Comparison between groups					
	CT	AN	C		n	SMD [95% CI]	p-value	I^2 (%)	p-value
TEE (kcal.day^{-1})	1782 [1574 ; 1990]	1912 [1501 ; 2323]	2018 [1940 ; 2095]	CT *vs.* C	3	1.13 [0.16 ; 2.10]	0.022	62.1	0.071
				CT *vs.* AN	NA	NA	NA	NA	NA
				AN *vs.* C	NA	NA	NA	NA	NA
RMR (kcal.day^{-1})	1104 [1062 ; 1147]	908 [854 ; 962]	1276 [1216 ; 1336]	CT *vs.* C	8	1.30 [0.81 ; 1.78]	<0.001	64.0	0.007
				CT *vs.* AN	6	1.35 [1.00 ; 1.71]	<0.001	12.9	0.33
				AN *vs.* C	5	2.05 [1.69 ; 2.41]	<0.001	0.0	0.80
RMR/FFM (kcal.day^{-1}.kg^{-1})	33.35 [30.09 ; 36.61]	27.19 [25.27 ; 29.12]	31.62 [29.07 ; 34.17]	CT *vs.* C	5	-0.47 [-1.01 ; 0.06]	0.083	51.5	0.083
				CT *vs.* AN	3	1.33 [0.79 ; 1.87]	<0.001	0.0	0.42
				AN *vs.* C	2	0.84 [0.27 ; 1.40]	0.004	0.0	0.61
RQ	0.85 [0.82 ; 0.87]	0.87 [0.83 ; 0.91]	0.83 [0.81 ; 0.85]	CT *vs.* C	5	-0.03 [-0.52 ; 0.46]	0.89	32.4	0.21
				CT *vs.* AN	4	-0.88 [-2.09 ; 0.33]	0.15	80.1	0.002
				AN *vs.* C	3	-1.13 [-2.73 ; 0.46]	0.16	84.6	0.002
AEE (kcal.day^{-1})	911 [458 ; 1365]	1002 [817 ; 1187]	840 [482 ; 1199]	CT *vs.* C	3	-0.10 [-0.71 ; 0.50]	0.74	13.2	0.32
				CT *vs.* AN	NA	NA	NA	NA	NA
				AN *vs.* C	NA	NA	NA	NA	NA
PAL	1.58 [1.36 ; 1.80]	2.14 [1.90 ; 2.38]	1.57 [1.41 ; 1.72]	CT *vs.* C	3	-0.31 [-1.55 ; 0.93]	0.62	81.0	0.005
				CT *vs.* AN	NA	NA	NA	NA	NA
				AN *vs.* C	NA	NA	NA	NA	NA

AEE: activity energy expenditure; AN: subjects with anorexia nervosa; C: normal-weight control participants; CI: Confidence Interval; CT: constitutionally thin subjects; FFM: fat-free mass; PAL: physical activity level; RMR: resting metabolic rate; RQ: respiratory quotient; SMD: standardized mean difference; TEE: total energy expenditure

Table 8: Results of the meta-analysis on biochemical parameters in CT, AN and C

	Mean [Min ; Max]			Comparison between groups					
	CT	**AN**	**C**		**n**	**SMD [95% CI]**	**p-value**	**I^2 (%)**	**p-value**
Fasting FT3 ($pmol.L^{-1}$)	4.16 [3.99 ; 4.34]	2.88 [2.69 ; 3.06]	4.12 [3.78 ; 4.47]	CT *vs.* C	12	-0.42 [-0.81 ; -0.03]	0.033	65.0	0.001
				CT *vs.* AN	10	2.90 [2.09 ; 3.70]	<0.001	85.0	<0.001
				AN *vs.* C	10	2.70 [1.92 ; 3.49]	<0.001	84.8	<0.001
Fasting Cortisol ($nmol.L^{-1}$)	371.0 [237.3 ; 504.8]	598.4 [406.3 ; 790.4]	427.3 [193.8 ; 660.8]	CT *vs.* C	3	0.66 [0.22 ; 1.10]	0.003	0.0	0.62
				CT *vs.* AN	4	-1.39 [-2.02 ; -0.75]	<0.001	45.0	0.14
				AN *vs.* C	3	-0.65 [-1.04 ; -0.25]	0.001	0.0	0.71
24h mean Cortisol ($nmol.L^{-1}$)	229.5 [219.4 ; 239.5]	398.5 [361.5 ; 435.4]	263.1 [251.9 ; 274.3]	CT *vs.* C	8	0.55 [0.16 ; 0.93]	0.005	47.2	0.066
				CT *vs.* AN	6	-2.56 [-3.85 ; -1.26]	<0.001	92.3	<0.001
				AN *vs.* C	6	-1.84 [-2.84 ; -0.84]	<0.001	87.8	<0.001
Fasting IGF-1 ($ug.L^{-1}$)	260.5 [243.2 ; 277.9]	148.7 [134.1 ; 163.4]	260.5 [244.1 ; 276.9]	CT *vs.* C	9	0.07 [-0.52 ; 0.65]	0.83	78.4	<0.001
				CT *vs.* AN	7	2.12 [1.13 ; 3.10]	<0.001	87.4	<0.001
				AN *vs.* C	7	2.40 [1.40 ; 3.39]	<0.001	85.4	<0.001
Fasting E2 ($ng.L^{-1}$)	62.3 [52.7 ; 72.0]	12.3 [10.6 ; 14.0]	54.8 [47.4 ; 62.3]	CT *vs.* C	10	-0.12 [-0.36 ; 0.11]	0.30	4.2	0.40
				CT *vs.* AN	8	2.02 [1.36 ; 2.69]	<0.001	78.2	<0.001
				AN *vs.* C	9	1.47 [1.00 ; 1.93]	<0.001	68.7	0.001
Fasting GH ($mIU.L^{-1}$)	4.10 [1.33 ; 6.88]	9.14 [6.96 ; 11.33]	10.01 [0.00 ; 22.78]	CT *vs.* C	2	0.19 [-0.50 ; 0.89]	0.59	33.0	0.22
				CT *vs.* AN	4	-0.59 [-1.09 ; -0.10]	0.019	45.4	0.14
				AN *vs.* C	2	-0.81 [-1.26 ; -0.37]	<0.001	0.0	0.37
24h mean GH ($mIU.L^{-1}$)	7.72 [5.66 ; 9.78]	9.06 [1.80 ; 16.32]	6.30 [4.06 ; 8.54]	CT *vs.* C	5	-0.08 [-0.36 ; 0.20]	0.58	0.0	0.46
				CT *vs.* AN	5	-0.93 [-1.72 ; -0.14]	0.021	83.0	<0.001
				AN *vs.* C	5	-1.02 [-1.93 ; -0.11]	0.027	85.5	<0.001
Fasting DHEAS ($ug.L^{-1}$)	1480 [994 ; 1965]	1791 [1454 ; 2127]	1776 [1184 ; 2367]	CT *vs.* C	3	0.28 [-0.01 ; 0.57]	0.059	0.0	0.84
				CT *vs.* AN	3	-0.28 [-0.60 ; 0.04]	0.086	13.0	0.32
				AN *vs.* C	3	0.02 [-0.50 ; 0.55]	0.93	65.0	0.057
Fasting FSH ($IU.L^{-1}$)	5.51 [3.63 ; 7.40]	2.84 [2.07 ; 3.60]	4.76 [3.11 ; 6.42]	CT *vs.* C	4	-0.16 [-0.46 ; 0.15]	0.32	0.0	0.71
				CT *vs.* AN	4	1.10 [0.32 ; 1.88]	0.006	75.2	0.007
				AN *vs.* C	4	0.63 [0.23 ; 1.04]	0.002	22.4	0.28
Fasting LH ($IU.L^{-1}$)	10.08 [8.15 ; 12.00]	1.31 [0.65 ; 1.97]	8.56 [6.83 ; 10.29]	CT *vs.* C	4	-0.15 [-0.45 ; 0.16]	0.35	0.0	0.54
				CT *vs.* AN	4	2.04 [1.04 ; 3.04]	<0.001	80.0	0.002
				AN *vs.* C	4	1.29 [0.72 ; 1.87]	<0.001	50.8	0.11

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	Mean [Min ; Max]			Comparison between groups					
	CT	AN	C		n	SMD [95% CI]	p-value	I^2 (%)	p-value
Fasting SHBG ($nmol.L^{-1}$)	70.5 [45.4 ; 95.5]	83.4 [43.9 ; 122.9]	87.5 [41.3 ; 133.7]	CT *vs.* C	4	0.24 [-0.15 ; 0.62]	0.23	0.0	0.56
				CT *vs.* AN	4	-0.29 [-0.68 ; 0.10]	0.15	20.2	0.29
				AN *vs.* C	4	-0.10 [-0.63 ; 0.43]	0.72	51.6	0.10
Fasting Testosterone ($nmol.L^{-1}$)	1.55 [1.26 ; 1.84]	1.54 [0.89 ; 2.19]	1.98 [1.39 ; 2.57]	CT *vs.* C	3	0.48 [0.16 ; 0.81]	0.004	0.0	0.87
				CT *vs.* AN	3	0.14 [-0.47 ; 0.76]	0.65	59.3	0.086
				AN *vs.* C	3	0.63 [0.14 ; 1.11]	0.011	35.6	0.21
Fasting FTI (ratio testosterone/SHBG)	0.600 [0.465 ; 0.735]	0.760 [0.629 ; 0.891]	0.741 [0.599 ; 0.883]	CT *vs.* C	2	0.30 [-0.17 ; 0.78]	0.21	0.0	0.41
				CT *vs.* AN	2	-0.27 [-0.70 ; 0.16]	0.21	0.0	0.39
				AN *vs.* C	2	0.00 [-0.42 ; 0.42]	1.00	0.0	1.00
Fasting Glucose ($mmol.L^{-1}$)	4.52 [4.28 ; 4.75]	3.96 [3.57 ; 4.35]	4.64 [4.40 ; 4.88]	CT *vs.* C	5	0.16 [-0.21 ; 0.52]	0.41	0.0	0.52
				CT *vs.* AN	3	1.11 [0.54 ; 1.67]	<0.001	0.0	0.94
				AN *vs.* C	2	1.18 [0.63 ; 1.73]	<0.001	0.0	0.55
Fasting Insulin ($mIU.L^{-1}$)	5.81 [4.88 ; 6.74]	8.30 [1.27 ; 15.33]	7.55 [6.54 ; 8.56]	CT *vs.* C	3	0.54 [0.00 ; 1.07]	0.050	0.0	0.58
				CT *vs.* AN	2	0.11 [-1.06 ; 1.29]	0.90	64.9	0.091
				AN *vs.* C	NA	NA	NA	NA	NA
Fasting HOMA ([fasting insulin × fasting glucose]/22.5)	1.21 [0.93 ; 1.50]	1.37 [0.63 ; 2.10]	1.49 [1.03 ; 1.94]	CT *vs.* C	3	0.26 [-0.27 ; 0.78]	0.34	0.0	0.51
				CT *vs.* AN	2	0.51 [-0.18 ; 1.20]	0.15	0.0	0.33
				AN *vs.* C	NA	NA	NA	NA	NA
Fasting Triglyceride ($mg.dL^{-1}$)	69.1 [56.4 ; 81.7]	NA	66.3 [59.9 ; 72.7]	CT *vs.* C	4	-0.07 [-0.46 ; 0.33]	0.73	0.0	0.76
				CT *vs.* AN	NA	NA	NA	NA	NA
				AN *vs.* C	NA	NA	NA	NA	NA
Fasting FT4 ($pmol.L^{-1}$)	16.76 [15.75 ; 17.77]	14.79 [13.23 ; 16.34]	17.57 [15.32 ; 19.82]	CT *vs.* C	2	0.21 [-0.41 ; 0.83]	0.50	46.3	0.17
				CT *vs.* AN	2	0.88 [0.50 ; 1.26]	<0.001	0.0	0.70
				AN *vs.* C	2	1.04 [0.55 ; 1.53]	<0.001	19.7	0.27
Fasting Vitamin D ($ug.L^{-1}$)	21.35 [18.34 ; 24.37]	23.62 [20.74 ; 26.51]	26.29 [23.76 ; 28.83]	CT *vs.* C	3	0.36 [0.07 ; 0.66]	0.015	0.0	0.75
				CT *vs.* AN	2	-0.12 [-0.44 ; 0.19]	0.44	0.0	0.58
				AN *vs.* C	2	0.24 [-0.07 ; 0.56]	0.12	0.0	0.86

AN: subjects with anorexia nervosa; C: normal-weight control participants; CT: constitutionally thin subjects; DHEAS: dehydroepiandrosterone sulfate; E2: estradiol; FSH: follicle-stimulating hormone; FT3: free triiodothyronine; FT4: free thyroxine; FTI: free testosterone index; GH: growth hormone; HOMA: homeostasis model assessment; IGF-1: insulin-like growth factor 1; LH: luteinizing hormone; NA: not applicable (calculation unavailable due to lack of data); SHBG: sex hormone-binding globulin; SMD: standardized mean difference

Table 9: Summarize of all the results of from systematic and meta-analysis approaches

	Meta-analysis results	CT *vs.* C			CT *vs.* AN		
		CT < C	**C < CT**	**CT = C**	**CT < AN**	**AN < CT**	**CT = AN**
BMI	AN<CT<C	**29** [1, 3–11, 18–20, 22–25, 27, 32, 33, 35–38, 40–44]				**6** [7, 11, 24, 26, 34, 42]	**13** [3, 5, 8–10, 18, 20–23, 25, 32, 35]
Weight	AN<CT<C	**17** [1, 4–7, 18–20, 22, 25, 27, 30, 32, 33, 35, 42, 44]				**3** [7, 26, 34]	**9** [5, 18, 20–22, 25, 32, 35, 42]
Height	CT=C ; AN=CT ; trend for AN<C	**2** [19, 33]		**15** [1, 4, 7, 9, 18, 20, 21, 23, 25, 27, 30–32, 35, 44]			**10** [7, 9, 18, 20, 21, 23, 25, 31, 32, 35]
% FM	AN<CT<C	**19** [1, 3–6, 8–11, 18–20, 23, 24, 27, 38, 40, 43, 44]		**3** [22, 25, 33]		**10** [3, 5, 8, 9, 11, 20, 22–24, 35] + **1** [25] trend	**3** [10, 18, 21]
FFM	CT=AN<C	**9** [4–6, 10, 18, 19, 27, 33, 43] + **1** [3] trend		**1** [20]			**7** [3, 5, 10, 18, 20, 21, 35]
BMC	NA	**1** [33]					**1** [35]
Total BMD	CT<C	**3** [4, 27, 35]					**1** [35]
TEI	AN<C=CT			**7** [1, 4–6, 8, 33, 43]		**2** [5, 8]	
% Carbohydrate	C=CT		**1** [33]	**6** [1, 4–6, 43, 44]			**1** [5]
% Fat	C=CT	**1** [33]	**1** [6] trend	**5** [1, 4, 5, 43, 44]			**1** [5]
% Protein	CT=C			**7** [1, 4–6, 33, 43, 44]	**1** [5]		
Snacking	NA		**3** [1, 6, 43]				
Fasting leptin	AN<CT<C	**4** [1, 6, 9, 35]		**3** [5, 38, 43]		**6** [5, 9, 26, 34, 35, 38]	

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	Meta-analysis results	CT *vs.* C			CT *vs.* AN		
		CT < C	**C < CT**	**CT = C**	**CT < AN**	**AN < CT**	**CT = AN**
24h mean leptin	AN<CT<C	**6** [3, 4, 11, 23–25]		**1** [8]		**6** [3, 8, 11, 23–25]	
Fasting total ghrelin	CT=C<AN			**2** [6, 22]	**3** [22, 26, 34]		
24h mean total ghrelin	C<AN ; C=CT ; CT=AN	**1** [8]		**1** [23]	**2** [8, 23]		
Fasting acylated ghrelin	CT=C			**1** [6]			
TEE	CT<C	**2** [6, 43]		**2** [1, 5]			**1** [5]
RMR	AN<CT<C	**7** [1, 3, 5, 6, 27, 33, 43]		**4** [7, 18, 20, 32]		**5** [5, 18, 20, 21, 32] + **1** [3] trend	**1** [7]
RMR/FFM ratio	AN<C ; AN<CT ; trend for C<CT		**2** [5, 18] + **1** [6] only males	**5** [1, 4, 20, 27, 43]		**4** [5, 18, 20, 21]	
RQ	C=CT=AN			**7** [1, 4, 5, 7, 18, 20, 43]			**4** [7, 18, 20, 21]
AEE	C=CT			**3** [1, 4, 5]	**1** trend [5]		
PAL	C=CT	**2** [19, 43]		**2** [4, 5]	**1** trend [5]		
Fasting FT3	AN<C<CT			**14** [1, 3–6, 8, 9, 11, 22–25, 32, 43]		**10** [3, 5, 8, 9, 11, 22–25, 32]	
Fasting cortisol	CT<C<AN			**1** [9]	**3** [9, 26, 34]		
24h mean cortisol	CT<C<AN			**8** [1, 3, 4, 8, 11, 23–25]	**7** [3, 8, 11, 22–25]		
Fasting IGF-1	AN<C=CT	**1** [6] trend for females		**12** [1, 3–5, 8, 9, 11, 22–25, 43] + **1** [6] only males		**8** [3, 8, 9, 11, 22–25]	**1** [5]
Fasting E2	AN<C=CT		**1** [22]	**10** [1, 3, 4, 8, 9, 11, 23–25, 27]		**8** [3, 8, 9, 11, 22–25]	

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	Meta-analysis results	CT *vs.* C			CT *vs.* AN		
		CT < C	**C < CT**	**CT = C**	**CT < AN**	**AN < CT**	**CT = AN**
Fasting GH	CT=C ; CT<AN ; AN<C			**1** [9]	**3** [9, 26, 34]		**1** [35]
24h mean GH	C=CT<AN			**6** [3, 8, 11, 22–24]	**4** [8, 11, 23, 24]		**1** [3]
Fasting DHEAS	C=AN ; trend for CT<C and CT<AN			**3** [3, 9, 31]	**1** [9]		**2** [3, 31]
Fasting FSH	AN<C=CT			**3** [3, 8, 25]		**2** [8, 25]	**1** [3]
Fasting LH	AN<C=CT			**3** [3, 8, 25]		**3** [3, 8, 25]	
Fasting SHBG	CT=AN=C			**4** [8, 9, 23, 31]	**1** [23] + **1** [31] trend		**2** [8, 9]
Fasting testosterone	AN=CT<C			**2** [3, 25]			**2** [3, 25]
Fasting FTI	CT=C=AN			**2** [8, 9]			**2** [8, 9]
Fasting glucose	AN<CT=C			**6** [4, 6, 27, 32, 38, 43]		**1** [32]	**2** [26, 34]
Fasting insulin	CT<C ; CT=AN	**1** [43] + **1** [6] trend for females		**2** [4, 38] + **1** [6] only males			**2** [26, 34]
Fasting HOMA	C=CT ; AN=CT			**3** [4, 6, 38]			**1** [34]
Fasting triglycerides	C=CT			**5** [1, 4, 6, 27, 43]			
Fasting FT4	AN<CT=C			**3** [3, 32, 43]		**1** [32] + **1** [3] trend	
Fasting Vitamin D	CT=AN ; AN=C ; CT<C			**2** [4, 9]			**1** [9]

AEE: activity energy expenditure; AN: subjects with anorexia nervosa; C: normal-weight control participants; CT: constitutionally thin subjects; BMC: bone mineral content; BMD: bone mineral density; BMI: body mass index; DHEAS: dehydroepiandrosterone sulfate; E2: estradiol; FFM: fat-free mass; FM: fat mass; FSH: follicle-stimulating hormone; FT3: free triiodothyronine; FT4: free thyroxine; FTI: free testosterone index; GH: growth hormone; HOMA: homeostasis model assessment; IGF-1: insulin-like growth factor 1; LH: luteinizing hormone; NA: not applicable; PAL: physical activity level; RMR: resting metabolic rate; RQ: respiratory quotient; SHBG: sex hormone-binding globulin; TEE: total energy expenditure; TEI: total energy intake

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AXE 2 : Phénotypage du tissu musculaire de personnes présentant une maigreur constitutionnelle

ÉTUDE 4 : DÉVELOPPEMENT ET VALIDATION DE DEUX NOUVELLES MÉTHODES D'ANALYSE EN HISTOLOGIE MUSCULAIRE

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Two new reliable immunohistochemical methods for simultaneous identification of capillaries, the three types of fibers and basal lamina in human skeletal muscle

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Keywords: Capillary supply, Immunohistochemistry, Combined staining method, Muscle fiber type, CD31

Abstract

Capillary network of skeletal muscle has a crucial role in oxygen supply and is strongly associated with the phenotype and metabolic profile of muscle fibers. Abundant literature has explored capillarization of skeletal muscle in different populations and in response to different interventions. Capillary and fiber type identification techniques have considerably evolved over the last decades, but to the best of our knowledge, no validated immunohistochemical method has yet been developed to simultaneously identify capillaries (using CD31), the three different muscle fiber types and basal lamina. Nine human muscle biopsies of *vastus lateralis* were stained using 5 different methods to test: the reliability of different CD31 antibodies for capillary identification, the reliability between single section or serial sections methods, and the intra-experimenter reproducibility in visual detection of capillaries. High reliability for the different antibodies directed against capillaries was observed for capillary contacts (CC) measurements (intra-class correlations (ICC) [$ICC_{95\%}$] of 0.89 [0.72;0.96] for type I fibers, 0.93 [0.81;0.97] for type IIA fibers, 0.88 [0.71;0.96] for type IIX fibers, 0.95 [0.86;0.98] for all fiber types) as well as a high level of similarity between single and serial sections methods. A strong similarity in capillary analysis between the different methods was obtained for each sample measurements. Analysis of Lin's concordance correlation coefficients and Bland and Altman's graphics showed a strong intra-experimenter reproducibility. This article proposes two time- and tissue-sparing immunohistochemical methods to accurately assess a complete fiber typing (type I, IIA, and IIX) along with muscle capillarization on a single muscle section.

Introduction

Human muscle is highly heterogeneous and composed of different types of fibers: slow oxidative fibers (type I fibers), fast oxidative glycolytic fibers (type IIA fibers) and fast glycolytic fibers (type IIX fibers) (Schiaffino and Reggiani 2011). This classification of muscle fiber types relied on different methods. Since the '70s, it has been assessed using the myofibrillar adenosinetriphosphatase (mATPase) method (Brooke and Kaiser 1970; Johnson et al. 1973; Essén et al. 1975; Denis et al. 1986; Proctor et al. 1995; Gavin et al. 2015), based on mATPase sensitivity to pH. In the '90s, the development of the first monoclonal antibodies directed against myosin heavy chains (MHC) gradually replaced mATPase staining (Schiaffino et al. 1989; Gorza 1990; Kadi et al. 1998). In 2012, Bloemberg & Quadrilatero performed a fine muscle fiber typing (type I, IIA, and IIX fibers) on a single muscle section (Bloemberg and Quadrilatero 2012). Furthermore, capillary supply is also a major determinant of muscle phenotype, and techniques of capillaries identification have also progressed a lot. First techniques to identify capillaries with the combination of amylase and periodic acid schiff staining (Andersen 1975) have been progressively superseded by the use of the *Ulex europaeus* agglutinin 1 (UEA 1) lectin, anti-collagen type IV, anti-von Willebrand factor (Parsons et al. 1993; Madsen and Holmskov 1995; Qu et al. 1997), and then by the CD31 antibody (Charles et al. 2006; Merlet et al. 2019) which recognizes the platelet endothelial cell adhesion molecule-1 (PECAM-1) – a transmembrane glycoprotein expressed by vascular endothelial cells. Capillary supply is commonly assessed through global indexes independent of the fiber type (such as the capillary density (CD) or the capillary to fiber ratio (C/F)) but is also frequently assessed through local indexes in a fiber-type specific manner (such as capillary contacts per fiber (CC), CC per fiber cross-sectional area (CAFA) or capillary to fiber perimeter exchange index (CFPE)) (Hepple et al. 1997; Hepple 1997; Harris 2005; Charles et al. 2006). The capillary network can even be further analyzed using morphometric indexes such as the capillary tortuosity index (CapTor) (Vincent et al. 2010;

Ravelojaona et al. 2015) or the ratio between the length of contact with the muscle fiber (LC) to the perimeter of muscle fiber (LC/PF) (Charifi et al. 2004; Merlet et al. 2019). To date, analyses of local capillary indexes require serial cross-sectioning, which present several limitations. First, serial cross-sectioning is tissue-consuming while human tissues are difficult to obtain and most of the time collected in reduced quantity. Serial cross-sectioning analyses are also time-consuming as the same fibers must be clearly identified among hundreds of others over several cross-sections. This also presents risks of bias due to the changes of fiber types and capillaries along muscle fibers. From these limitations, the need for simultaneous identification of fiber types and capillaries had already been raised more than thirty years ago (Rosenblatt et al. 1987; Paljärvi and Naukkarinen 1990; Eržen and Maravić 1993), even suggesting that immunohistochemical techniques could provide further possibilities. In more recent publications, new immunofluorescence techniques succeeded to stain capillaries, type I fibers, type II fibers, and laminin (a protein component of basal lamina) on a single muscle section (Snijders et al. 2016, 2017; Nederveen et al. 2016, 2018; Tan et al. 2018; Moro et al. 2019). However, these techniques did not distinguish type IIA from type IIX muscle fibers. Type IIX fibers present a specific phenotype – for review see (Schiaffino and Reggiani 2011). For instance, they present a lower capillarization than type IIA fibers (Andersen 1975; Larsson et al. 1999; Campos et al. 2002; Gavin et al. 2015), and in response to training their proportion decreases, contrary to the proportion of type IIA fibers, which sometimes increases (Gavin et al. 2015; Kosek et al. 2006; Mohr et al. 1997). Therefore, in training studies, it is of high interest to separately analyze type IIA from type IIX fibers. To the best of our knowledge, only one publication reported the staining of the three muscle fiber types and basal lamina using immunohistochemical techniques along with the staining of capillaries in humans (Al-Shammari et al. 2019) – but using UEA 1 instead of CD31 antibody, which is known to be one of the most specific and sensitive markers of endothelial cells (Duscha et al. 1999). Thus, we aimed to propose accurate and reliable

immunohistochemical methods identifying basal lamina, capillaries with CD31 antibody, as well as type I, IIA and IIX muscle fibers, on a single muscle section.

Materials and methods

The present study used muscle samples from a previous protocol (Galusca et al. 2018) that was conducted in accordance with the Helsinki Declaration and approved by the local research and ethics committee of Saint-Étienne – France. All subjects gave written informed consent prior to inclusion in the study and the protocol was registered at ClinicalTrials.gov as NCT01224561.

Muscle biopsies

Biopsies from the *vastus lateralis* were performed under local anesthesia by a specialized surgeon in nine participants. A biopsy of approximately 140 mg of muscle was collected at one-third of the distance from the upper margin of patella to the anterior superior iliac spine with Weil-Blakesley forceps. A part of the sample containing well-identified fascicles was well oriented, included in an embedding medium (Cryomount, Histolab, Göteborg, Sweden), frozen in liquid nitrogen-precooled isopentane, and stored either in an ultra-low temperature freezer or in a liquid nitrogen tank. Serial 10-µm thick transverse sections were cut at -18°C using a cryostat (CM1950, Leica biosystems, Wetzlar, Germany), mounted on five serial glass slides, air-dried at room temperature, and stored at -20°C.

Immunohistochemical stains

As displayed in Tab.1, five slides were stained with 5 different cocktails of antibodies, respectively referred to as method 1 (M1), method 2 (M2), method 3 (M3), method 4 (M4), and method 5 (M5). M1

and M3 aimed to identify on a single muscle section: capillaries, complete fiber type distribution, and laminin. M1 consisted of a two-step procedure using a mouse monoclonal CD31 antibody (clone JC70A, IgG1 isotype, Dako, Agilent Technologies, Santa Clara, USA) whereas M3 consisted of a single-step procedure using a rabbit polyclonal CD31 antibody (Ab28364, IgG isotype, Abcam, Cambridge, UK) (see Tab.2). The two-step M1 procedure consisted of CD31 application in a first step, followed by the application of a cocktail of the other antibodies in a second separate step (Tab.2). The single-step M3 procedure consisted of the application of CD31 in the same cocktail as the other antibodies. M2 and M4 aimed to identify capillaries and laminin (without fiber type staining), both using a single-step procedure. M2 used the mouse monoclonal CD31 antibody (Clone JC70A, Dako) whereas M4 used the rabbit polyclonal CD31 antibody (Ab28364, Abcam) for identification of capillaries. M5 only aimed to identify fiber type distribution, using a single-step procedure adapted from Bloemberg and Quadrilatero's protocols (Bloemberg and Quadrilatero 2012). All the staining procedures were verified using negative controls to ensure appropriate staining specificity. For all details about staining procedures of the different methods, see Tab.1 and Tab.2.

Tab.1: Information on primary and secondary antibodies used in the different methods

Design		Primary antibody								Secondary antibody				
Protocol	**Method**	**Identification**	**Antibody**	**Reference**	**Species**	**Clonality**	**Isotype**	**Dilution**	**Source**	**Reference**	**Species**	**Isotype**	**Source**	**Dilution**
Two-step	M1	Capillaries	Anti-CD31	Anti-CD31 – JC70A	Mouse	Mono	IgG1	1:40	Dako	546 – A-21123	GaM	IgG1	ThermoFisher Alexa Fluor®	1:300
		Type I fibers	Anti-MHCI	BA-F8 – AB_10572253	Mouse	Mono	IgG2b	1:100	DSHB	350 – A-21140	GaM	IgG2b		
		Type IIA fibers	Anti-MHC (all but IIX)	BF-35 – AB_2274680	Mouse	Mono	IgG1	1:100	DSHB	488 – A-21121	GaM	IgG1		
		Laminin	Anti-laminin	2E8 – AB_2134060	Mouse	Mono	IgG2a	1:100	DSHB	633 – A-21136	GaM	IgG2a		
Single-step	M2	Capillaries	Anti-CD31	Anti-CD31 – JC70A	Mouse	Mono	IgG1	1:40	Dako	546 – A-21123	GaM	IgG1	ThermoFisher Alexa Fluor®	1:300
		Laminin	Anti-laminin	2E8 – AB_2134060	Mouse	Mono	IgG2a	1:100	DSHB	633 – A-21136	GaM	IgG2a		
Single-step	M3	Capillaries	Anti-CD31	Anti-CD31 – Ab28364	Rabbit	Poly	IgG	1:30	Abcam	546 – A11035	GaR	IgG	ThermoFisher Alexa Fluor®	1:300
		Type I fibers	Anti-MHCI	BA-F8 – AB_10572253	Mouse	Mono	IgG2b	1:100	DSHB	350 – A-21140	GaM	IgG2b		
		Type IIA fibers	Anti-MHC (all but IIX)	BF-35 – AB_2274680	Mouse	Mono	IgG1	1:100	DSHB	488 – A-21121	GaM	IgG1		
		Laminin	Anti-laminin	2E8 – AB_2134060	Mouse	Mono	IgG2a	1:100	DSHB	633 – A-21136	GaM	IgG2a		
Single-step	M4	Capillaries	Anti-CD31	Anti-CD31 – Ab28364	Rabbit	Poly	IgG	1:30	Abcam	546 – A11035	GaR	IgG	ThermoFisher Alexa Fluor®	1:300
		Laminin	Anti-laminin	2E8 – AB_2134060	Mouse	Mono	IgG2a	1:100	DSHB	633 – A-21136	GaM	IgG2a		
Single-step	M5	Type I fibers	Anti-MHCI	BA-F8 – AB_10572253	Mouse	Mono	IgG2b	1:100	DSHB	350 – A-21140	GaM	IgG2b	ThermoFisher Alexa Fluor®	1:300
		Type IIA fibers	Anti-MHC (all but IIX)	BF-35 – AB_2274680	Mouse	Mono	IgG1	1:100	DSHB	488 – A-21121	GaM	IgG1		

DSHB: Developmental studies hybridoma bank, GaM: Goat anti-Mouse, GaR: Goat anti-Rabbit, MHC: Myosin heavy chain

Tab.2: Protocols of the single- and two-step procedures

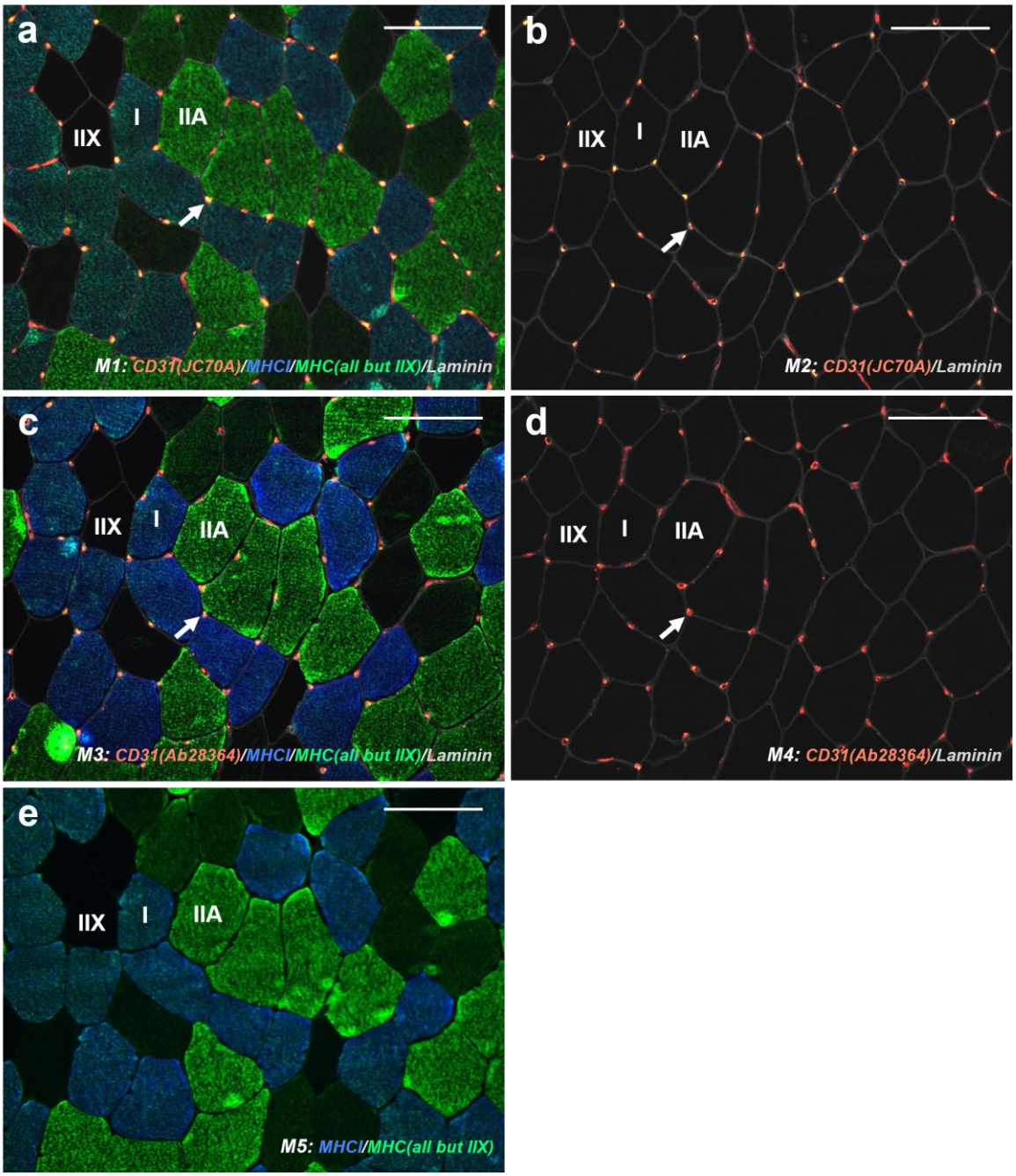
M1: two-step procedure (CD31 application followed by the cocktail of the other antibodies in a second step)	**Time**	**M2, M3, M4, and M5: single-step procedure (CD31 directly applied in the cocktail of the other antibodies)**	**Time**
Air dry sections at room temperature	40 min	Air dry sections at room temperature	40 min
Fixation in acetone bath	15 min	Fixation in acetone bath	15 min
Sections encircling with hydrophobic pen	10 min	Sections encircling with hydrophobic pen	10 min
PBS wash	3×5 min	PBS wash	3×5 min
Blocking with PBS and GS 10% + BSA 10%	20 min	Blocking with PBS and GS 10% + BSA 10%	20 min
Apply primary antibody: CD31 JC70A	60 min	Apply first antibody cocktail: BA-F8 and/or BF-35 and/or 2E8 and/or CD31 Ab28364 and/or CD31 JC70A (See Tab.1)	60 min
PBS wash	3×5 min	PBS wash	4×5 min
Blocking with PBS and GS 10% + BSA 10%	20min	Blocking with PBS and GS 10%+ BSA 10%	30 min
Apply appropriate secondary antibody: 546 IgG1	60 min (dark)	Apply appropriate secondary antibody cocktail: 350 IgG2b and/or 488 IgG1 and/or 546 IgG1 and/or 546 IgG and/or 633 IgG2a (See Tab.1)	60 min (dark)
PBS wash	4×5 min	PBS wash	3×5 min
Blocking with PBS and GS 10% + BSA 10%	30 min	Mounting with ProLong™ Gold Antifade	
Apply primary antibody cocktail: BA-F8 + BF-35 + 2E8	60 min (dark)		
PBS wash	4×5 min		
Blocking with PBS and GS 10% + BSA 10%	30 min		
Apply appropriate secondary antibody cocktail: 350 IgG2b + 488 IgG1 + 633 IgG2a	60 min (dark)		
PBS wash Mounting with ProLong™ Gold Antifade (P36934 ThermoFisher Scientific, Waltham, USA)	3×5 min		
TOTAL	**8 hours 10 min**		**4 hours 45 min**

BSA: Bovine serum albumin, GS: Goat serum, M: Method, PBS: Phosphate buffered saline

Image acquisition and analysis

Muscle sections were observed under optical microscopy (DM2000, Leica microsystems, Wetzlar, Germany) with a ×20 objective, connected to a 5 Megapixels camera with active cooling (DFC450 C, Leica microsystems, Wetzlar, Germany). Leica software (LAS version 4.3.0, Leica microsystems, Wetzlar, Germany) was used for image acquisition. The microscope was equipped with a fluorescence illumination system (Lumen 200, Prior Scientific, Cambridge, UK), and with blue (Excitation: bandpass filter (BP):

360/40 nm, Emission: BP 470/40 nm), green (Excitation: BP 480/40 nm, Emission: BP 527/30 nm), orange-red (Excitation: 545/30 nm, Emission: BP 610/75 nm), and deep red (Excitation: BP: 620/60 nm, Emission: BP 700/75 nm) filter cubes. ImageJ software (NIH, Bethesda, USA) was used to merge channels and to perform image analysis. Nine regions on each muscle section were captured in order to be representative of a large part of the muscle section. Thus, a significant number of muscle fibers could be identified on each muscle biopsy. Owing to serial cross-sectioning, the exact same muscle fibers were analyzed between the 5 different methods, for each muscle sample (see Fig.1). The number of capillary contacts per fiber (as previously defined as "CC" (Hepple et al. 1997; Harris 2005)) was assessed for M1, M2, M3, and M4 – given that M5 was only used for fiber typing but not for direct capillaries identification. For further details on CC measurements, see the review of Harris that reported the different capillary indexes used in literature (Harris 2005). CC analysis was performed on 50 type I, 50 type IIA and 50 type IIX fibers - except for two muscle biopsies for which only 34 and 35 type IIX fibers were analyzed due to their low proportion. Analyses of CC were performed on single merged images for M1 and M3. For M2 and M4 that only stained capillaries and basal lamina, CC analyses required the use of a serial cross-section to identify muscle fiber type of the cells in contact with the capillaries, therefore we used M5 staining in this case to assess muscle fiber type. The same experimenter repeated twice all measurements of each image to assess intra-experimenter reproducibility of capillaries identification. The two measurements were spaced in time to avoid any memory bias of the experimenter.



Immunohistochemical staining using M1 (Method 1) a), M2 (Method 2) b), M3 (Method 3) c), M4 (Method 4) d), and M5 (Method 5) e). Type I fibers are blue (BA-F8), type IIA fibers are green (BF-35), type IIX fibers are black (unstained), and initially deep red laminin (2E8) has been colored in grey. Capillaries are orange red (arrow points) using JC70A antibody from mouse on a) and b), and using Ab28364 antibody from rabbit on c) and d)

M: Method, MHC: Myosin heavy chain, the arrows point to a capillary, scale bars: 100 µm

Fig.1 Immunohistochemical staining of human skeletal muscle

Statistics

Statistical analyses and graphics were carried out using Stata software, Version 15 (StataCorp, College Station, USA) and GraphPad software (version 5.0, Prism, San Diego, USA). Continuous data were expressed as mean ± standard deviation (SD). The assumption of normality was assessed using the Shapiro-Wilk test. To measure the inter-methods and intra-experimenter agreement, intra-class correlation coefficients (ICC) and Lin's concordance correlation coefficients (CCC) were estimated, and were interpreted according to usual rules of thumbs (Altman 1991): <0.2 (negligible), 0.2-0.4 (low/weak consistency), 0.4-0.6 (moderate agreement), 0.6-0.8 (substantial/good agreement), and >0.8 (excellent agreement). The results were also presented using Bland and Altman's plots (Fig.3).

Results and discussion

We aimed to propose new reliable time- and tissue-sparing immunohistochemical staining protocols to accurately identify capillaries, muscle fiber types (I, IIA, IIX), and basal lamina, on a single muscle section. Based on works from the last decade (Bloemberg and Quadrilatero 2012; Snijders et al. 2016, 2017; Nederveen et al. 2016, 2018; Tan et al. 2018; Moro et al. 2019), we proposed here two new staining methods: M1 and M3 (as detailed in Tab. 1 and Tab.2).

First, the concordance between JC70A and Ab28364 antibodies both directed against PECAM-1 had to be estimated. JC70A is a mouse monoclonal antibody and Ab28364 is a rabbit polyclonal antibody, developed by different companies and with different isotypes (see Tab.1 and Material and methods). Thus, differences in capillaries identification could have been expected. From a visual point of view, Fig.1 shows slightly sharper staining with JC70A monoclonal antibody compared to Ab28364 polyclonal antibody. This observation led us to question a potential overestimation of capillaries using the

polyclonal antibody, even if the experimenters who are familiar with capillary analyses should probably not confound capillaries with artefacts. An analysis of ICC was performed to assess concordance between CC measurements from these different antibodies. Tab.3 showed high values of ICC between methods for type I fibers (ICC [$ICC_{95\%}$]: 0.89 [0.72;0.96]), type IIA fibers (ICC [$ICC_{95\%}$]: 0.93 [0.81;0.97]), type IIX fibers (ICC [$ICC_{95\%}$]: 0.88 [0.71;0.96]), and for all fiber types (ICC [$ICC_{95\%}$]: 0.95 [0.86;0.98]); thus demonstrating a high degree of similarity in capillaries identification. Observation of individual values may also be of clinical interest. As displayed in Fig.2, a strong similarity in CC measurements was observed for each muscle sample using the different methods. This reinforces again the strong similarity in capillaries identification using JC70A or Ab28364 antibodies, even at an individual level.

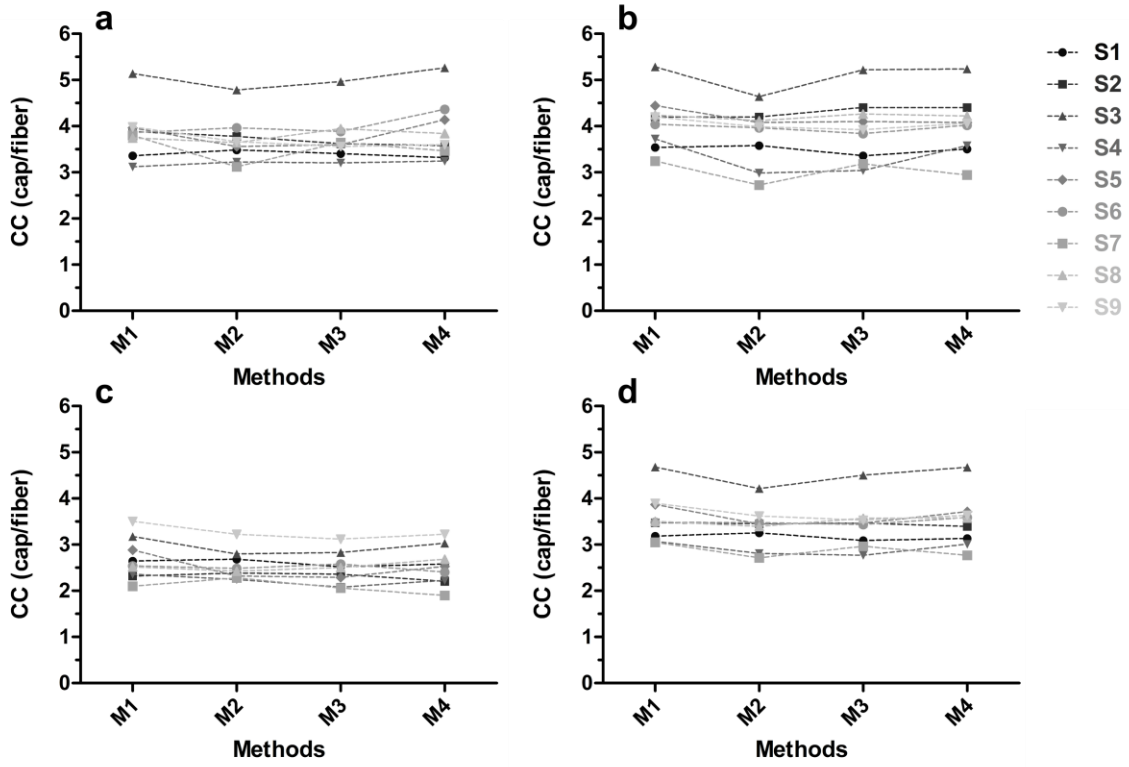
Tab.3: Capillary supply assessments between the different methods

Types of fibers	**Capillary contacts (CC)**				
	M1 (cap/fiber)	**M2** (cap/fiber)	**M3** (cap/fiber)	**M4** (cap/fiber)	**ICC**
Type I fibers	3.87 ± 0.56	3.69 ± 0.48	3.76 ± 0.50	3.87 ± 0.64	0.89 [0.72;0.96]
Type IIA fibers	4.10 ± 0.59	3.81 ± 0.61	3.92 ± 0.68	4.00 ± 0.64	0.93 [0.81;0.97]
Type IIX fibers	2.67 ± 0.44	2.54 ± 0.32	2.48 ± 0.34	2.53 ± 0.41	0.88 [0.71;0.96]
All types	3.58 ± 0.52	3.37 ± 0.44	3.42 ± 0.49	3.50 ± 0.54	0.95 [0.86;0.98]

cap/fiber: capillaries per muscle fiber, CC: Capillary contacts, ICC: Intra-class correlations, M: Method

Concordance between single and serial cross-section capillary identification also had to be assessed. Differences could have been expected between methods that require a single section analysis (M1 and M3) compared to methods that required a serial cross-section analysis (M2 and M4). As observed in Fig.1, capillaries seem to be less apparent in M1 and M3 because of the colors of surrounding fibers, compared to M2 and M4. Indeed, capillaries seem to be very easy to detect in M2 and M4 for which fiber distribution was observed in a separate slide (M5). This first visual observation tends to suggest that capillarization might be underestimated in M1 and M3. However, capillaries should still be well

detected when carefully analyzing large-format images on a large screen, despite colors of the surrounding fibers. Indeed, results showed a high degree of concordance between methods for fibers of type I (ICC: 0.89), type IIA (ICC: 0.93), type IIX (ICC: 0.88), and for all fiber types (ICC: 0.95). In addition, high reliability was also observed in CC values of each individually observed sample (Fig.2), despite small variations. This small variability could be partly explained by the distance (a few tens or hundreds of micrometers) between muscle sections that were stained by the different methods. Although this is supposed to introduce a minor bias, the fact remains that capillaries can evolve slightly within a few micrometers. This small variability could also be attributed to a random error related to experimenter's reproducibility in the detection of capillaries, which led us to question intra-experimenter reproducibility.



Measurements of capillary contacts for each sample between methods in type I fibers a), type IIA fibers b), type IIX fibers c), and all types of fibers d)

cap/fiber: capillaries per muscle fiber, CC: Capillary contacts, M: Method, S: Sample

Fig.2 Individual values between methods by type of fibers

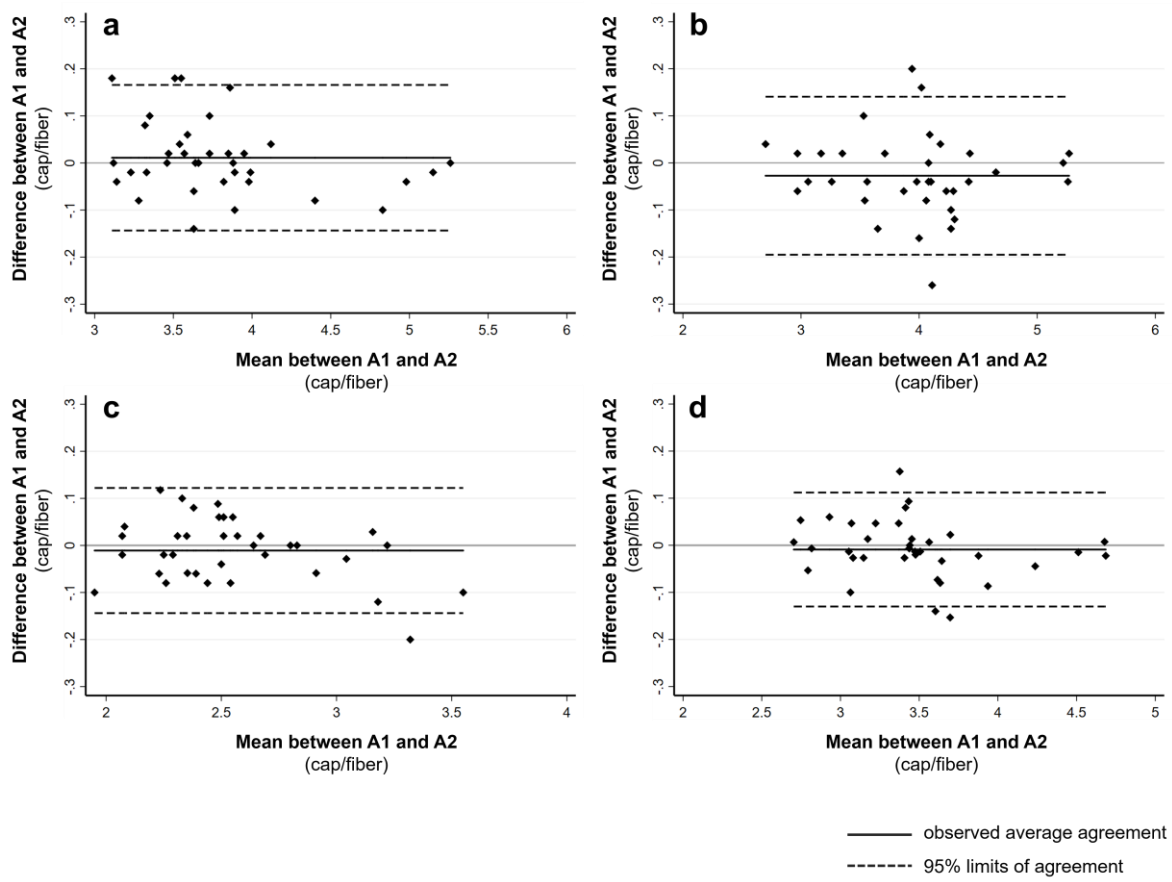
Lin's CCC were calculated between two separate analysis (A1 and A2) of the same images and performed by the same experimenter, in order to test intra-experimenter reproducibility. As displayed in Tab.4, Lin's CCC were systematically higher than 0.96 for each type of fibers and each method. Considering all types of fibers, Lin's CCC [$CCC_{95\%}$] of 0.996 [0.990;1.002] was observed for M1, 0.989 [0.974;1.004] for M2, 0.987 [0.970;1.005] for M3, 0.994 [0.986;1.002] for M4, and 0.992 [0.987;0.997] for all methods, thus demonstrating a high degree of intra-experimenter reproducibility.

Tab.4: Intra-experimenter reproducibility for the different methods

Types of fibers	Capillary contacts (CC)														
	M1			M2			M3			M4			All methods		
	A1 (cap/fiber)	A2 (cap/fiber)	Lin's CCC	A1 (cap/fiber)	A2 (cap/fiber)	Lin's CCC	A1 (cap/fiber)	A2 (cap/fiber)	Lin's CCC	A1 (cap/fiber)	A2 (cap/fiber)	Lin's CCC	A1 (cap/fiber)	A2 (cap/fiber)	Lin's CCC
Type I	3.87 ± 0.56	3.88 ± 0.57	0.997 [0.994;1.001]	3.69 ± 0.48	3.68 ± 0.51	0.994 [0.987;1.002]	3.76 ± 0.50	3.68 ± 0.56	0.966 [0.921;1.010]	3.87 ± 0.64	3.90 ± 0.63	0.996 [0.989;1.002]	3.80 ± 0.53	3.78 ± 0.55	0.989 [0.982;0.996]
Type IIA	4.10 ± 0.59	4.12 ± 0.59	0.985 [0.963;1.007]	3.81 ± 0.61	3.81 ± 0.64	0.986 [0.967;1.006]	3.92 ± 0.68	3.94 ± 0.69	0.992 [0.979;1.004]	4.00 ± 0.64	4.07 ± 0.63	0.992 [0.983;1.001]	3.96 ± 0.61	3.99 ± 0.62	0.989 [0.982;0.996]
Type IIX	2.67 ± 0.44	2.67 ± 0.48	0.992 [0.982;1.002]	2.54 ± 0.32	2.55 ± 0.37	0.967 [0.932;1.003]	2.48 ± 0.34	2.48 ± 0.38	0.981 [0.958;1.004]	2.53 ± 0.41	2.56 ± 0.39	0.986 [0.968;1.004]	2.55 ± 0.37	2.57 ± 0.39	0.984 [0.974;0.994]
All types	3.58 ± 0.52	3.58 ± 0.52	0.996 [0.990;1.002]	3.37 ± 0.44	3.38 ± 0.47	0.989 [0.974;1.004]	3.42 ± 0.49	3.40 ± 0.52	0.987 [0.970;1.005]	3.50 ± 0.54	3.54 ± 0.53	0.994 [0.986;1.002]	3.47 ± 0.48	3.48 ± 0.50	0.992 [0.987;0.997]

A: Analysis, cap/fiber: capillaries per muscle fiber, CC: Capillary contacts, CCC: Concordance Correlation Coefficients, M: Method

Bland and Altman's graphical analyses were also performed to assess reproducibility between the first analysis (A1) and the repeated analysis (A2) (Fig.3). Differences between A1 and A2 were plotted against means between A1 and A2, for type I, IIA, IIX fibers, and for all types of fibers (Fig.3), in order to assess global agreement between A1 and A2. First, Fig.3 shows that data were homogeneously dispersed. Then, the average agreement was very close to zero for all fiber types, meaning that A1 and A2 measurements give the same results, without systematic error. Bland and Altman's method also defines the 95% limits of agreement to help in the analysis of random error. Based on CC values already existing in the literature (Proctor et al. 1995; Gavin et al. 2005; Kryger and Andersen 2007; Groen et al. 2014; Verdijk et al. 2016; Galusca et al. 2018; Moro et al. 2019), we had *a priori* defined acceptable intervals of 0.4 capillary per fiber. Fig.3 shows the limits of agreement between 0.1 to 0.2 capillary per fiber. Based on clinical and biological considerations, limits of agreement were therefore acceptable, since 95% of the differences between one measurement to the other were included in a range < 0.2 capillary per fiber. Finally, both Lin's CCC and Bland and Altman's graphics showed a high degree of intra-experimenter reproducibility.



Comparison between analysis 1 (A1) and analysis 2 (A2) of capillary contacts (CC) for type I fibers a), type IIA fibers b), type IIX fibers c), and all types of fibers d)

A: Analysis, cap/fiber: capillaries per muscle fiber

Fig.3 Graphics of Bland and Altman of intra-experimenter reproducibility in capillary contacts analysis

Although we propose here two reliable methods (M1 and M3) to simultaneously identify muscle capillaries, the three types of fiber and basal lamina, some limitations can be pointed out. The disadvantage of M1 using CD31 mouse monoclonal antibody was the requirement to perform the staining in two steps, because of the cross-reactivity with BF-35. The main limitation of M3 using CD31 Ab28364 rabbit polyclonal antibody was a slightly lower sharpness compared with M1, likely due to antibody polyclonality. Therefore, we recommend to an untrained experimenter to use M1 (CD 31 JC70A) for easier detection of capillaries. Yet, high values of ICC were obtained between the different methods,

and CD31 Ab28364 was already used in the literature (Snijders et al. 2016, 2017; Nederveen et al. 2016, 2018; Tan et al. 2018). On the other hand, the single-step M3 procedure was also found reliable and might be relevant for fast staining by trained experimenters. Another limitation might be the small individual variability observed in Fig.2. Nevertheless, this no longer exists by calculating the means of several individuals, which is usually done in clinical studies (Tab.3). Based on the present results, these limitations should be tempered considering the high reliability observed between the different methods.

Our initial objective was to develop a method allowing easy identification of capillaries with simultaneous staining of the three types of fibers and basal lamina. The statistical analyses were therefore conducted on the CC local index – a fiber-type dependent index, and results showed strong reliability in the identification of capillaries. Thus, the two immunohistochemical techniques M1 and M3 can be used for capillaries counting of both global indexes (CD, C/F) and local indexes (CC, CAFA, CFPE). While reliability has been verified for such indexes that only implies capillaries identification, it remains to be confirmed for indexes related to the geometry of capillaries – such as LC/PF or CapTor. We would prefer the M1 procedure to perform morphological analyses of capillaries, since these kinds of analyses require a high level of accuracy. Indeed, from our visual observations, the M1 procedure allows providing sharp images with high quality and almost no artefact.

In perspective, it seems also important to consider the recent literature reporting an increasing number of semi- or fully automatic computational programs that are designed to perform faster and more objective analyses. Most of the time, these kinds of programs allow analyses of muscle fibers distribution and/or cross-sectional areas of fibers (Bergmeister et al. 2016; Wen et al. 2018; Kastenschmidt et al. 2019; Desgeorges et al. 2019; Encarnacion-Rivera et al. 2020), but some free open-source programs (ImageJ, Fiji, CellProfiler…) are even able to perform some capillary analyses (Smith and Barton 2014; Mayeuf-

Louchart et al. 2018; Al-Shammari et al. 2019; Sanz et al. 2019). In addition to the staining methods described in the present article, it could be very useful to consider the use of such automated programs to further reduce the time for experimentation and analysis. However, although these methods undoubtedly represent the future of histomorphometry, today they require, by the users' own admission, high quality and artifact-free biopsies, which is not always the case with the muscle biopsies of patients, for example (Sanz et al. 2019).

In conclusion, we propose here two new reliable, time-efficient, and timesaving immunohistochemical methods (referred to as M1 and M3 in Tab.1 and Tab.2) for capillary supply analysis in human skeletal muscle. These immunohistochemical methods allow simultaneous identification of capillaries, type I, IIA, IIX fibers, and basal lamina. The main advantage of the M1 procedure is its accuracy facilitating the identification of capillaries by an unexperienced experimenter or even potentially by an automated program, and the main advantage of the M3 procedure is its ability to perform fast staining in a single step (< 5 hours). A high degree of similarity in capillaries measurements for the different methods was found, and a high degree of intra-experimenter reproducibility was observed. The major benefit of these single section methods is the suppression of the time-consuming analysis of serial sectioning analyses. With these methods, all the necessary information for a fine fiber typing (3 different types of muscle fibers), assessment of global and local indexes of capillarization, and measurement of muscle fibers morphometry (such as cross-sectional area thanks to the staining of basal lamina) just have to be “read” on the merged picture.

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Conflict of interest: None

Ethics approval: The present study used muscle samples from a previous study (Galusca et al. 2018) that was conducted in accordance with the Helsinki Declaration and that was approved by the local research and ethics committee of Saint-Étienne – France

Consent to participate: All subjects gave written informed consent prior to inclusion in the study

Author contributions: JV: Conceptualization, JV, DT, LF, CH, DC, NG, BG: Data curation, project administration, resources, visualization, supervision, JV, MB: Writing – original draft, review & editing, MB, AB, JV: Methodology, Investigation, Validation, BP: Statistical analysis

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ÉTUDE 5 : CARACTÉRISATION DU PHÉNOTYPE MUSCULAIRE CHEZ DES FEMMES ET DES HOMMES PRÉSENTANT UNE MAIGREUR CONSTITUTIONNELLE ET EFFET D'UNE SURNUTRITION SUR LEUR PHÉNOTYPE MUSCULAIRE

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Graphical abstract

Skeletal muscle of females and males with constitutional thinness: a low intramuscular lipid content and oxidative profile

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Novelty: •Low intramuscular triglycerides and glycogen content in skeletal muscle of constitutionally thin individuals; •Low oxidative capacity, low capillary supply, and fiber hypotrophy in skeletal muscle of

constitutionally thin participants; •Increase in intramuscular triglycerides in constitutional thinness in response to overfeeding

Keywords: Constitutional thinness; Skeletal muscle; Intramuscular triglycerides; Oxidative capacity; Muscle glycogen; Capillary supply

Abstract

Constitutional thinness (CT) is a non-pathological state of underweight. The present study aimed to explore skeletal muscle energy storage in individuals with constitutional thinness and to further characterize muscle phenotype at baseline and in response to overfeeding. Thirty subjects with CT (15 females, 15 males) and 31 normal-weight control subjects (16 females, 15 males) participated in the study. Histological and enzymological analyses were performed on muscle biopsies before and after overfeeding. In skeletal muscle of CT participants compared to controls, it was observed a lower content in intramuscular triglycerides for type I ($p<0.01$, -17%) and type IIA ($p<0.05$, -14%) muscle fibers, a lower glycogen content for type I fibers ($p<0.01$, -6%) and type IIA fibers ($p<0.05$, -5%), a specific fiber type distribution, a marked muscle hypotrophy ($p<0.001$, -20%), a low capillary-to-fiber ratio ($p<0.001$, -19%), and a low citrate synthase activity ($p<0.05$, -18%). In response to overfeeding, CT participants increased their intramuscular triglycerides content in type I ($p<0.01$, +10%) and type IIA ($p<0.01$, +9%) muscle fibers. CT individuals seem to present an unusual muscle phenotype and different adaptations to overfeeding compared to normal-weight participants, suggesting a specific energy metabolism and muscle adaptations. NCT02004821

Introduction

While the potential existence of thinness due to a constitutional origin was first suggested in 1933 (Grafe 1933), the scientific literature remains quite limited more than 85 years later with only about 40 heterogeneous studies investigating it. Given this lack of medical and scientific interest, this constitutional thinness (CT) is poorly recognized, diagnosed, and documented. Despite an increasing number of clinical consultations, it remains difficult to provide appropriate medical support and to help CT individuals overcome their weight gain resistance. People with CT present a low body mass index (BMI) (< 18.5 $kg.m^{-2}$), no eating disorder-related traits as anorexia nervosa (AN) or any other eating disorders, and no over-exercising or associated diseases that could induce underweight (Scalfi et al. 1992; Bosy-Westphal et al. 2004; Tagami et al. 2004; Bossu et al. 2007; Marra et al. 2009, 2019; Hasegawa et al. 2011; Estour et al. 2014, 2017; Germain et al. 2014, 2016; Galusca et al. 2015, 2018; Riveros-McKay et al. 2019; Margaritelis et al. 2019; Ling et al. 2020). Despite a potentially more fractionated energy intake with smaller portioned-meals and more snacking (Germain et al. 2014; Ling et al. 2020), individuals with CT have a similar total daily energy intake compared to normal-weight controls (Germain et al. 2007, 2014; Estour et al. 2014). They also have similar proportions in macronutrient intake (Bossu et al. 2007; Galusca et al. 2018) and do not seem to present any signs of impairments in their gastrointestinal fat absorption, such as steatorrhea (Ling et al. 2019). Individuals with CT nevertheless present an unusual phenotype of impaired bone quality with a low bone mineral density (Galusca et al. 2008, 2018; Fernández-García et al. 2009; Hasegawa et al. 2011), a low bone mass, and a diminished breaking strength, despite an apparently normal bone turnover (Galusca et al. 2008). These data obtained in young adults (< 36 years) suggest a potential increased risk of osteoporosis with aging in CT population, even if it remains to be shown. Given the well-known strong connection between bone and muscle tissues, usually called the "functional muscle-bone unit" (Fricke and Schoenau 2007), skeletal muscle

alterations might also be expected in CT. In addition, two studies found a significantly higher resting metabolic rate (RMR) to fat-free mass (FFM) ratio in both CT females (Bossu et al. 2007) and CT males (Marra et al. 2019) compared to normal-weight controls, underlying a potential increased muscle metabolism in CT individuals of each gender. However, RNA profiling in skeletal muscle reported that fat storage-inducing transmembrane 1 and 2 (respectively FITM1 and FITM2) genes involved in triglycerides metabolism were downregulated in CT females, on top of an unusual and untypical skeletal muscle phenotype (Galusca et al. 2018). In light of these findings, it seems of major interest to investigate the storage and use of muscle energy substrates to better understand and help CT individuals. To the best of our knowledge, no studies have been conducted to date on muscle energy storage (i.e. intramuscular triglycerides (IMTG) and glycogen content (Essén et al. 1975)) and its metabolic implications in CT. Clinically, the main issue for medical practitioners remains to provide appropriate strategies to help CT patients gain weight, as it has been discussed for a long time (Wissmer 1953; Aubertin 1953; Passmore et al. 1955; Genest et al. 1955; Apfelbaum and Sachet 1982). More recently, a 4-week fat overfeeding of 630 extra kcal/day failed to increase bodyweight in CT women compared to controls but interestingly increased RMR-to-FFM ratio in CT participants contrary to controls (Germain et al. 2014). We, therefore, hypothesized that specific adaptations in muscle metabolism of CT individuals could partly explain their weight gain resistance. The aim of the present study was first to investigate muscle energy storage and skeletal muscle phenotype in a large subset of both females and males with CT, compared to normal-weight control volunteers at baseline. The second objective was to explore muscle storage and muscle phenotype in response to overfeeding containing proteins, carbohydrates, and fats, in CT participants compared to normal-weight controls.

Materials and methods

The present clinical investigation was a large-scale study developed in partnership with the Nestlé Institute of Health Sciences (NIHS). This investigation was conducted in accordance with the Helsinki Declaration, last updated in 2013 and was registered in clinical-trial.gov as NCT02004821. The local research and ethics committee of Saint-Étienne – France approved the study and all subjects gave written informed consent prior to inclusion in the study.

Subjects

Sixty-one subjects participated in the study: 30 CT subjects (15 females and 15 males) and 31 normal-weight controls (16 females and 15 males). CT females were recruited with a BMI between 13 and 17.5 kg.m-2, CT males with a BMI between 13 and 18.5 kg.m-2, and control females and males with a BMI between 20 and 25 kg.m-^{2}. The inclusion criteria for all participants were: age between 18 and 35 years (mean age of 23.1 ± 2.9 years for controls and 25.5 ± 4.5 years for CT participants), stable weight for at least 3 months, no over-exercising (according to the MOSPA questionnaire (Iqbal et al. 2006)), agreement to a potential weight gain of 2 kg (< 10% of bodyweight), no chronic or congenital diseases, and no medications. Normal-weight control subjects were recruited by advertising and participants with CT were recruited among outpatients consulting for bodyweight gain desire at the division of Endocrinology, Diabetes, Metabolism and Eating Disorders of the CHU Saint-Étienne, France. The specific inclusion criteria of CT participants were: no AN traits or other eating disorders confirmed by psychiatric evaluation and validated psychological questionnaires (Dutch eating behavior questionnaire (DEBQ) (van Strien et al. 1986), eating disorder inventory (EDI) (Garner et al. 1983), eating disorder examination questionnaire (EDE) (Cooper and Fairburn 1987), and body shape questionnaire (BSQ) (Cooper et al.

1987)), stable bodyweight for at least 3 months and also throughout the post-pubertal period, no amenorrhea, normal insulin-like growth factor 1 (IGF-1), estradiol, free triiodothyronine (FT3), cortisol, and non-blunted leptin. The exclusion criteria for all participants were: eating disorders (DSM-IV) or other psychiatric conditions, intensive physical activity (more than 3 sessions of physical activity per week), vegetarians or lactose-intolerant subjects, significant alcohol or tobacco consumption (> 10 glasses of wine per week or > 10 cigarettes per day), severe progressive disorder, and surgical history or treatment deemed incompatible with the study. For further details, see the complete design of the protocol (Ling et al. 2016).

Overfeeding protocol

All participants (CT and control groups) had to complete an extra consumption of 600 kcal per day for 2 weeks, by adding one bottle of Renutryl® Booster (300 mL) to their usual diet, every evening. One bottle of Renutryl® Booster contained 30 g of proteins, 72 g of carbohydrates, 21 g of fats, minerals, and oligo-elements. Twenty percent of the energy contained in one bottle came from proteins, 48.5% from carbohydrates and 31.5% from fats (Supplementary Table S1). To the best of our knowledge, only one publication investigated the effects of an overfeeding in CT individuals (Germain et al. 2014). This 4-week fat overfeeding of 630 extra kcal per day showed an increase in bodyweight of CT participants which did not persist after the first 2 weeks (Germain et al. 2014; Ling et al. 2016). It, therefore, appeared that an overfeeding of fat exclusively might not be the best strategy to increase bodyweight of CT participants, and we hypothesized that a more balanced nutritional composition could be more relevant. In addition, these previous observations suggested that a 2-week overfeeding could be a better duration than 4 weeks in order to observe changes in CT. Thus, the duration of overfeeding was reduced to 2 weeks in the present study while maintaining the same range of extra calories (630 kcal per day in our

previous study (Germain et al. 2014), and 600 kcal per day in the present one). This shorter duration was also designed to maximize our confidence in compliance and recruitment of participants. Except for the extra consumption of one bottle of Renutryl® Booster per day after dinner in free-living conditions, participants were asked not to modify their regular lifestyle (baseline diet and physical activities) during the whole study. Instead of the previously used real food (Germain et al. 2014), the present overfeeding consisted of a multi-nutrient liquid in order to facilitate the ingestion of extra calories. Throughout the protocol, participants were regularly in contact with the investigators to check compliance and avoid compensatory behaviors. Compliance of overfeeding was defined as an increase in food intake of at least 450 kcal/day, a positive change in urine urea, and no increase in physical activity level (PAL) (Ling et al. 2020). Only participants who complied with overfeeding were included in post-overfeeding statistical analyses, i.e. 22 normal-weight and 24 CT participants.

Anthropometry, body composition, and physical activity level

Bodyweight was measured with a digital scale (ProDoc, PD200M, Detecto, Webb City, USA) to the nearest 0.1 kg and body height was recorded with a standard wall-mounted stadiometer to the nearest 0.1 cm. Fat mass (FM) and lean mass measurements were assessed by Dual-energy X-ray Absorptiometry (LUNAR, DPX-L). PAL was measured in free-living conditions for 5 days with an accelerometer (ActiHeart®, CamNtech, Cambridge, UK).

Muscle biopsies collection

A biopsy of 100 to 150 mg of the vastus lateralis muscle was performed with an incision by a specialized-surgeon at one-third of the distance from the upper margin of patella to the anterior superior iliac spine, using Weil-Blakesley forceps under local anesthesia. The biopsy was performed on the right leg just

before overfeeding and on the left leg at the end of overfeeding. Samples were divided to perform histological and enzymatic analyses. A piece of the sample containing well-identified fascicles was oriented under a stereo microscope, included in an embedding medium (Cryomount, Histolab, Göteborg, Sweden), frozen in isopentane cooled to its freezing point in liquid nitrogen and stored at -80°C. Serial 10 μm thick transverse sections were cut at -18°C using a cryostat (CM1950, Leica Biosystems, Wetzlar, Germany), mounted on glass slides, air-dried at room temperature, and stored at -20°C. Before staining, frozen slides were air-dried at room temperature for 45 minutes.

Histological staining (Figure 1)

Muscle fiber type and muscle capillarization

Slides were fixed in acetone for 15 minutes, washed in a phosphate-buffered saline solution (PBS) for 3×5 minutes and then blocked in a PBS solution containing 10% of goat serum (GS) and 10% of bovine serum albumin (BSA) for 20 minutes. CD31 (Dako, M0823, Agilent Technologies, Santa Clara, USA) primary antibody directed against platelet endothelial cell adhesion molecule (PECAM-1) was used to identify muscle capillaries. The antibody was diluted (1/40) in PBS-10% GS-10%BSA and applied for 1 hour. After a PBS wash (3×5 minutes), sections were blocked again with a PBS-10%GS-10%BSA solution and then incubated with an appropriate conjugated secondary antibody (1/300, Alexa Fluor A-21123, ThermoFisher Scientific, Waltham, USA) for 1 hour. After another washing (4×5 minutes in PBS) and blocking (30 minutes in PBS-10%GS-10%BSA) baths, sections were incubated in a cocktail of primary antibodies (DSHB, Iowa City, USA) diluted (1/100) in PBS-10%GS-10%BSA: BF-35, BA-F8 and 2E8 for 1 hour. BA-F8 antibody specifically reacts with type I fibers while BF-35 strongly reacts with type IIA fibers, slightly with type I fibers and not at all with type IIX fibers. 2E8 antibody reacts with laminin. After the last washing (4×5 minutes in PBS) and blocking (30 minutes in PBS-10%GS-10%BSA), sections were

incubated with a cocktail of appropriate conjugated secondary antibodies diluted (1/300, ThermoFisher Scientific, Waltham, USA) in PBS-10%GS-10%BSA during 1 hour. After 3 consecutive 5-min washes in PBS, slides were mounted with a mounting medium ProLong™ Gold antifade (P36934, ThermoFisher Scientific, Waltham, USA).

Oil Red O staining

Oil red O (ORO) staining was used to identify IMTG. ORO stock solution was prepared by adding 0.5 g of ORO (O0625 Sigma-Aldrich, Saint-Louis, USA) for 100 mL of 60% triethyl phosphate (538728, Sigma-Aldrich, Saint-Louis, USA). Before the staining, a 36% triethyl phosphate working solution containing 12 mL of ORO stock solution for 8 mL of distilled water was prepared and filtered with Whatman Grade 42 filter paper to remove crystallized ORO. Serial transverse sections were fixed in a 3.7% formaldehyde (F1635, Sigma-Aldrich, Saint-Louis, USA) solution for 1 hour, rinsed in distilled water and incubated in the ORO working solution for 30 minutes. Slides were then rinsed in distilled water, subsequently rinsed with running tap water for 10 minutes and mounted with Aquatex.

Periodic Acid Schiff staining

Periodic acid Schiff (PAS) staining was used to identify muscle glycogen. Serial transverse sections were fixed in Carnoy's solution (60% ethanol, 30% chloroform, 10% glacial acetic acid) for 10 minutes, rinsed in distilled water, treated with 1% periodic acid for 5 minutes, rinsed in distilled water, incubated in Schiff's reagent (477601, Carlo Erba, Cornaredo, Italy) for 15 minutes at room temperature, and rinsed again. Slides were then dehydrated in alcohol baths (95°, 100°, 100°), treated with xylene and mounted with Eukitt.

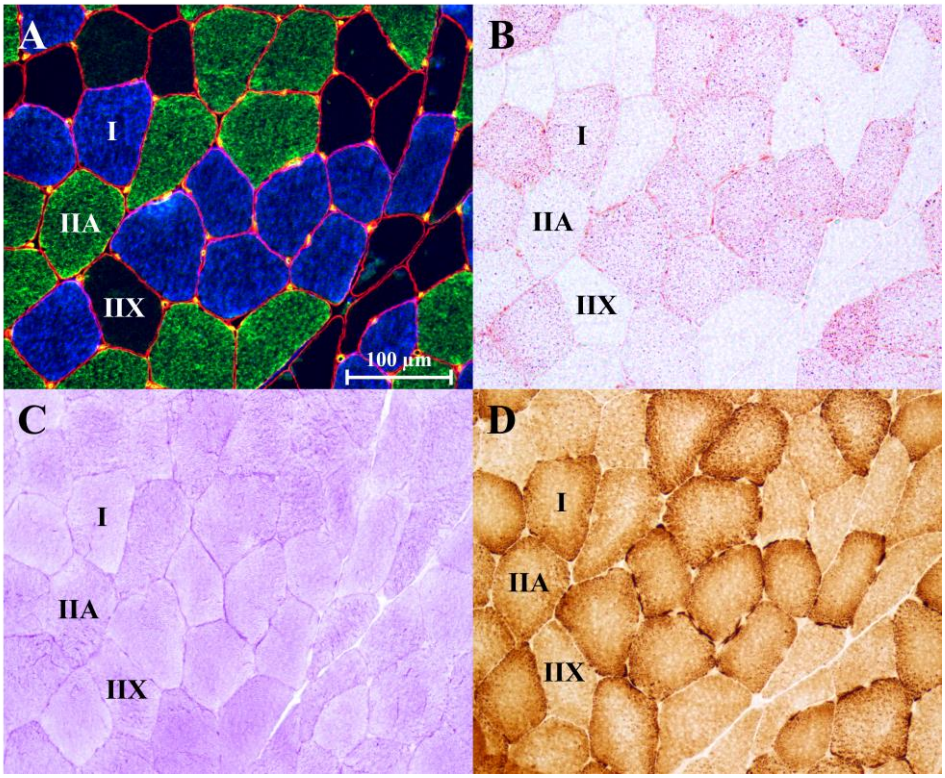
Cytochrome C Oxidase activity evaluation

Serial transverse sections were incubated for 2 hours at 37°C in a solution of 3,3'-diaminobenzidine (DAB) (D5637, Sigma-Aldrich, Saint-Louis, USA) (previously prepared in a phosphate buffer), cytochrome C (C2506, Sigma-Aldrich, Saint-Louis, USA) saccharose, catalase (C100, Sigma-Aldrich, Saint-Louis, USA), rinsed in distilled water, dehydrated in alcohol baths (95°, 100°, 100°), treated with xylene and mounted with Eukitt.

Image analyses

Image acquisition was performed with a slide scanner (Axio Scann.Z1, Carl Zeiss, Munich, Germany) and image analyses were achieved with ImageJ software (NIH, Bethesda, USA). Concerning the immunohistochemical technique, different pictures of the same region obtained with different appropriate filters allowed identifying the different types of fibers on a merged picture (Figure 1). Type I, IIA, and IIX fibers were identified and counted to determine fiber type composition. The few co-expressing fibers were classified into a main type (I, IIA or IIX), depending on the fiber type they were the most similar to. This choice was based on under-representation of co-expressing fibers in untrained young adults (Staron et al. 2000) and aimed to perform a fast and large-scale fiber typing (an average of 1421 fibers per biopsy were individually analyzed). The measurement of fiber cross-sectional areas (CSA) was performed on 150 fibers of each fiber type. The muscle fibers analyzed were randomly selected in different regions of the muscle section. For type IIX fibers, due to their low number, up to 150 fibers were measured when possible. An overall mean muscle fiber CSA was also calculated as the sum of the products between percentages and mean areas of each type of fibers. The percentage of area occupied by each fiber type was calculated as the product between the proportion and the mean area of each fiber type divided by the overall mean CSA. Muscle capillarization was examined with global indexes

(capillary density (CD), capillary to fiber (C/F) ratio) and local indexes (capillary contact (CC), CC with sharing factor (SF), capillary to fiber-perimeter exchange (CFPE), capillary contact per fiber area (CAFA)) (Harris 2005). For locales indexes, 40 fibers of each fiber type (or slightly less in case of poor muscle tissue quality) were assessed, randomly chosen. Concerning IMTG, glycogen, and cytochrome C oxidase (COx) histological analyses, measurements of optical densities were performed by converting images to grayscale before quantifying the mean grey intensity of each muscle fiber. Due to the low prevalence of type IIX fibers, these parameters were only measured in type I and type IIA fibers (50 fibers of each fiber type, randomly chosen). Results were expressed in arbitrary units (AU). An overall index representing each substrates load in the whole muscle section was calculated as the product of optical densities and percentages of area occupied by each fiber type. Since the optical density value of a fiber is the average grey density value of each pixel of the fiber, the measurement does not depend on the fiber size.



Immunohistochemical serial cross-section staining of muscle fiber type (A), intramuscular triglycerides (IMTG) (B), glycogen content (C), and cytochrome C oxidase (COx) activity (D). On image A, type I fibers are blue, type IIA fibers are green, type IIX fibers are black (unstained) and laminin is red. Thanks to cross-sectioning, it was possible to identify the corresponding muscle fibers between the fiber type staining (A) and the other stainings (B, C, D).

Figure 1: Staining of skeletal muscle fibers

Enzyme activities evaluations

The enzyme activity of enolase (ENO), β-hydroxyacyl-CoA dehydrogenase (β-HAD), citrate synthase (CS), second (CII) and fourth (CIV) respiratory chain complexes, lactate dehydrogenase (LDH), myokinase (MK) and creatine kinase (CK) were measured just before and at the end of the overfeeding protocol. Muscle samples underwent a total protein extraction in mannitol buffer with mixing (3×15 seconds) and centrifugation (20 minutes – 650 G), repeated two times to optimize the extraction. Mannitol buffer was

composed of D-Mannitol 225 mM, Tris-HCl 7.7 mM, ethylenediaminetetraacetic acid (EDTA) 0.1 mM and saccharose 75 mM. Protein concentrations were measured for each sample using a protein assay kit (10752735, Thermo Scientific™ Pierce™ BCA™, ThermoFisher Scientific, Waltham, USA) and readjusted to 1 µg.µl-1. Measurements of the maximal slope of enzyme reactions (i.e. the maximal variation of optical density per minute) were performed by spectrophotometry with a microplate reader (CLARIOstar®, BMG LABTECH, Ortenberg, Germany) at 37°C, with appropriate reaction mediums and substrates in excess. Enzyme activities were expressed in international unit (IU) per mg protein (µmol.min-1.mg-1).

The evaluation of clinical characteristics and myocellular assessments were performed pre and post-overfeeding.

Statistics

The statistical analyses were carried out using Stata statistical software (version 13, StataCorp, College Station, USA) and the graphs were obtained using GraphPad Prism 5.0 software. The tests were two-sided, with a type-I error set at 5%. Hedges' g effect size (ES) was estimated and presented with 95% confidence interval. Continuous data were expressed as mean ± standard deviation (SD). The assumption of normality was studied using the Shapiro-Wilk test. The following effects – Group, Sex and Group×Sex interaction at baseline – were estimated using multivariable analyses (i.e. multiple linear regression) taking into account possible confounders such as age and PAL. The normality of residuals was studied as aforementioned. When appropriate, a logarithmic transformation was used to achieve the normality of dependent variables. Multivariable analyses adjusted for age and PAL were also performed to evaluate Group effect by Gender sub-groups, before overfeeding. The relationships between continuous parameters at baseline were assessed using correlation coefficients (Pearson or Spearman, in accordance

with statistical distribution), applying a Sidak's type I error correction for multiple comparisons. These correlations' results were illustrated with a color-coded heatmap. Finally, to examine the effect of the 2-week overfeeding intervention, random-effects models (i.e. linear mixed models) were performed to measure group, time-point evaluation, and their interaction effects, taking into account between and within-participant variability (subject as random-effect) and adjusting analyses for age, PAL and Sex. As discussed for non-repeated data analyses, the normality of residuals was studied and when appropriate, a logarithmic transformation was used to achieve normality.

Results

Baseline analysis

Anthropometry and body composition

As it was intended, weight and BMI of the CT group was largely lower compared to controls ($p<0.001$, respectively -30% and -26%) (Table 1), and FM percentage was also found lower ($p<0.001$). Total FM, total lean mass and right leg lean mass (site of the pre-overfeeding muscle biopsy) were all found lower in CT group compared to controls ($p<0.001$; respectively -48%, -22% and -29%). PAL was lower in CT group compared to controls ($p=0.006$, ES: 0.37 [0.11; 0.63]). This result was also observed for CT males ($p=0.008$, ES: 0.52 [0.14; 0.89]), but was not significant for CT females ($p=0.69$, ES: 0.07 [-0.30; 0.45]) (Table 1).

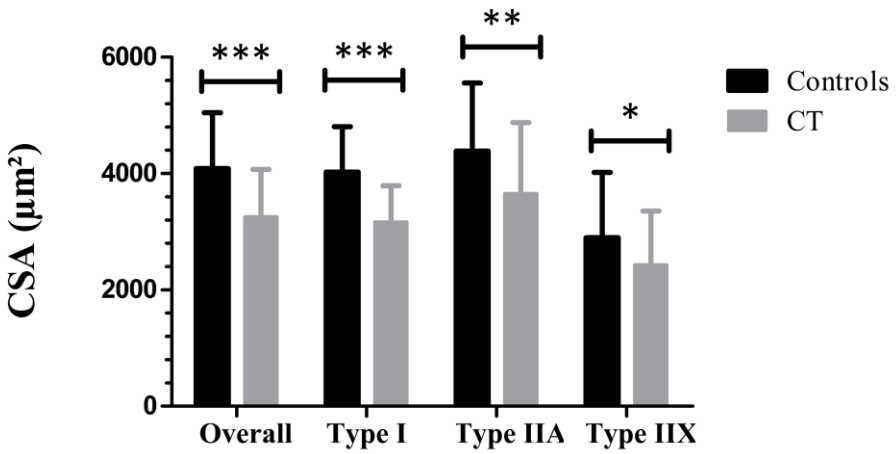
Fiber type distribution and CSA

Percentages of type I and type IIA fibers were found similar between groups but CT participants had higher percentages of type IIX muscle fibers (Table 1). Proportions of fiber types were also expressed as the percentage of area occupied by these fibers, a better estimate of muscle volume occupied by the different types of fibers in muscle. In CT group, this parameter was found lower in type I fibers (31.9 ± 14.0% in the CT group vs. 38.0 ± 11.4% in controls, p=0.044, ES: 0.28 [0.01; 0.55]), similar in type IIA fibers (52.4 ± 11.2% in the CT group vs. 51.3 ± 9.5% in controls, p=0.77, ES: 0.04 [-0.23; 0.31]) and tended to be higher in type IIX fibers (15.7 ± 10.3% in the CT group vs. 10.7 ± 9.6% in controls, p=0.051, ES: -0.27 [-0.54; 0.00]). Overall mean fiber CSA index was lower in CT group compared to controls (p<0.001, ES: 0.58 [0.31; 0.85], -20%) (Figure 2). This lower muscle fiber area was found in type I (p<0.001, ES: 0.76 [0.49; 1.03], -21%), type IIA (p=0.001, ES: 0.46 [0.19; 0.73], -17%), and type IIX fibers (p=0.013, ES: 0.39 [0.09; 0.69], -16%) of CT individuals. In CT males compared to control males, CSA was found lower in overall mean index (p=0.004, -27%), in type I fibers (p<0.001, -30%), IIA fibers (p=0.019, -22%), and IIX fibers (p=0.025, -26%). In CT females, CSA was found significantly lower in overall mean index (p=0.017, -16%) and type I fibers (p=0.008, -12%), but did not reach significance in type IIA fibers (p=0.13, -16%) and IIX fibers (p=0.36, -21%) (Table 1).

IMTG storage, glycogen storage, and COx histological enzyme activity

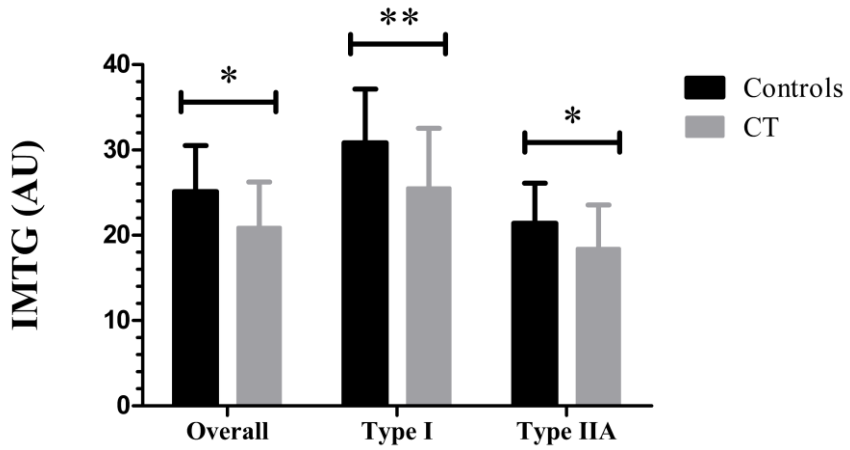
IMTG was lower in the CT group compared to controls in overall index (p=0.014, ES: 0.34 [0.07; 0.62], -17%), type I fibers (p=0.002, ES: 0.44 [0.18; 0.70], -17%), and type IIA fibers (p=0.048, ES: 0.27 [0.00; 0.53], -14%) (Figure 3). In CT females compared to control females, IMTG was found lower in overall index (p=0.049, ES: 0.40 [0.00; 0.80], -21%) and type I fibers (p=0.010, ES: 0.53 [0.14; 0.92], -20%) but not significantly in type IIA fibers (p=0.11, ES: 0.32 [-0.07; 0.71], -22%). In CT males, p-values were not found

<0.05 in overall index (p=0.39, ES: 0.17 [-0.22; 0.56], -9%), type I fibers (p=0.15, ES: 0.27 [-0.11; 0.64], -12%) and type IIA fibers (p=0.65, ES: 0.08 [-0.29; 0.46], -5%). Glycogen content was lower in CT group compared to controls, in type I (p=0.008, ES: 0.37 [0.10; 0.63], -6%) and IIA fibers (p=0.015, ES: 0.33 [0.07; 0.60], -5%). A trend was observed in glycogen overall index (p=0.071, ES: 0.25 [-0.02; 0.52], -2%). Compared to control females, CT females had a lower glycogen content in overall index (p=0.023, ES: 0.47 [0.07; 0.87], -8%), type I fibers (p=0.034, ES: 0.43 [0.04; 0.83], -8%), and type IIA fibers (p=0.045, ES: 0.41 [0.01; 0.81], -7%). In CT males, glycogen content was lower in type I (p=0.048, ES: 0.38 [0.00; 0.75], -6%) and type IIA (p=0.077, ES: 0.34 [-0.04; 0.71], -4%) fibers but similar in overall index (p=0.40, ES: 0.16 [-0.23; 0.55], +1%). COx activity histologically measured was found similar between CT and control groups in overall index, type I fibers and type IIA fibers (Table 1).



CSA: cross-sectional area; CT: subjects with constitutional thinness – * p<0.05, ** p<0.01, ***p<0.001

Figure 2: Cross-sectional areas of muscle fibers in controls (n=31) and subjects with constitutional thinness (n=30) at baseline



AU: arbitrary unit; CT: subjects with constitutional thinness; IMTG: intramuscular triglycerides – * $p<0.05$, ** $p<0.01$

Figure 3: Intramuscular triglycerides content in controls (n=31) and subjects with constitutional thinness (n=30) at baseline

Table 1: Characteristics of the population, fiber type distribution, cross-sectional areas, intramuscular triglycerides content, glycogen content and cytochrome C oxidase activity at baseline

	Controls			CT			Multivariable Analysis					
	Females (n=16)	Males (n=15)	Total (n = 31)	Females (n=15)	Males (n=15)	Total (n = 30)	Group Effect		Sex Effect		Group × Sex	
							p-value	Hedges' g	p-value	Hedges' g	p-value	Hedges' g
Age (years)	22.4 ± 2.8	23.9 ± 2.9	23.1 ± 2.9	27.4 ± 4.6 [c]	23.6 ± 3.8	25.5 ± 4.5	0.018	-0.31 [-0.57; -0.06]	0.24	-0.15 [-0.41; 0.10]	0.006	0.37 [0.11; 0.62]
Height (m)	1.65 ± 0.06	1.80 ± 0.07	1.73 ± 0.10	1.61 ± 0.06	1.76 ± 0.04 [a]	1.68 ± 0.09	0.013	0.33 [0.07; 0.58]	<0.001	1.22 [0.97; 1.48]	0.80	0.03 [-0.22; 0.29]
Weight (kg)	62.9 ± 4.8	74.8 ± 7.3	68.6 ± 8.5	42.8 ± 4.5 [c]	53.8 ± 2.9 [c]	48.3 ± 6.7	<0.001	1.71 [1.45; 1.97]	<0.001	1.10 [0.84; 1.36]	0.85	0.03 [-0.23; 0.28]
BMI ($kg.m^{-2}$)	23.0 ± 1.1	23.0 ± 1.2	23.0 ± 1.1	16.5 ± 0.8 [c]	17.4 ± 0.8 [c]	17.0 ± 0.9	<0.001	2.63 [2.37; 2.88]	0.088	0.22 [-0.03; 0.48]	0.13	-0.20 [-0.46; 0.06]
PAL	1.72 ± 0.18	1.81 ± 0.26	1.76 ± 0.22	1.60 ± 0.22	1.55 ± 0.24 [b]	1.57 ± 0.22	0.006	0.37 [0.11; 0.63]	0.76	0.04 [-0.22; 0.30]	0.11	0.21 [-0.05; 0.47]
FM (%)	31.6 ± 4.0	20.9 ± 6.4	26.4 ± 7.5	23.3 ± 2.9 [c]	15.5 ± 2.3 [a]	19.4 ± 4.8	<0.001	0.69 [0.43; 0.95]	<0.001	-1.11 [-1.37; -0.85]	0.55	-0.08 [-0.34; 0.18]
Total FM (kg)	20.6 ± 3.3	16.4 ± 5.9	18.6 ± 5.1	10.6 ± 1.7 [c]	8.8 ± 1.5 [b]	9.7 ± 1.8	<0.001	1.02 [0.76; 1.28]	0.001	-0.45 [-0.71; -0.19]	0.40	-0.11 [-0.37; 0.15]
Total lean mass (kg)	42.1 ± 3.5	58.1 ± 6.0	49.8 ± 9.4	32.8 ± 3.3 [c]	45.3 ± 2.2 [c]	39.1 ± 6.9	<0.001	1.17 [0.92; 1.43]	<0.001	1.79 [1.53; 2.05]	0.29	0.14 [-0.12; 0.40]
Right leg lean mass (kg)	6.8 ± 0.5	9.3 ± 1.1	8.0 ± 1.5	4.7 ± 0.6 [c]	6.7 ± 0.5 [c]	5.7 ± 1.2	<0.001	1.37 [1.12; 1.63]	<0.001	1.54 [1.28; 1.80]	0.56	0.08 [-0.18; 0.33]
Fiber type distribution (%)												
Type I	39.4 ± 7.4	36.3 ± 12.0	37.9 ± 9.9	38.1 ± 14.6	28.0 ± 5.8 [0.056]	33.0 ± 12.0	0.11	0.21 [-0.05; 0.47]	0.027	-0.29 [-0.55; -0.04]	0.75	0.04 [-0.22; 0.30]
Type IIA	48.7 ± 9.4	47.2 ± 8.8	48.0 ± 9.0	44.3 ± 8.6 [0.080]	47.7 ± 11.9	46.0 ± 10.3	0.21	0.16 [-0.10; 0.42]	0.66	0.06 [-0.20; 0.32]	0.55	-0.08 [-0.34; 0.18]
Type IIX	12.0 ± 9.1	16.5 ± 11.4	14.1 ± 10.4	17.6 ± 13.2	24.3 ± 11.8 [0.079]	20.9 ± 12.8	0.033	-0.28 [-0.54; -0.02]	0.11	0.21 [-0.05; 0.47]	0.99	0.00 [-0.26; 0.26]
CSA (μm^2)												
Overall	3490 ± 478	4819 ± 898	4086 ± 960	2931 ± 471 [a]	3515 ± 952 [b]	3256 ± 818	<0.001	0.58 [0.31; 0.85]	<0.001	0.66 [0.39; 0.93]	0.15	0.20 [-0.07; 0.47]
Type I	3714 ± 456	4414 ± 932	4028 ± 781	3278 ± 576 [b]	3077 ± 672 [c]	3167 ± 627	<0.001	0.76 [0.49; 1.03]	0.034	0.29 [0.02; 0.57]	0.16	0.19 [-0.08; 0.47]
Type IIA	3642 ± 596	5301 ± 1057	4385 ± 1172	3043 ± 556	4149 ± 1389 [a]	3658 ± 1218	0.001	0.46 [0.19; 0.73]	<0.001	0.75 [0.48; 1.02]	0.36	0.13 [-0.15; 0.40]
Type IIX	2338 ± 872	3872 ± 829	2896 ± 1127	1842 ± 716	2858 ± 833 [a]	2434 ± 925	0.013	0.39 [0.09; 0.69]	<0.001	0.73 [0.43; 1.03]	0.22	0.19 [-0.12; 0.49]

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	Controls			CT			Multivariable Analysis					
	Females (n=16)	Males (n=15)	Total (n = 31)	Females (n=15)	Males (n=15)	Total (n = 30)	Group Effect		Sex Effect		Group × Sex	
							p-value	Hedges' g	p-value	Hedges' g	p-value	Hedges' g
IMTG content (AU)												
Overall	27.1 ± 4.1	22.7 ± 6.0	25.1 ± 5.4	21.3 ± 6.1 [a]	20.6 ± 4.8	20.9 ± 5.3	0.014	0.34 [0.07; 0.62]	0.087	-0.24 [-0.51; 0.04]	0.28	-0.15 [-0.42; 0.12]
Type I	33.6 ± 4.9	28.0 ± 6.5	30.9 ± 6.3	26.7 ± 7.5 [a]	24.6 ± 6.6	25.6 ± 7.0	0.002	0.44 [0.18; 0.70]	0.031	-0.29 [-0.56; -0.03]	0.41	-0.11 [-0.37; 0.16]
Type IIA	22.4 ± 3.8	20.3 ± 5.5	21.4 ± 4.7	17.5 ± 5.7	19.3 ± 4.6	18.4 ± 5.1	0.048	0.27 [0.00; 0.53]	0.83	-0.03 [-0.29; 0.23]	0.31	-0.14 [-0.40; 0.13]
Glycogen content (AU)												
Overall	78.4 ± 8.2	84.2 ± 12.0	81.0 ± 10.3	72.0 ± 8.9 [a]	84.9 ± 13.5	79.2 ± 13.2	0.071	0.25 [-0.02; 0.52]	0.001	0.46 [0.19; 0.73]	0.89	-0.02 [-0.29; 0.25]
Type I	70.2 ± 9.2	80.3 ± 15.8	75.1 ± 13.6	64.4 ± 7.9 [a]	75.2 ± 12.9 [a]	70.4 ± 12.1	0.008	0.37 [0.10; 0.63]	0.001	0.49 [0.22; 0.75]	0.42	0.11 [-0.16; 0.37]
Type IIA	84.1 ± 8.2	92.2 ± 15.3	88.0 ± 12.6	78.4 ± 9.5 [a]	88.2 ± 14.2 [0.077]	83.8 ± 13.1	0.015	0.33 [0.07; 0.60]	0.004	0.40 [0.14; 0.67]	0.75	0.04 [-0.22; 0.31]
COx activity (AU)												
Overall	100.5 ± 9.4	101.7 ± 17.4	101.1 ± 13.3	102.6 ± 11.0	105.6 ± 11.8	104.3 ± 11.4	0.32	-0.14 [-0.41; 0.13]	0.54	0.08 [-0.19; 0.35]	0.46	-0.10 [-0.37; 0.17]
Type I	112.4 ± 9.0	113.9 ± 18.4	113.1 ± 14.1	118.1 ± 13.5	119.1 ± 17.0	118.7 ± 15.3	0.17	-0.19 [-0.45; 0.08]	0.77	0.04 [-0.23; 0.31]	0.98	0.00 [-0.27; 0.26]
Type IIA	91.9 ± 9.4	99.5 ± 16.9	95.6 ± 13.9	92.2 ± 10.5	101.4 ± 10.2	97.3 ± 11.2	0.72	-0.05 [-0.31; 0.22]	0.013	0.34 [0.07; 0.61]	0.61	-0.07 [-0.33; 0.20]

BMI: body mass index; COx: cytochrome C oxidase; CSA: cross-sectional area; CT: subjects with constitutional thinness; FM: fat mass; IMTG: intramuscular triglycerides; PAL: physical activity level

[a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$ between controls and subjects with constitutional thinness, within gender, from multivariable analysis

Capillary supply and enzyme activity

As displayed in Table 2, capillary density was not found different between CT and control groups. C/F ratio was lower in CT subjects compared to controls ($p<0.001$, ES: 0.64 [0.36; 0.91], -19%). CC was found lower in CT group in type I ($p<0.001$, ES: 0.65 [0.38; 0.91], -18%), type IIA ($p=0.002$, ES: 0.44 [0.17; 0.71], -16%) and type IIX fibers ($p=0.008$, ES: 0.42 [0.11; 0.72], -10%). CC taking into account SF, a better estimate of capillary supply, was also found lower in all fiber types of CT individuals (Table 2). CFPE was found lower in the CT group in type I ($p=0.026$, ES: 0.31 [0.04; 0.58], -7%) and type IIA fibers ($p=0.024$, ES: 0.31 [0.04; 0.58], -8%) but not in type IIX ($p=0.21$, ES: 0.19 [-0.11; 0.49], -4%). CAFA was not found different in any of the muscle fiber types. In CT group, CS and MK activities were both found 18% lower compared to controls (respectively $p=0.010$, ES: 0.36 [0.09; 0.62] and $p=0.009$, ES: 0.37 [0.10; 0.65]). Trends toward a higher CII activity ($p=0.078$, ES: -0.26 [-0.56; 0.03], +4%) and lower CK activity ($p=0.076$, ES: 0.25 [-0.03; 0.52], -5%) were observed in CT group. β-HAD, ENO, CIV, and LDH activities were found similar between groups. In all muscle analyses at baseline, no Group×Sex interactions were observed. Analysis of correlations between enzyme activities and energy storage in CT group and control group are detailed in Figure 4. CS activity was positively correlated with IMTG overall index ($R=0.41$ and $p=0.033$) and with IMTG in type IIA fibers ($R=0.59$ and $p<0.001$) in the control group whereas in CT, CS was correlated with glycogen overall content ($R=0.52$ and $p=0.009$), with glycogen in type I fibers ($R=0.65$ and $p<0.001$), and with glycogen in type IIA fibers ($R=0.45$ and $p=0.027$). In the control group, CS was found positively correlated with COx activity in type IIA fibers ($R=0.38$, $p=0.040$) and tended to be with overall COx activity ($R=0.34$, $p=0.081$). ENO activity was negatively correlated with COx overall activity ($R= -0.42$, $p=0.026$) (Figure 4) in controls. In CT group, ENO was positively correlated with overall COx activity ($R=0.41$ and $p=0.045$), tended to be with type I fibers COx activity ($R=0.39$, $p=0.062$), and

was significantly correlated with type IIA fibers COx activity (R=0.63 and p<0.001). Glycogen overall content was also correlated with COx activity in type IIA fibers (R=0.46, p=0.016) in CT (Figure 4).

Table 2: Capillary supply and enzyme activities at baseline

	Controls			CT			Multivariable Analysis					
	Females (n=16)	Males (n=15)	Total (n = 31)	Females (n=15)	Males (n=15)	Total (n = 30)	Group Effect		Sex Effect		Group × Sex	
CAPILLARIZATION – Global Indexes							p-value	Hedges' g	p-value	Hedges' g	p-value	Hedges' g
CD	313.1 ± 54.4	317.5 ± 44.5	314.9 ± 49.8	299.6 ± 58.5	309.8 ± 55.5	305.2 ± 56.0	0.32	0.14 [-0.14; 0.42]	0.46	0.10 [-0.17; 0.38]	0.49	-0.10 [-0.37; 0.18]
C/F	1.08 ± 0.18	1.50 ± 0.34	1.25 ± 0.33	0.90 ± 0.18 [a]	1.10 ± 0.25 [b]	1.01 ± 0.24	<0.001	0.64 [0.36; 0.91]	<0.001	0.70 [0.42; 0.98]	0.40	0.12 [-0.16; 0.39]
CAPILLARIZATION – Local Indexes												
TYPE I FIBER												
CC	3.73 ± 0.51	4.41 ± 0.71	4.05 ± 0.69	3.20 ± 0.63 [a]	3.45 ± 0.69 [b]	3.34 ± 0.67	<0.001	0.65 [0.38; 0.91]	0.001	0.45 [0.19; 0.72]	0.81	0.03 [-0.24; 0.30]
CC with SF	1.26 ± 0.22	1.60 ± 0.33	1.42 ± 0.32	1.06 ± 0.23 [a]	1.20 ± 0.28 [b]	1.13 ± 0.26	<0.001	0.65 [0.38; 0.91]	<0.001	0.54 [0.27; 0.81]	0.82	0.03 [-0.24; 0.30]
CFPE (CC/Pe) (10^{-3})	16.17 ± 2.06	17.14 ± 1.50	16.62 ± 1.86	14.74 ± 2.49	16.05 ± 2.07 [0.083]	15.46 ± 2.32	0.026	0.31 [0.04; 0.58]	0.027	0.31 [0.04; 0.57]	0.50	-0.09 [-0.36; 0.18]
CAFA (CC/CSA) (10^{-3})	1.10 ± 0.19	1.04 ± 0.14	1.07 ± 0.17	1.08 ± 0.20	1.18 ± 0.21	1.14 ± 0.21	0.15	-0.20 [-0.47; 0.07]	0.82	0.03 [-0.24; 0.30]	0.21	-0.17 [-0.44; 0.10]
TYPE IIA FIBER												
CC	3.30 ± 0.64	4.51 ± 0.80	3.87 ± 0.94	2.79 ± 0.48	3.62 ± 0.63 [b]	3.25 ± 0.70	0.002	0.44 [0.17; 0.71]	<0.001	0.76 [0.50; 1.03]	0.37	0.12 [-0.15; 0.39]
CC with SF	1.10 ± 0.24	1.62 ± 0.34	1.34 ± 0.39	0.91 ± 0.16	1.28 ± 0.26 [a]	1.11 ± 0.29	0.003	0.42 [0.16; 0.69]	<0.001	0.84 [0.57; 1.10]	0.42	0.11 [-0.16; 0.38]
CFPE (CC/Pe) (10^{-3})	14.23 ± 1.87	15.87 ± 2.33	14.99 ± 2.22	12.78 ± 2.09	14.55 ± 1.49 [0.059]	13.76 ± 1.96	0.024	0.31 [0.04; 0.58]	0.002	0.45 [0.18; 0.72]	0.74	-0.04 [-0.31; 0.22]
CAFA (CC/CSA) (10^{-3})	0.99 ± 0.14	0.88 ± 0.15	0.94 ± 0.15	0.97 ± 0.19	0.93 ± 0.15	0.95 ± 0.17	0.96	-0.01 [-0.28; 0.26]	0.17	-0.19 [-0.46; 0.08]	0.16	-0.19 [-0.46; 0.08]
TYPE IIX FIBER												
CC	2.24 ± 0.46	2.84 ± 0.40	2.46 ± 0.52	1.85 ± 0.47	2.47 ± 0.52 [a]	2.21 ± 0.58	0.008	0.42 [0.11; 0.72]	<0.001	0.64 [0.34; 0.94]	0.94	-0.01 [-0.31; 0.29]
CC with SF	0.72 ± 0.16	0.98 ± 0.14	0.81 ± 0.19	0.59 ± 0.16	0.82 ± 0.20 [a]	0.73 ± 0.21	0.007	0.43 [0.12; 0.73]	<0.001	0.69 [0.39; 0.99]	0.79	0.04 [-0.26; 0.34]
CFPE (CC/Pe) (10^{-3})	11.80 ± 1.54	11.51 ± 1.74	11.70 ± 1.58	10.79 ± 2.22	11.55 ± 1.71	11.24 ± 1.93	0.21	0.19 [-0.11; 0.49]	0.48	0.11 [-0.20; 0.41]	0.28	-0.16 [-0.47; 0.14]
CAFA (CC/CSA) (10^{-3})	1.12 ± 0.35	0.76 ± 0.15	0.99 ± 0.34	1.13 ± 0.33	0.93 ± 0.18	1.01 ± 0.27	0.58	-0.08 [-0.38; 0.22]	0.004	-0.45 [-0.75; -0.15]	0.16	-0.21 [-0.52; 0.09]

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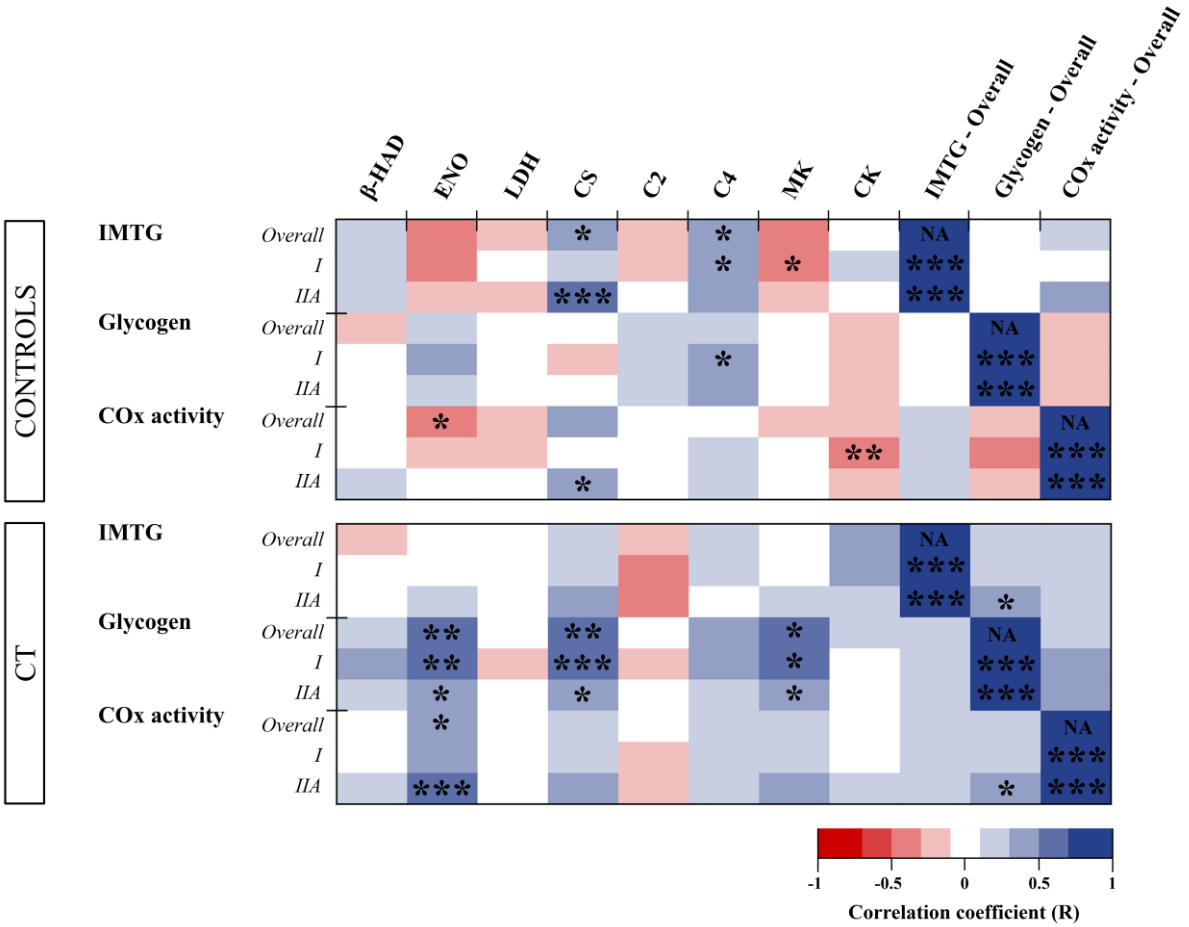
	Controls			CT			Multivariable Analysis					
	Females (n=16)	Males (n=15)	Total (n = 31)	Females (n=16)	Males (n=15)	Total (n = 31)	Group Effect		Sex Effect		Group × Sex	
							p-value	Hedges' g	p-value	Hedges' g	p-value	Hedges' g
Enzyme activities – (IU.mg protein^{-1})												
β-HAD (10^{-3})	198 ± 33	199 ± 40	198 ± 36	179 ± 28	186 ± 23	182 ± 25	0.11	0.22 [-0.05; 0.49]	0.66	0.06 [-0.21; 0.33]	0.83	-0.03 [-0.30; 0.24]
ENO	1.50 ± 0.22	1.96 ± 0.42	1.71 ± 0.40	1.35 ± 0.34	1.86 ± 0.33	1.63 ± 0.42	0.13	0.21 [-0.06; 0.48]	<0.001	0.77 [0.50; 1.04]	0.38	0.12 [-0.15; 0.39]
CS (10^{-3})	52.1 ± 12.0	55.0 ± 13.4	53.5 ± 12.5	37.0 ± 9.5 [a]	49.3 ± 16.0	43.7 ± 14.6	0.010	0.36 [0.09; 0.62]	0.052	0.27 [0.00; 0.53]	0.43	-0.11 [-0.38; 0.16]
CII (10^{-3})	289 ± 22	293 ± 26	291 ± 24	311 ± 25	298 ± 16	304 ± 21	0.078	-0.26 [-0.56; 0.03]	0.49	-0.10 [-0.40; 0.19]	0.36	0.14 [-0.16; 0.43]
CIV (10^{-3})	33.5 ± 9.0	37.9 ± 17.6	35.6 ± 13.6	31.3 ± 8.1	33.0 ± 11.9	32.2 ± 10.2	0.27	0.15 [-0.12; 0.42]	0.35	0.13 [-0.14; 0.39]	0.54	0.08 [-0.19; 0.35]
LDH (10^{-3})	443 ± 43	414 ± 80	430 ± 64	446 ± 55	414 ± 48	428 ± 53	0.62	-0.07 [-0.34; 0.21]	0.052	-0.27 [-0.54; 0.00]	0.92	-0.01 [-0.29; 0.26]
MK	1.53 ± 0.44	1.91 ± 0.53	1.70 ± 0.51	1.19 ± 0.39 [0.067]	1.56 ± 0.43 [a]	1.39 ± 0.45	0.009	0.37 [0.10; 0.65]	0.003	0.42 [0.15; 0.69]	0.66	0.06 [-0.21; 0.33]
CK	11.3 ± 0.6	10.6 ± 1.4	11.0 ± 1.1	10.3 ± 1.1 [b]	10.5 ± 1.4	10.4 ± 1.2	0.076	0.25 [-0.03; 0.52]	0.40	-0.11 [-0.39; 0.16]	0.14	-0.20 [-0.48; 0.07]
β-HAD/CS ratio	4.00 ± 1.09	3.71 ± 0.68	3.87 ± 0.92	5.02 ± 1.15	4.09 ± 1.25	4.54 ± 1.27	0.035	-0.29 [-0.56; -0.02]	0.062	-0.26 [-0.53; 0.01]	0.58	0.08 [-0.19; 0.35]
ENO/CS ratio	30.4 ± 8.3	38.4 ± 16.2	34.1 ± 13.0	37.5 ± 8.8	40.2 ± 10.6	39.0 ± 9.7	0.12	-0.21 [-0.48; 0.06]	0.10	0.22 [-0.04; 0.49]	0.35	0.13 [-0.14; 0.39]
CIV/CII ratio (10^{-3})	119 ± 35	140 ± 51	128 ± 43	104 ± 28	116 ± 42	111 ± 36	0.28	0.16 [-0.13; 0.45]	0.16	0.21 [-0.09; 0.50]	0.50	0.10 [-0.20; 0.39]

β-HAD: β-hydroxyacyl-CoA dehydrogenase; CII and CIV: second and fourth respiratory chain complexes; CAFA: capillary contact per fiber area; CC: capillary contact; CD: capillary density; C/F capillary to fiber ratio; CFPE: capillary to fiber-perimeter exchange; CK: creatine kinase; CS: citrate synthase; CSA: cross-sectional area; CT: subjects with constitutional thinness; ENO: enolase; LDH: lactate dehydrogenase; MK: myokinase; Pe: Perimeter; SF: sharing factor

Units: CD (cap.mm^{-2}), C/F (cap.fib^{-1}), CC (cap.fib^{-1}), CC with SF (cap.fib^{-1}), CFPE (cap.fib^{-1}.µm^{-1}), CAFA (cap.fib^{-1}.µm^{-2})

[a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$ between controls and subjects with constitutional thinness, within gender, from multivariable analysis



β-HAD: β-hydroxyacyl-CoA dehydrogenase; CII and CIV: second and fourth respiratory chain complexes; CK: creatine kinase; COx: cytochrome C oxidase; CS: citrate synthase; CT: subjects with constitutional thinness; ENO: enolase; IMTG: intramuscular triglycerides; LDH: lactate dehydrogenase; MK: myokinase; NA: Not Applicable (correlation within a single variable) – * p<0.05, ** <0.01, *** <0.001

Figure 4: Heatmap of correlations between intramuscular triglycerides, glycogen content, cytochrome C oxidase activity, and enzyme assessments in controls (n=31) and subjects with constitutional thinness (n=30) at baseline

Effect of overfeeding

After 2 weeks of overfeeding, both control and CT groups gained weight ($p<0.001$, 68.6 ± 8.5 kg pre vs. 69.4 ± 9.5 kg post-overfeeding in controls and $p<0.001$, 48.3 ± 6.7 kg pre vs. 48.5 ± 6.5 kg post-overfeeding in CT individuals, $p=0.23$, ES: -0.12 [-0.31; 0.07], for Group×Time interaction) (Table 3). CSA of each muscle fibers was found unchanged in response to overfeeding in both groups. For IMTG indexes, no Group×Time interactions were observed. Yet, the mixed model showed an increase in IMTG of CT group in overall index ($p=0.001$, ES: 0.47 [0.19, 0.75], +11%), type I fibers ($p=0.004$; ES: 0.40 [0.13; 0.68], +10%) and type IIA fibers ($p=0.007$, ES: 0.38 [0.11; 0.65], +9%). All indexes of IMTG were found unchanged in the control group. Glycogen content, COx activity, and capillary supply were found unchanged with overfeeding for all indexes in both control and CT groups, except for C/F ratio (see Table 3). Statistical analysis of CII and CIV enzyme activities showed an absence of significance in Group×Time interaction for CII activity, and a trend for CIV activity ($p=0.078$, ES: 0.18 [-0.02; 0.38]). Yet, CT group tended to increase CII activity ($p=0.054$, ES: 0.31 [0.00; 0.62], +5%) and CIV activity ($p=0.052$, ES: 0.29 [0.00; 0.58], +17%) whereas CII and CIV activities were found unchanged in control group. No effects of overfeeding were observed for β-HAD, ENO, CS, LDH, MK and CK enzyme activities in both groups.

Table 3: Effect of overfeeding

	Controls		CT		Mixed model	
	Pre overfeeding	Post overfeeding	Pre overfeeding	Post overfeeding	Group × Time	
Characteristics of the population					**p-value**	**Hedges' g**
Weight (kg)	68.6 ± 8.5	69.4 ± 9.5 [c]	48.3 ± 6.7	48.5 ± 6.5 [c]	0.23	-0.12 [-0.31; 0.07]
BMI (kg.m^{-2})	23.0 ± 1.1	23.3 ± 1.2 [c]	17.0 ± 0.9	17.1 ± 0.9 [c]	0.46	-0.07 [-0.26; 0.12]
FM (%)	26.4 ± 7.5	26.3 ± 6.8	19.4 ± 4.8	19.2 ± 4.8	0.063	0.18 [-0.01; 0.37]
Total FM (kg)	18.6 ± 5.1	18.8 ± 5.0 [b]	9.7 ± 1.8	9.5 ± 1.7 [a]	0.71	0.04 [-0.15; 0.23]
Total lean mass (kg)	49.8 ± 9.4	50.5 ± 9.4 [c]	39.1 ± 6.9	39.3 ± 7.0 [a]	0.031	-0.21 [-0.40; -0.02]
CSA (µm²)						
Overall	4086 ± 960	3994 ± 1163	3256 ± 818	3478 ± 766	0.087	0.17 [-0.02; 0.37]
Type I	4028 ± 781	3801 ± 855	3167 ± 627	3308 ± 619	0.12	0.15 [-0.04; 0.35]
Type IIA	4385 ± 1172	4384 ± 1419	3658 ± 1218	3781 ± 1076	0.38	0.09 [-0.11; 0.28]
Type IIX	2896 ± 1127	2727 ± 937	2434 ± 925	2368 ± 809	0.67	0.05 [-0.17; 0.27]
IMTG content (AU)						
Overall	25.1 ± 5.4	25.7 ± 6.5	20.9 ± 5.3	23.2 ± 4.1 [b]	0.16	0.14 [-0.06; 0.34]
Type I	30.9 ± 6.3	31.4 ± 6.5	25.6 ± 7.0	28.2 ± 5.8 [b]	0.17	0.14 [-0.06; 0.33]
Type IIA	21.4 ± 4.7	22.5 ± 6.2	18.4 ± 5.1	20.1 ± 3.9 [b]	0.30	0.10 [-0.09; 0.30]
Glycogen content (AU)						
Overall	81.0 ± 10.3	85.4 ± 12.4	79.2 ± 13.2	80.0 ± 11.7	0.57	-0.06 [-0.25; 0.14]
Type I	75.1 ± 13.6	78.5 ± 13.1	70.4 ± 12.1	72.1 ± 10.7	0.85	-0.02 [-0.21; 0.18]
Type IIA	88.0 ± 12.6	88.9 ± 12.5	83.8 ± 13.1	84.8 ± 12.1	0.79	0.03 [-0.17; 0.22]
COx activity (AU)						
Overall	101.1 ± 13.3	103.6 ± 12.1	104.3 ± 11.4	101.2 ± 14.5	0.20	-0.13 [-0.33; 0.07]
Type I	113.1 ± 14.1	114.1 ± 14.7	118.7 ± 15.3	113.2 ± 18.6	0.16	-0.14 [-0.34; 0.06]
Type IIA	95.6 ± 13.9	98.1 ± 10.1	97.3 ± 11.2	94.3 ± 13.8	0.20	-0.13 [-0.33; 0.07]
CAPILLARIZATION – Global Indexes						
CD	314.9 ± 49.8	307.0 ± 66.1 [0.090]	305.2 ± 56.0	307.6 ± 58.5	0.30	0.11 [-0.09; 0.31]
C/F	1.25 ± 0.33	1.23 ± 0.39	1.01 ± 0.24	1.09 ± 0.24 [a]	0.041	0.21 [0.01; 0.41]
CAPILLARIZATION – Local Indexes						
TYPE I FIBER						
CC	4.05 ± 0.69	3.94 ± 1.09	3.34 ± 0.67	3.45 ± 0.62	0.30	0.10 [-0.09; 0.30]
CC with SF	1.42 ± 0.32	1.41 ± 0.48	1.13 ± 0.26	1.19 ± 0.25	0.43	0.08 [-0.12; 0.27]
CFPE (CC/Pe) (10^{-3})	16.62 ± 1.86	16.49 ± 3.12	15.46 ± 2.32	15.41 ± 1.97	0.68	0.04 [-0.15; 0.24]
CAFA (CC/CSA) (10^{-3})	1.07 ± 0.17	1.08 ± 0.21	1.14 ± 0.21	1.09 ± 0.17	0.69	-0.04 [-0.23; 0.16]
TYPE IIA FIBER						
CC	3.87 ± 0.94	3.99 ± 1.13	3.25 ± 0.70	3.44 ± 0.79	0.39	0.09 [-0.11; 0.28]
CC with SF	1.34 ± 0.39	1.43 ± 0.48	1.11 ± 0.29	1.20 ± 0.32 [0.062]	0.68	0.04 [-0.15; 0.24]
CFPE (CC/Pe) (10^{-3})	14.99 ± 2.22	14.91 ± 2.59	13.76 ± 1.96	13.83 ± 2.08	0.51	0.07 [-0.13; 0.26]
CAFA (CC/CSA) (10^{-3})	0.94 ± 0.15	0.90 ± 0.14	0.95 ± 0.17	0.92 ± 0.17	0.68	0.04 [-0.15; 0.24]

(end of the table on the next page)

	Controls		CT		Mixed model	
	Pre overfeeding	Post overfeeding	Pre overfeeding	Post overfeeding	Group × Time	
					p-value	Hedges' g
TYPE IIX FIBER						
CC	2.46 ± 0.52	2.29 ± 0.55 $^{0.093}$	2.21 ± 0.58	2.09 ± 0.42	0.55	0.07 [-0.15; 0.29]
CC with SF	0.81 ± 0.19	0.77 ± 0.20	0.73 ± 0.21	0.69 ± 0.15	0.80	0.03 [-0.19; 0.25]
CFPE (CC/Pe) (10^{-3})	11.70 ± 1.58	11.03 ± 1.83 $^{0.085}$	11.24 ± 1.93	10.90 ± 1.97	0.63	0.05 [-0.17; 0.27]
CAFA (CC/CSA) (10^{-3})	0.99 ± 0.34	0.92 ± 0.21	1.01 ± 0.27	1.01 ± 0.32	0.87	0.02 [-0.20; 0.24]
Enzyme activities – (IU.mg protein^{-1})						
β-HAD (10^{-3})	198 ± 36	205 ± 29	182 ± 25	193 ± 37	0.77	0.03 [-0.17; 0.23]
ENO	1.71 ± 0.40	1.82 ± 0.45	1.63 ± 0.42	1.76 ± 0.71	0.92	0.01 [-0.19; 0.21]
CS (10^{-3})	53.5 ± 12.5	57.6 ± 18.3	43.7 ± 14.6	46.4 ± 14.8	0.93	-0.01 [-0.21; 0.19]
CII (10^{-3})	291 ± 24	299 ± 40	304 ± 21	317 ± 30 $^{0.054}$	0.51	0.07 [-0.14; 0.29]
CIV (10^{-3})	35.6 ± 13.6	33.3 ± 10.5	32.2 ± 10.2	37.7 ± 12.8 $^{0.052}$	0.078	0.18 [-0.02; 0.38]
LDH (10^{-3})	430 ± 64	437 ± 66	428 ± 53	430 ± 53	0.67	-0.04 [-0.25; 0.16]
MK	1.70 ± 0.51	1.86 ± 0.63	1.39 ± 0.45	1.46 ± 0.60	0.57	-0.06 [-0.26; 0.14]
CK	11.0 ± 1.1	11.4 ± 1.1	10.4 ± 1.2	10.8 ± 1.4	0.95	0.01 [-0.19; 0.21]
β-HAD/CS ratio	3.87 ± 0.92	3.93 ± 1.42	4.54 ± 1.27	4.40 ± 1.04	0.72	-0.04 [-0.24; 0.16]
ENO/CS ratio	34.1 ± 13.0	34.5 ± 13.0	39.0 ± 9.7	39.6 ± 13.7	0.69	-0.04 [-0.24; 0.16]
CIV/CII ratio (10^{-3})	128 ± 43	116 ± 37	111 ± 36	123 ± 36	0.15	0.16 [-0.06; 0.38]

β-HAD: β-hydroxyacyl-CoA dehydrogenase; BMI: body mass index; CII and CIV: second and fourth respiratory chain complexes; CAFA: capillary contact per fiber area; CC: capillary contact; CD: capillary density; C/F capillary to fiber ratio; CFPE: capillary to fiber-perimeter exchange; CK: creatine kinase; COx: cytochrome C oxidase; CS: citrate synthase; CSA: cross-sectional area; CT: subjects with constitutional thinness; ENO: enolase; FM: fat mass; IMTG: intramuscular triglycerides; LDH: lactate dehydrogenase; MK: myokinase; Pe: Perimeter; SF: sharing factor

Units: CD (cap.mm^{-2}), C/F (cap.fib^{-1}), CC (cap.fib^{-1}), CC with SF (cap.fib^{-1}), CFPE (cap.fib^{-1}.µm^{-1}), CAFA (cap.fib^{-1}.µm^{-2})

[a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$ between pre and post-overfeeding within controls and subjects with constitutional thinness, from mixed model analysis

Discussion

The main objective of the present study was to investigate muscle energy substrates and muscle phenotype of CT subjects compared to control ones, at baseline and in response to overfeeding. The present work seems to be the first to report low IMTG stores in CT. Individuals with CT presented lower IMTG stores for overall index (p=0.014, -17%), type I (p=0.002, -17%), and type IIA (p=0.048, -14%) muscle fibers, compared to controls. Moreover, CT females seemed to be more affected than CT males

by this low IMTG content. Indeed, CT females showed significantly lower IMTG in overall index and type I fibers than controls (respectively -21% and -20%), unlike CT males whose IMTG content was not significantly lower than controls (respectively -9% and -12%). The main reason for exploring IMTG in CT was the downregulation of FITM1 and FITM2 genes previously observed (Galusca et al. 2018). FITM1 is specific to skeletal muscle whereas FITM2 is expressed in many tissues (Kadereit et al. 2008), both playing a role in the partitioning of newly synthesized triglycerides into lipids droplets (Kadereit et al. 2008). Indeed, overexpression of FITM2 results in IMTG accumulation (Miranda et al. 2011). Thus, present results are consistent with our initial hypothesis and existing literature. From these low IMTG levels observed in CT, low β-HAD enzyme activity could have been expected. Although CT subjects presented much lower values of β-HAD activity; significance was not achieved (182 ± 25 IU.mg protein-1 in CT group vs. 198 ± 36 IU.mg protein-1 in controls, p=0.11, -8%), likely due to the high variability of this type of enzyme assessments. The low IMTG content of CT individuals observed for the first time here might be in agreement with their low muscle oxidative profile (Galusca et al. 2018), but should be further investigated in future works. A lower expression in CT of perilipins (PLIN) that have many different roles in the regulation of IMTG synthesis and lipid droplets growth (Morales et al. 2017) would for instance support present observations. It could also be of interest to assess AMP-activated protein kinase (AMPK), the key "fuel sensing" system playing a crucial role in the activation of fatty acid metabolism (Hardie 2004), and its signaling cascades (in particular acetyl-CoA carboxylase (ACC), malonyl-CoA and carnitine palmitoyltransferase-1 (CPT1) (Winder 2001; Saha and Ruderman 2003)).

On the other hand, CT participants also showed a lower glycogen content compared to controls in type I (p=0.008, -6%) and IIA fibers (p=0.015, -5%), and a trend towards a lower glycogen content in overall index (p=0.071). This lower glycogen content was found in overall index, type I fibers and type IIA fibers for CT females (respectively -8%, -8%, and -7%), but only for type I fibers in CT males (-6%) despite a

tendency for type IIA fibers (-4%). Clinically, these results should however be interpreted with caution since, although significant, the differences remain small. Besides, ENO enzyme of glycolysis was not found significantly lower in skeletal muscle of CT subjects. Carbohydrate supplementation and physical training that are known to impact muscle glycogen content (Hearris et al. 2018) would not, however, explain the results since CT and control participants had normal energy intakes with the same distribution of macronutrients and were all recruited without engagement in regular intense physical activity (according to the MOSPA questionnaire, and no more than 3 sessions of physical activity per week) (Ling et al. 2016, 2019). PAL, measured for the first time in CT males in the present study, was found lower compared to control males. In contrast, PAL in CT females was found similar to control females, as already reported (Bossu et al. 2007; Galusca et al. 2018). Although unexpected, these results further refute the potential implication of high energy expenditure through over-exercising in the low BMI of CT participants, since on the contrary, CT participants presented a similar PAL (females) or a lower PAL (males) compared to controls.

As a second main result, CT subjects displayed an unusual muscle phenotype. Firstly, a muscle hypotrophy was observed for all fiber types of CT individuals, in agreement with previous findings (Galusca et al. 2018). In CT, CSA was found -21%, -17% and -16% lower, for type I, IIA and IIX fibers respectively, with strong statistical power. Overall mean CSA was also largely lower (<0.001, -20%) in CT. In view of the statistical results, muscle hypotrophy was even more pronounced in CT males than in CT females (-30%, -22%, -26% and -27% for males and -12%, -16%, -21% and -16% for females, in type I, IIA, IIX fibers and overall mean CSA respectively). This may suggest the presence of a minimum threshold in fiber size of "healthy" individuals who are not suffering from myopathy. A lower percentage of area occupied by type I fibers (p=0.044) was observed in CT and fiber type composition revealed a higher

percentage of type IIX fibers in CT (p=0.033), suggesting a low oxidative profile. Fiber distribution should however be considered with caution given the high variability in the biopsied fibers of a 150 mg sample.

In addition, present results showed a lower capillary supply in CT. C/F ratio, a global index of capillary supply, was found lower in subjects with CT. The normal CD observed in CT group was easily explained by their low C/F ratio which tended to decrease CD, concomitantly with their smaller fibers that tended to increase CD. Concerning local indexes, the low capillarization of CT subjects affected all fiber types, but with a higher statistical power in type I oxidative fibers. Since capillaries are determinant factors of dioxygen and nutrients supply, present results support once more a low oxidative capacity in CT. Yet, CAFA index (CC/CSA), which reflects a supply diffusion distance, contrasted with other local indexes: no statistical differences were found between CT subjects and controls. This, therefore, raises the hypothesis that the low capillary supply observed in CT individuals might only reflect an adaptation to their small fiber size. In addition, capillaries do not only provide oxygen supply but are also implied in the control of muscle mass. Indeed, satellite cells are localized close to blood vessels and are correlated with muscle capillarization (Mounier et al. 2011). Then, it could also be relevant to explore whether satellite cells would be less abundant in CT.

CS enzyme activity was found lower in CT participants (p=0.010) even if no alterations of the mitochondrial respiratory chain were demonstrated here. Indeed, CIV activity determined with spectrophotometry and COx activity determined with histological methods were both found similar to controls in CT participants. A trend toward a higher CII activity (p=0.078) was however observed. Previous observations on the same cohort however showed a tendency toward lower first complex of the respiratory chain (CI) and CII activities using oxygraphy on fresh skeletal muscle in CT (Ling et al. 2019), while a higher CII activity but similar CI activity compared to controls were observed in white adipose

tissue (Ling et al. 2019). Further studies are needed to question mitochondrial respiration regarding these differing results. Rapid muscle regeneration of ATP was also explored in the present study and results showed a lower MK activity ($p=0.009$) and a tendency for CK activity to be low ($p=0.076$) in CT. Thus, CT people could display impairments in their anaerobic alactic metabolism involving ADP-to-ATP conversions and hydrolysis of phosphocreatine which both provide rapid regeneration of energy. Analysis of correlations also showed different profiles between CT and control groups (Figure 4). In the control group, CS activity was unsurprisingly positively correlated with IMTG storage and COx activity, and COx activity was negatively associated with ENO activity. In contrast, for CT participants, CS was positively correlated with glycogen content, and COx activity was positively associated with ENO and glycogen content. These correlations need to be interpreted with caution regarding their moderate-to-large correlation coefficients but would suggest an unusual muscle metabolic profile in CT individuals, mostly based on carbohydrate metabolic pathways to support mitochondrial oxidative activity. Although some studies suggest a similar fasting respiratory quotient at rest between CT and normal-weight individuals (Scalfi et al. 1992; Bossu et al. 2007; Marra et al. 2007, 2019; Germain et al. 2014; Galusca et al. 2018; Ling et al. 2019); this substrates utilization remains to be further questioned during daily activities and exercise. These enzyme results should be cautiously interpreted given the technical limitations: enzyme assessments were performed on homogenates of muscle samples and were therefore inevitably impacted by fiber type distribution. Yet, these homogenates are still representative of the enzyme efficiency of the whole muscle sample.

Altogether, present results suggest a low muscle oxidative profile in CT. In future investigations, it could be of major interest to question the potential downregulation of key regulators of oxidative metabolism – such as peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) (Liang and Ward 2006) – to further explore this untypical metabolic profile in CT.

For the first time, results showed that present overfeeding succeeded in making people with CT gain weight ($p<0.001$). This result however needs to be interpreted with caution from a clinical point of view: CT individuals only went from 48.3 ± 6.7 kg pre to 48.5 ± 6.5 kg post-overfeeding. To the best of our knowledge, the present study was the first to investigate the effect of an overfeeding on skeletal muscle tissue in CT compared to controls. Based on literature, different results could have been expected. For instance, recent studies in normal-weight individuals showed contrasting results on skeletal muscle in response to 8 weeks of high-fat overfeeding. Toledo et al. observed an increase in lipid droplet content and CS mRNA expression but no changes in COx mRNA expression (Toledo et al. 2018); whereas Covington et al. observed no modifications of IMTG content (Covington et al. 2017). In the present study, normal-weight controls did not modify their IMTG stores for any of the indexes, but in contrast, CT individuals increased their IMTG in overall index ($p=0.001$, +11%), type I fibers ($p=0.004$; +10%) and type IIA fibers ($p=0.007$, +9%). If this increase might have been facilitated by their low IMTG at baseline, it remains that CT individuals showed an increase in their IMTG content. CII and CIV activities also tended to be increased in the CT group in response to overfeeding (respectively $p=0.054$, +5% and $p=0.052$, +17%), which might be related to their increase in IMTG. IMTG accumulation might be associated with higher mitochondrial oxidation from lipid substrates, but it would therefore be difficult to explain the absence of an increase in both β-HAD and CS activities. CII and CIV results must be interpreted with caution given the absence of significant Group×Time interactions (respectively $p=0.51$, 0.07 [-0.14; 0.29] and $p=0.078$, 0.18 [-0.02; 0.38]). The other analyses such as glycogen content, capillary supply, CS or COx activities, do not seem to be particularly modified by the overfeeding for either CT or control participants.

From a methodological point of view, some limitations in the present study should be considered. Food intake was recorded using self-reported 2×7-day food diaries in free-living conditions, pre and post-overfeeding (Ling et al. 2016). As previously published (Ling et al. 2020), baseline food intake was not

lower in CT individuals compared to controls, and both CT and control groups increased food intake with overfeeding. However, food intake assessed through self-reporting (using GENI software and SUVIMAX study) is known to be subjective. More objective methods should be used in the future. In addition, the diet has been reported during the 2nd week of overfeeding, and not during the whole period. PAL was measured by ActiHeart® providing estimations of PAL, based on its capacity to detect and quantify movements. This accelerometer is not necessarily the most accurate and appropriate to evaluate all types of physical activities and sedentary lifestyles. It probably led to underestimations of PAL, especially for spontaneous and repeated muscle contractions in daily life, such as fidgeting. Yet, literature shows a growing interest in potential implications of fidgeting in CT (Marra et al. 2007; Pasanisi et al. 2013; Germain et al. 2014), which should be more investigated in future studies.

To conclude, this was the first study that performed skeletal muscle biopsies in a large sample of both females and males with CT. The results provided evidence of low IMTG and glycogen stores, low CSA reflecting muscle hypotrophy, high proportions of type IIX fibers, low capillary supply, and specific enzyme features including a low CS activity in CT participants. Most of the results suggested a low muscle oxidative profile in CT that might be indicative of low aerobic capacity. In response to overfeeding, few modifications specific to CT groups were observed, except a moderate increase in IMTG, CII activity, and CIV activity, that have however to be interpreted with caution. Present findings raised the question of the functional consequences in real conditions of these histological and enzymological observations. It would be of particular interest to evaluate aerobic and anaerobic capacities, substrates oxidation, and metabolic flexibility in CT. From a clinical approach, the fact remains that CT individuals have a very low BMI, and physical activity combined with a diet may help people with CT gain weight. Thus, a better knowledge of CT subjects' physical abilities might make it possible to provide better care for this population.

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Supplementary Table S1: Composition of the Renutryl® Booster overfeeding

Renutryl® Booster (300 ml)		
Energy	kcal	600
Fat	g	21
31.5% kcal		
Carbohydrate	g	72
48.5 % kcal		
of which sugars	g	21
of which lactose	g	<1.5
Protein	g	30
20 % kcal		
Minerals		
Sodium	mg	285
Potassium	mg	720
Calcium	mg	687
Phosphate	mg	459

Renutryl® Booster is a hyper-caloric (2 kcal/mL) oral nutritional supplement providing macronutrients and micronutrients. Four flavors (vanilla, coffee, caramel, and strawberry) were proposed to the participants

PARTIE III – DISCUSSION GÉNÉRALE

Recontextualisation

Alors que plusieurs centaines de milliers d'études cliniques relatives à l'obésité ont été publiées à ce jour, la MC reste extrêmement peu explorée. Une quarantaine d'études cliniques seulement ont investigué ce trouble pondéral, souvent qualifié de situation « miroir » à l'obésité. Pourtant, les premiers écrits scientifiques relatant l'existence d'une maigreur d'origine physiologique sont anciens puisqu'ils remonteraient, d'après nos recherches, aux années 1930 (Grafe 1933). Les articles relatifs à la MC débutent généralement en définissant la MC comme une condition physiologique naturelle de forte maigreur sans causes pathologiques ou troubles alimentaires. Le manque de recul clinique souligne cependant la nécessité d'explorer davantage cette population particulière. Par ailleurs, le manque de connaissances engendre un manque de reconnaissance de la MC à l'origine d'une stigmatisation sociale forte et d'un manque de considération médicale. La revue de littérature a également mis en avant le risque accru d'erreur de diagnostic différentiel entre MC et AM (Estour et al. 2014, 2018). Finalement, nous avons observé que la définition même de la MC apparaissait très ambiguë et plurielle d'un point de vue tant sémantique que clinique. La revue de littérature a également permis de donner un premier aperçu des critères de diagnostic de la MC utilisés dans la littérature, révélant là encore des approches très hétérogènes.

Fort de ces constats, le premier axe de ce travail de doctorat s'est porté sur la définition et le diagnostic de la MC, permettant finalement d'établir un arbre décisionnel d'aide au diagnostic de la MC. Cet arbre décisionnel s'est fondé sur la revue systématique des critères de diagnostic utilisés pour l'inclusion des participants MC dans les études cliniques publiées à ce jour. Si cet outil d'aide au diagnostic a constitué une première étape dans la caractérisation de la MC, il est néanmoins apparu logique, dans une seconde étape, de procéder à l'analyse des résultats de ces études cliniques. Au regard de notre champ d'expertise et des différents résultats issus de l'**axe 1** suggérant un métabolisme musculaire spécifique

dans la MC, les travaux de thèse se sont portés, dans un **2nd axe**, sur l'exploration cellulaire du tissu musculaire de sujets présentant une MC. Étant donné certaines limites méthodologiques des techniques immunohistochimiques actuellement utilisées, une étude préliminaire de mise au point méthodologique a été réalisée dans un premier temps afin de faciliter l'approche histologique musculaire.

Si l'ensemble des résultats a été discuté dans chacune des publications, il nous semble pertinent dans cette partie de discuter ceux qui nous apparaissent comme étant les plus importants et pouvant apporter de nouveaux éléments dans l'approche clinique et physiologique de la MC.

Discussion

Le **1er axe** de ce travail de thèse a tout d'abord mis en exergue une forte hétérogénéité des critères de diagnostic de la MC utilisés par les études cliniques, soulignant l'importance d'harmoniser nos approches. Cela constitue non seulement un enjeu majeur en termes de diagnostic clinique, mais également en ce qui concerne la possibilité de comparer les résultats des différentes études cliniques. Avant même de discuter la définition et les critères de diagnostic de la MC, il nous semble important de discuter les enjeux sémantiques de l'appellation même de la « maigreur constitutionnelle ». L'**étude 1** a effectivement révélé une approche sémantique non consensuelle entre les études parmi les 35 études finalement inclues dans l'analyse (689 références retenues après élimination des doublons, 199 études exclues sur la base de leur titre, 164 sur la base de leur résumé, 291 après lecture de l'article). Pourtant, il semble primordial d'harmoniser la terminologie relative à la MC, non seulement pour faciliter la communication scientifique, mais également afin que les moteurs de recherche bibliographique puissent référencer et identifier les études relatives à la MC. En effet, une étude sur cinq n'utilise aucune terminologie spécifique (Scalfi et al. 1992; Hinney et al. 1997; Fernández-García et al. 2009) tandis que les critères utilisés semblent correspondre à un contexte de MC. L'identification bibliographique de ces références se révèle dès lors très complexe. Si la nécessité d'adopter une terminologie commune apparaît évidente, deux appellations différentes émergent cependant de la littérature (**étude 1**) : « constitutional thinness » (minceur constitutionnelle) et « constitutional leanness » (maigreur constitutionnelle) (Bossu et al. 2007; Galusca et al. 2018; Ling et al. 2019). Il est à noter que le terme « leanness » se réfère à la « maigreur », ce qui suggère une forte quantité de MM, et par conséquence une faible quantité de masse grasse. Néanmoins, l'approche méta-analytique de notre **étude 2** a montré la situation opposée : les sujets MC présenteraient une MM extrêmement diminuée pour un pourcentage de masse grasse relativement élevé au regard de leur sous-poids important. D'après notre

étude 2 centrée sur l'analyse de la composition corporelle, il semble donc que l'appellation de la littérature anglophone « constitutional leanness » soit peu appropriée. L'appellation « constitutional thinness » nous semble ainsi préférable. À notre connaissance, une seule désignation est utilisée dans la littérature française (« maigreur constitutionnelle ») et cette désignation apparaît ainsi relativement inappropriée vis-à-vis des résultats de composition corporelle de l'**étude 2** que nous venons de mentionner. La question se pose donc : faut-il cesser d'utiliser la dénomination de « maigreur » constitutionnelle pour lui préférer, par exemple, l'appellation peu élégante de « sous-poids » constitutionnel ? Notons cependant qu'un tel changement risquerait d'induire une confusion supplémentaire.

Au-delà de la terminologie, l'**étude 1** a également révélé des approches diverses en ce qui concerne la définition de la notion de maigreur. Les paramètres et les seuils utilisés sont apparus nombreux dans la littérature. De façon surprenante, l'**étude 1** a même montré qu'une étude sur cinq ne semble pas donner de critère spécifique pour définir la notion de maigreur dans ses critères d'inclusion (Miljic et al. 2006; Marra et al. 2007; Santonicola et al. 2012; Pasanisi et al. 2013). Bien que l'utilisation de l'IMC connaisse de nombreuses limitations (Maffetone et al. 2017), ce critère très simple à mettre en place nous semble constituer une première approche pertinente de la notion de sous-poids. Soixante-trois pour cent des études systématiquement analysées (**étude 1**) ont utilisé un seuil d'IMC pour définir la maigreur, avec des seuils cependant très variables allant de 16.5 kg/m² (Bossu et al. 2007; Germain et al. 2009; Galusca et al. 2015) à 20.0 kg/m² (Petretta et al. 1997; Margaritelis et al. 2019). Malgré le caractère très arbitraire de la définition d'un seuil « strict », nous recommandons de ne pas aborder la possibilité d'une MC pour un IMC excédant 18.5 kg/m², d'après les seuils de maigreur utilisés par l'OMS (World Health Organization Expert Committee 1995; World Health Organization 2019). Si certaines études ont utilisé comme critère de maigreur un faible pourcentage de masse grasse (Diaz et al. 1992; Margaritelis et al. 2019), nous

déconseillons cependant fortement l'utilisation de ce critère. En effet, notre **étude 2** a montré, au contraire, que la MC serait caractérisée par des pourcentages de masse grasse relativement élevés au regard de leur important sous-poids. Les sujets MC présentent des pourcentages de masse grasse très largement supérieurs aux patients AM (**étude 2**). Ainsi, un pourcentage de masse grasse extrêmement faible pourrait au contraire aller davantage dans le sens d'un diagnostic d'AM plutôt que de MC. D'autres études ont également utilisé une classification de l'état de maigreur par rapport à des silhouettes (Slof et al. 2003; Mazzeo et al. 2004). Nous déconseillons également l'utilisation de cette méthode étant donné l'inclusion de sujets présentant des IMC de 20.3 kg/m² chez les femmes (Slof et al. 2003) et de 22.5 kg/m² chez les hommes (Mazzeo et al. 2004) dans les deux études l'ayant mise en place. L'utilisation de cette méthode semble donc incompatible avec un diagnostic de MC. Au-delà de la considération de la maigreur à l'instant t de l'étude clinique ou de la consultation, l'historique du sous-poids nous semble être également un critère d'importance, notamment dans le diagnostic différentiel entre AM et MC. L'**étude 1** a montré que 71 % des études requièrent une stabilité pondérale sans amaigrissement soudain dans l'histoire pondérale lors de l'inclusion des sujets MC. Contrairement à l'AM pour laquelle une cassure de la courbe pondérale est généralement nettement observée à la déclaration de la maladie, la MC semble se caractériser par un état de sous-poids ayant toujours été présent sans déclenchement soudain (Bossu et al. 2007; Estour et al. 2014, 2017).

Ainsi que cela a été souligné en revue de la littérature de ce travail de thèse, le diagnostic différentiel entre MC et AM représente effectivement un enjeu majeur du diagnostic de la MC. L'**étude 1** rapporte que 91 % des études vérifient l'absence de troubles alimentaires, ce qui souligne l'importance de ce paramètre. Les méthodes d'évaluation de ce paramètre apparaissent en revanche diverses : différents questionnaires, différents types d'interview, et plus rarement, un dosage de marqueurs potentiels de sous-nutrition tels qu'IGF-1, β-estradiol, FT3, cortisol ou leptine. En 2017, il a été montré que le score de

restriction cognitive du questionnaire DEBQ constituait un marqueur présentant une forte spécificité et sensibilité dans la différenciation entre la MC et l'AM (Estour et al. 2017). Il est par ailleurs intéressant de constater que l'utilisation d'une méthode subjective et déclarative telle qu'un questionnaire permet de différencier ces deux populations. Si nous devions conseiller l'utilisation d'un questionnaire en particulier, et plus spécifiquement d'un critère spécifique, nous conseillerions ainsi l'utilisation de l'échelle de la restriction cognitive alimentaire du questionnaire DEBQ dans le diagnostic différentiel de la MC et de l'AM. Si cela est possible, il nous semble néanmoins important de réaliser un bilan biochimique pour s'assurer du diagnostic. En effet, la puissance statistique de la méta-analyse de notre **étude 3** a confirmé que le sujet MC présente un bilan sanguin extrêmement différent de celui du patient AM. Face au scepticisme encore présent vis-à-vis de la MC, y compris dans la sphère médicale, ces importantes différences de bilans biochimiques entre le sujet MC et le patient AM constituent un argument majeur de l'existence de la MC. Si certains experts rappellent qu'il est possible pour un patient AM de parvenir à duper les soignants lors de la consultation clinique, le bilan sanguin du patient ne peut pas masquer en revanche la carence nutritionnelle du patient AM. L'**étude 3** méta-analytique a effectivement révélé que de nombreux paramètres différenciaient la MC de l'AM, tels que les taux de FT3, cortisol, IGF-1, leptine, estradiol, GH, FSH, et LH. L'analyse a montré que les patients AM présentaient des taux plus faibles de FT3, IGF-1, leptine, estradiol, FSH et LH ainsi que des taux plus élevés de cortisol et GH que les sujets T et MC. Par ailleurs, en 2017, il a été montré que les marqueurs de la FT3 et de la leptine seraient particulièrement discriminants entre la MC et l'AM (Estour et al. 2017). La méta-analyse de notre **étude 3** a confirmé que les participants MC et T présentaient des taux similaires d'IGF-1, estradiol, GH, FSH, LH et SHBG. Concernant les taux de FT3 et de cortisol, l'**étude 3** a en revanche, pour la première fois, montré des résultats inattendus probablement dus à notre approche méta-analytique et à la taille importante de notre échantillon (jusqu'à n=1 218 participants MC, T et AM confondus sur

certaines variables). Alors que toutes les études rapportaient individuellement des taux similaires entre les sujets MC et T de FT3 et de cortisol, la méta-analyse a mis en évidence des niveaux de FT3 supérieurs et de cortisol inférieurs chez les sujets MC comparativement aux sujets T. Ainsi, le sujet MC présente des niveaux de FT3 et de cortisol si éloignés de ceux du sujet AM qu'ils apparaissent même différents du sujet T.

La FT3 nous semble être un marqueur auquel il faudrait s'intéresser de façon un peu plus approfondie. En effet, cette hormone semble être liée au statut nutritionnel puisque l'AM, par exemple, se caractérise par une diminution importante de FT3 (Miyai et al. 1975). Cette diminution de FT3 semble liée à la dénutrition puisque la renutrition entraîne une augmentation de FT3 chez des patientes AM et qu'une corrélation positive a été montrée entre les taux de FT3 et le pourcentage du poids idéal atteint après renutrition (Leslie et al. 1978). La baisse de FT3 dans le cadre de l'anorexie pourrait être un moyen adaptatif pour économiser l'énergie puisqu'une corrélation existe entre les taux de FT3 et le métabolisme de repos (Onur et al. 2005). Ainsi, l'augmentation discrète mais significative du taux de FT3 que nous avons mise en évidence dans notre **étude 3** chez le sujet MC pourrait en partie expliquer l'augmentation de la DER ajustée à la MM rapportée dans certaines études (Bossu et al. 2007; Marra et al. 2019; Ling et al. 2020) et participer au maintien d'un faible poids. Nos résultats montrent en effet que les taux de FT3 tendraient à être corrélés à la DER ($R=0.35$, $p=0.07$) chez les sujets MC, tandis que cette corrélation n'est pas obtenue chez les sujets T. En effet, ainsi que présenté dans plusieurs revues de littérature (Simonides and van Hardeveld 2008; Salvatore et al. 2014), des liens étroits existent entre les hormones thyroïdiennes et le muscle. Il a par exemple été montré que les hormones thyroïdiennes, et plus particulièrement la T3, étaient capables d'activer l'expression de la protéine 3 de découplage mitochondrial (uncoupling protein 3 (UCP3)) et de moduler à la hausse le métabolisme de repos (de Lange et al. 2001; Solanes et al. 2005). Si les résultats méta-analytiques des taux de FT3 et cortisol

soulèvent de nouveaux questionnements sur la physiologie du sujet MC, ils apparaissent toutefois comme des marqueurs de diagnostic particulièrement discriminants entre la MC et l'AM. À partir de ces différents éléments, nous conseillons très fortement l'utilisation d'un bilan sanguin dans le diagnostic de la MC. Si nous devions sélectionner les marqueurs qui nous apparaissent comme étant les plus discriminants à partir de notre **étude 3** et de l'étude d'Estour et collaborateurs (Estour et al. 2017), nous conseillerions le dosage de FT3, leptine et cortisol. S'il n'était possible de ne réaliser qu'un seul de ces dosages, nous privilégierons le dosage de FT3 dans la mesure où ce marqueur apparaît non seulement très discriminant, mais également assez accessible et peu coûteux.

La présence de cycles menstruels serait également un critère d'intérêt dans le diagnostic d'une MC chez la femme. D'après notre **étude 1**, 80 % des études ayant inclus des femmes ont vérifié la présence des menstruations pour l'inclusion de femmes MC, ce qui représente une forte prise en compte de ce critère. Néanmoins, le diagnostic différentiel entre la MC et l'AM ne peut pas être uniquement basé sur ce critère dans la mesure où le critère d'aménorrhée a disparu du diagnostic de l'AM dans la 5ème édition du DSM parue en mai 2013 (American Psychiatric Association 2013). Ce faisant, la présence des cycles menstruels chez les femmes MC est devenue un critère nécessaire mais non suffisant pour différencier la MC de l'AM.

Par ailleurs, il nous semble également important pour un diagnostic de MC de considérer l'absence de dépense énergétique accrue pouvant être due, par exemple, à un niveau d'activité physique (NAP) élevé. L'**étude 1** a pourtant mis en avant que moins de la moitié des études considère ce paramètre. De plus, lorsqu'il est considéré, ce critère de non pratique excessive d'activité physique est généralement simplement mentionné sans détails supplémentaires quant à la méthodologie utilisée (Scalfi et al. 1992; Bossu et al. 2007; Marra et al. 2007). Une hétérogénéité des seuils de durée, fréquence et intensité utilisés

pour définir ce qu'est une pratique physique « non excessive » est à noter également. De rares études ont explicité leur approche méthodologique dans l'évaluation de l'absence d'activité physique excessive et certaines (Germain et al. 2014; Galusca et al. 2015; Ling et al. 2016) rapportent avoir utilisé le questionnaire MOSPA (Iqbal et al. 2006), dont la pertinence méthodologique reste discutable. Ces observations soulignent non seulement la nécessité d'attester l'absence de dépense énergétique excessive *via* l'activité physique mais témoignent également du besoin d'harmoniser nos approches méthodologiques en ce sens avec des méthodes peut-être plus objectives que les questionnaires. L'eau doublement marquée pourrait être une méthode très intéressante et a d'ailleurs été utilisée lors d'une étude sur la MC (Bossu et al. 2007), mais elle souffre de son coût exorbitant et ne donne qu'une dépense énergétique globale. D'autres méthodes telles que celles basées sur l'accélérométrie permettent un bon compromis entre coût et précision de la mesure, et ont également pour avantage de permettre une analyse plus fine du NAP des sujets. Quelques études ont, à notre connaissance, analysé la dépense énergétique des sujets *via* l'utilisation d'accéléromètres (Germain et al. 2014; Galusca et al. 2018). Cependant, les données ont essentiellement été exploitées dans le but d'évaluer la dépense énergétique. Une seule étude (Galusca et al. 2018) aurait présenté des données relatives aux temps passés aux différentes intensités d'activité physique, en utilisant des seuils néanmoins discutables (Tremblay et al. 2017). Il nous semble qu'une étude plus fine des temps passés aux différentes intensités d'activité physique couplée à l'analyse des comportements sédentaires (temps passé allongé, incliné, assis) pourrait permettre une caractérisation plus précise du NAP des sujets MC.

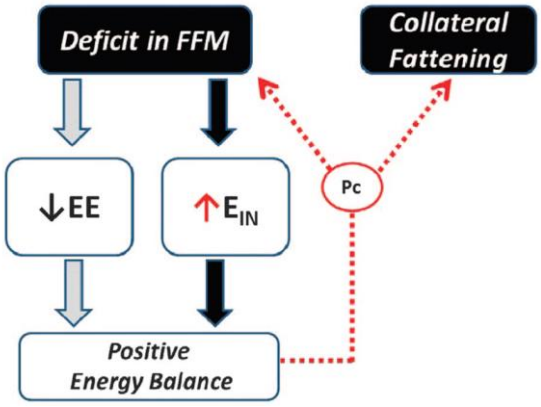
Enfin, un peu moins de la moitié des études analysées par l'**étude 1** font allusion de façon plus ou moins directe à la difficulté de prise de poids des sujets MC dans leur inclusion, même si les sujets sont souvent recrutés parmi les personnes maigres qui consultent en raison de leur souhait de prendre du poids (Bossu et al. 2007; Galusca et al. 2008; Germain et al. 2014; Ling et al. 2016; Estour et al. 2017). Néanmoins,

ce critère peut être ambigu puisque la MC n'est pas nécessairement systématiquement associée à un désir accru de prise de poids. La préoccupation pour le poids et l'alimentation de l'individu MC est similaire à celle du sujet T, contrairement au patient AM chez qui cette préoccupation est forte (Estour et al. 2017). À notre sens, si le désir marqué de prise de poids semble exclure le diagnostic d'une AM, l'absence de désir marqué de prise de poids ne constitue pas pour autant un critère suffisant pour réfuter la possibilité d'une MC.

Au-delà des aspects de diagnostic, ce travail de thèse s'est également dirigé vers l'analyse de la physiologie du sujet MC, en commençant par l'étude de l'anthropométrie et de la composition corporelle. À cette fin, l'**étude 3** a mené à l'identification de 1 212 références ; 402 doublons ont été éliminés, 381 articles ont été exclus à la lecture du titre ou du résumé et 390 références ont été exclues après lecture de l'article sur la base des critères d'éligibilité. Finalement, 39 études cliniques ont été inclues dans l'analyse. La revue systématique de ces études a mis en exergue la diversité des domaines d'études couverts par ces études ainsi que l'équivocité des résultats. Si la méta-analyse a logiquement confirmé le faible poids et le faible IMC caractérisant la MC, elle a cependant soulevé une différence significative de poids et d'IMC entre la MC et l'AM. Alors que 75 % et 68 % des études observaient respectivement un poids (Tolle et al. 2003; Marra et al. 2007; Pasanisi et al. 2013) et un IMC (Bossu et al. 2007; Germain et al. 2007; Estour et al. 2017) similaires entre les sujets MC et AM, la robustesse d'échantillon de la méta-analyse a cependant montré que les sujets MC présentaient un poids et un IMC légèrement mais significativement supérieurs aux patients AM. Bien que la MC et l'AM se caractérisent en effet par un état de sous-poids important, il semble que les patients AM soient encore plus sévèrement touchés par cet état de maigreur que les personnes MC, ce qui peut s'interpréter au regard du caractère pathologique de l'AM, mais également vis-à-vis d'un potentiel biais d'appariement pondéral à l'inclusion des sujets MC et AM dans les études.

De façon intéressante, l'**étude 2** a révélé que les participants MC ne présentaient pas d'importante diminution de leur pourcentage de masse grasse en dépit de leur état de sous-poids important. Ce résultat s'est basé sur l'analyse de 691 sujets (MC : n=205 ; T : n=228 ; AM : n=258). Bien que l'**étude 2** ait montré un pourcentage de masse grasse inférieur chez les sujets MC comparativement aux sujets T, le pourcentage de masse grasse est largement supérieur chez les sujets MC comparativement aux patients AM. En effet, l'analyse montre un pourcentage de masse grasse de l'ordre de 19 % chez les participantes MC alors qu'il avoisine les 11 % chez les patientes AM. Les valeurs des sujets MC apparaissent ainsi relativement dans les normes (Apfelbaum and Sachet 1982; Guy-Grand and Basdevant 1982; Jeukendrup and Gleeson 2010; Branco et al. 2018). Par ailleurs, une étude s'est spécifiquement intéressée à l'étude cellulaire du tissu adipeux chez les sujets MC (Ling et al. 2019). Cette étude a mis en évidence des adipocytes de faible taille avec une capacité respiratoire accrue chez les participants MC (Ling et al. 2019). Si la MC était effectivement caractérisée par un tissu adipeux relativement important (au regard du sous-poids important), dont la capacité oxydative serait de surcroît accrue, cela laisserait envisager une forte implication de la masse grasse dans la dépense énergétique de ces sujets. Notre **étude 2** montre également que, contrairement au pourcentage de masse grasse, la MM des individus MC serait en revanche très diminuée, dans des proportions similaires à celle des patientes AM. Les valeurs absolues de MM seraient même légèrement inférieures chez les participantes MC *vs.* AM (33.9 *vs.* 34.7 kg – **étude 2**), malgré un seuil de significativité non atteint. Finalement, la MC serait ainsi caractérisée par une MM très diminuée et une masse grasse au contraire très peu diminuée au regard du sous-poids important. Étant donné les nombreuses implications métaboliques de la masse grasse et de la MM (Nelson et al. 1992; Bosy-Westphal et al. 2009; Müller et al. 2009), les observations rapportées par l'**étude 2** questionnent plus spécifiquement les caractéristiques métaboliques et physiologiques des

sujets MC. Cette observation peut notamment faire penser au concept du « collateral fattening » (**Figure 27**) décrit et développé par les travaux de Dulloo et collaborateurs (Dulloo et al. 2016, 2018).



EE : dépense énergétique, E_{IN} : apports énergétiques, FFM : masse maigre, Pc : caractéristique « lean-to-fat partitioning » de l'individu

Issu de publication (Dulloo et al. 2016)

FIGURE 27 – CONCEPT DU « COLLATERAL FATTENING »

Le concept de « collateral fattening » veut qu'un déficit de MM se traduise non seulement par une dépense énergétique plus faible impliquant des besoins énergétiques moindres pour le maintien du poids, mais également par l'activation d'une boucle de rétroaction qui entraîne une augmentation des apports énergétiques dans une tentative de rétablissement de la MM (par le biais d'une caractéristique de « lean-to-fat partitioning » propre à l'individu) et pouvant résulter en un « collateral fattening » (**Figure 27**). Bien que Dulloo décrive ce concept de « collateral fattening » dans un contexte d'autorégulation de la composition corporelle pendant la récupération de poids (régimes « yoyo ») bien différent d'un contexte de MC, on pourrait imaginer qu'un mécanisme similaire puissent expliquer la composition corporelle du sujet MC. Une éventuelle résistance à l'anabolisme musculaire chez le sujet MC pourrait entretenir cette boucle d'autorégulation et ainsi entretenir le « collateral fattening » (**Figure 27**). L'**étude 2** mène ainsi à de nouveaux questionnements quant à de potentielles caractéristiques de

« lean-to-fat partitioning » spécifiques chez cette population mais soulève également la nécessité d'investiguer finement la balance énergétique des sujets MC.

Si la méta-analyse de notre **étude 3** confirme la normalité des apports énergétiques de ces sujets, tant quantitativement que qualitativement, la DET serait en revanche plutôt réduite chez les sujets MC *vs.* T, bien que ce résultat nécessite d'être confirmé par un plus grand nombre d'études. La DER absolue est également observée plus faible chez les sujets MC *vs.* T, ce qui apparaît cohérent étant donné la faible MM des sujets MC. Néanmoins, une fois ramenée à la MM, la DER tend à être supérieure chez les sujets MC *vs.* T (p=0.083) d'après notre approche méta-analytique (**étude 3**), ce qui suggérerait une importante capacité métabolique de la MM du sujet MC. Il est par ailleurs intéressant de noter que, pour une MM aussi diminuée que celle d'un patient AM, le sujet MC présente cependant une DER largement supérieure à celle d'un patient AM (**étude 3**). Ces différents éléments tendraient ainsi à confirmer la forte activité métabolique de la MM des sujets MC, renforçant encore davantage la nécessité d'investiguer le tissu musculaire, principale composante de la MM (Strugnell et al. 2014).

Finalement, cet **axe 1** a permis de proposer des recommandations quant à la définition et au diagnostic de la MC, basées sur des approches systématiques et méta-analytiques de la littérature. Qui plus est, l'**axe 1** a rapporté l'existence d'une physiologie atypique chez le sujet MC soulevant de nouveaux questionnements, notamment quant au métabolisme musculaire de cette population, abordés dans l'**axe 2** de ce travail de thèse.

L'**axe 2** de ce travail de doctorat s'est centré sur l'exploration du tissu musculaire de sujets MC comparativement à des sujets T, à l'état de base et en réponse à un protocole de surnutrition (**étude 5**). Cette exploration musculaire consistait à analyser 122 biopsies musculaires sur différents paramètres, y

compris le réseau microvasculaire. Une étude préliminaire (**étude 4**) a permis de développer et de valider de nouvelles méthodes visant non seulement à diminuer le temps d'analyse, mais également à réduire la quantité de tissu musculaire humain nécessaire et à augmenter le degré de précision. Ainsi qu'illustré par la **Figure 28**, l'**étude 4** a permis de valider statistiquement la fiabilité de deux nouvelles méthodes qui permettent la coloration simultanée des trois types de fibres musculaires (I, IIA, IIX), des capillaires et de la lame basale.

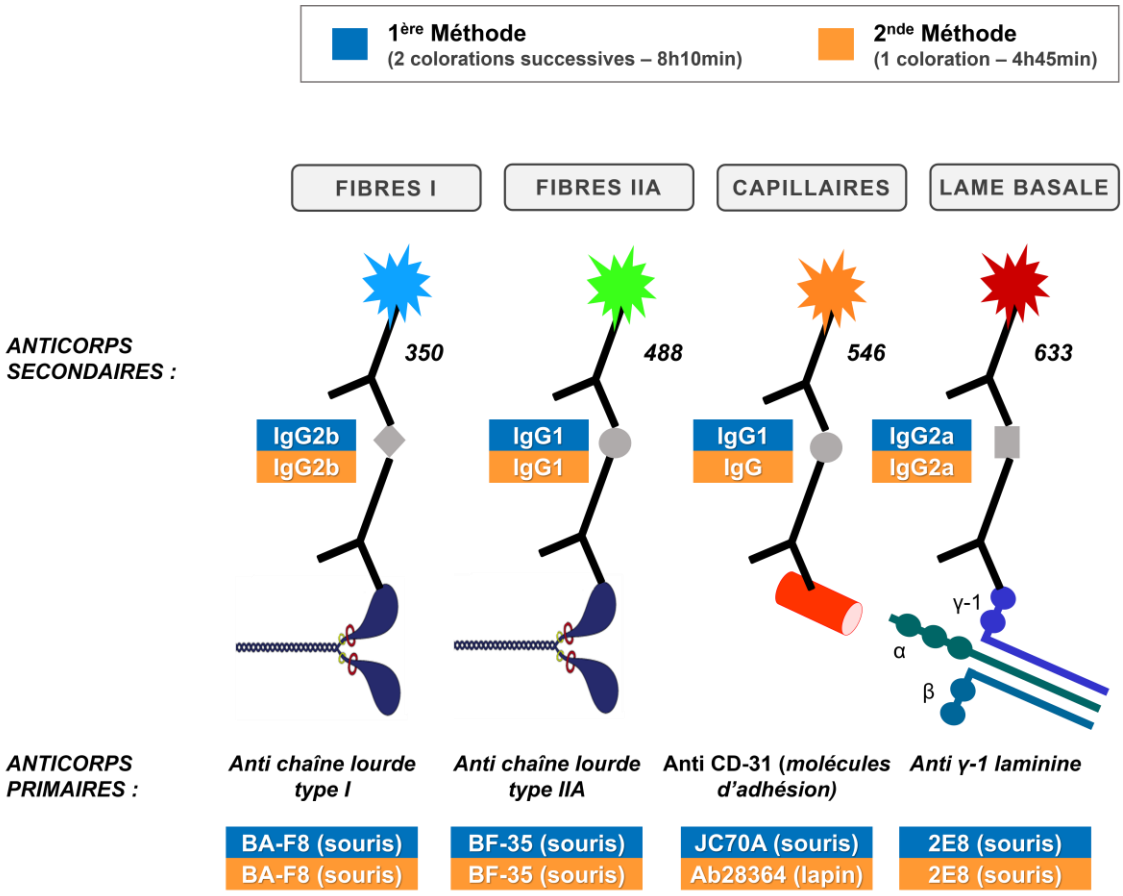


FIGURE 28 – PRINCIPE DES DEUX NOUVELLES METHODES DE COLORATIONS IMMUNOHISTOCHIMIQUES

L'étude statistique a notamment vérifié l'absence de biais vis-à-vis de l'anticorps anti-CD31 utilisé ainsi que vis-à-vis du caractère simultané (typologie et capillaires sur la même lame) ou séparé (typologie et capillaires sur lames différentes) des colorations. La reproductibilité de ces nouvelles méthodes a également été confirmée. Les deux nouvelles méthodes de colorations simultanées ne nécessitent qu'une seule coupe musculaire, ce qui permet d'éviter les inconvénients liés aux coupes sériées. Ces nouvelles méthodes réduisent la quantité de tissu humain nécessaire, qui est souvent difficile à obtenir et en petite quantité. Elles diminuent également le temps de coupe, réduisent la difficulté technique d'obtenir des coupes sériées, suppriment le temps nécessaire au repérage d'une même fibre musculaire entre les différentes coupes lors de l'analyse des images, et éliminent les risques d'imprécisions dus à l'évolution du tissu musculaire entre les différentes coupes sériées. L'ensemble des informations nécessaires à l'analyse typologique (fibres de type I, IIA, IIX), morphométrique (coloration de la lame basale) et de capillarisation est ainsi disponible sur une seule et même coupe musculaire. Cela nous a permis, par la suite, de traiter un nombre élevé de biopsies (122 échantillons musculaires – **étude 5**) de façon approfondie mais dans un temps raisonnable. Cette étude pourrait également amener vers d'autres optimisations que nous n'avons pas développées, mais qui pourraient à terme faciliter l'analyse du tissu musculaire. En effet, après avoir réussi à obtenir toute l'information nécessaire à l'analyse de la morphologie musculaire, de la typologie et de la capillarisation sur une même lame, la prochaine étape pourrait être d'aller vers une analyse automatisée de tous ces paramètres afin de gagner encore en temps et en précision d'analyse, ce qui a déjà été initié par différentes équipes (Al-Shammari et al. 2019; Sanz et al. 2019). Néanmoins, ce type d'analyse automatisée nécessite des biopsies d'extrêmement bonne qualité et sans artéfacts (Sanz et al. 2019), ce qui souligne la nécessité d'optimiser encore nos techniques.

L'**étude 5**, menée sur 30 sujets MC (15 femmes, 15 hommes) *vs.* 31 sujets T (16 femmes, 15 hommes), a tout d'abord permis de confirmer certains résultats observés par la première étude s'étant intéressée au tissu musculaire de sujets MC (10 femmes MC *vs.* 10 femmes T) (Galusca et al. 2018). En effet, l'**étude 5** a montré une forte hypotrophie musculaire avec une surface de section transversale des fibres musculaires qui est apparue très inférieure chez les sujets MC *vs.* T (fibres de type I : -21 %, IIA : -17 %, IIX : -16 %, moyenne : -20 %). Ce résultat s'est avéré particulièrement prononcé chez les hommes MC qui présentent une surface de section transversale de leurs fibres de type I étant 30 % inférieure à celle des sujets T. De façon concordante avec la première étude (Galusca et al. 2018), l'**étude 5** a également montré une typologie suggérant un profil faiblement oxydatif. Les résultats ont révélé de plus grandes proportions de fibres de type IIX et un pourcentage d'aire occupée par les fibres de type I plus faible chez les sujets MC que chez les sujets T. Une proportion élevée de fibres de type IIX peut être le signe d'un faible NAP. Ainsi les personnes paraplégiques présentent par exemple une très forte proportion de fibres de types IIX dans le vaste externe (Crameri et al. 2002), et il est fréquent d'observer, lors de programmes d'entraînement de quelques mois, une baisse de la proportion des fibres de type IIX (Coggan et al. 1992; Hikida et al. 2000; Bamman et al. 2007; Fry et al. 2014). Au regard de la plus forte proportion de fibres de type IIX observée chez nos sujets MC (**étude 5**), cela renforce encore davantage l'intérêt d'estimer précisément leur NAP.

Par ailleurs, il nous semble que la typologie musculaire ne reflète pas uniquement les capacités de force et d'endurance musculaires, mais pourrait également, dans le cas de la MC, jouer un rôle indirect sur la dépense énergétique du muscle squelettique. En effet, il est bien connu qu'une relation existe entre la masse musculaire et la DER (Zurlo et al. 1990) et dès 1994, l'équipe de Zurlo a également montré de façon assez surprenante et contre intuitive que la DER était significativement corrélée au pourcentage de fibres type II (Zurlo et al. 1994). Nous retrouvons également parmi les sujets MC de notre **étude 5**

une association significative (R=0.58, p=0.001) entre le pourcentage de surface occupée par les fibres de type II et la DER. Une des explications soulevées par Zurlo était alors « qu'une relative augmentation de la proportion des fibres de type II pouvait favoriser une utilisation moins efficace des substrats en favorisant la voie glycolytique » (Zurlo et al. 1994). Or, il est désormais connu qu'une partie importante de la chaleur produite par le muscle est due aux pompes SERCA (Arruda et al. 2003). Il a également été montré que la pompe SERCA1, présente dans les fibres de type II (Brandl et al. 1987), était régulée par la sarcolipine (SLN) et que cette protéine diminue le recaptage du calcium sans affecter l'activité ATPase de SERCA, étant ainsi à l'origine d'un découplage et d'une production de chaleur qui pourraient influencer la dépense énergétique journalière (Reis et al. 2002; Periasamy et al. 2017). Dans la mesure où des techniques immunohistochimiques permettent d'identifier facilement les patterns d'expression des différentes isoformes de la pompe SERCA (Fajardo et al. 2013), ce type de techniques pourrait permettre d'investiguer cette hypothèse chez nos sujets MC. Ainsi, le phénotype musculaire un peu plus rapide de nos sujets MC pourrait peut-être participer à une dépense énergétique supérieure de la MM ; dépense qui pourrait ne pas être évidente à l'état de repos mais apparaître lors d'un niveau de sollicitation plus élevé, soulignant ainsi l'intérêt d'explorer le métabolisme des personnes MC non plus seulement au repos mais à l'exercice également. Il est par ailleurs intéressant de noter que les hormones thyroïdiennes seraient en partie responsables de l'acquisition d'un phénotype musculaire rapide et stimuleraient l'expression de SERCA1 (Simonides and van Hardeveld 2008), ce qui fait écho à ce que nous avons présenté plus haut concernant les taux élevés de FT3 chez nos sujets MC. Nous avons de plus observé dans nos travaux une corrélation significative entre le pourcentage de surface occupée par les fibres de type II et la FT3 chez les sujets MC (R=0.52, p<0.01).

Concernant l'analyse du réseau micro vasculaire, les résultats de l'**étude 5** corroborent les observations de l'étude précédente (Galusca et al. 2018) : la majorité des indices capillaires analysés vont dans le sens

d'une plus faible capillarisation pour les différents types de fibres (I, IIA, IIX) chez les sujets MC *vs.* T, excepté pour l'indice de la densité capillaire retrouvé similaire. Pour la première fois cependant, l'**étude 5** a montré que l'indice rapportant les capillaires au contact de la fibre à l'aire de la fibre (CAFA) apparaissait similaire entre les sujets MC et T (indice non investigué dans l'étude précédente). Ainsi, l'indice CAFA, qui reflèterait la distance de diffusion entre les capillaires et la fibre musculaire, n'est pas diminué chez le sujet MC, contrairement aux autres indices capillaires. Ce résultat pourrait donc suggérer que la faible capillarisation « absolue » des sujets MC pourrait n'avoir aucune répercussion sur l'oxygénation des fibres, et ne constituerait finalement qu'une adaptation vis-à-vis des fibres musculaires de petite taille de ces sujets. Cependant, si l'apport en oxygène aux cellules ne représente peut-être pas un problème dans cette population ainsi qu'en attestent les indices de la densité capillaire et CAFA similaires entre les sujets MC et T, il n'en demeure pas moins que certains indices significativement plus faibles tels que l'indice CFPE pourraient potentiellement expliquer la faible masse musculaire et représenter un frein à la recherche d'une augmentation du poids des personnes MC par un travail de musculation par exemple. En effet, il a été démontré que les cellules satellites et les capillaires sont interdépendants (Christov et al. 2007; Verma et al. 2018) et une étude a même montré chez des sujets âgés soumis à un programme de musculation qu'une hypertrophie des fibres de type II n'était présente en réponse à l'entraînement que chez les sujets présentant un indice CFPE élevé au début de la prise en charge (Snijders et al. 2016). Ainsi, une faible capillarisation, même si elle n'impacte pas le transport de l'oxygène, pourrait potentiellement diminuer l'efficacité de la réponse des cellules satellites à un signal hypertrophique chez les individus MC. En outre, contrairement à l'indice LC/PF, l'indice CFPE n'est peut-être pas le meilleur indice pour refléter l'interface muscle/microcirculation et il semblerait que l'indice LC/PF pourrait être plus approprié (Charifi et al. 2004; Moro et al. 2019). Il serait ainsi intéressant, dans de futures études, d'explorer les liens entre les capillaires et les cellules satellites dans le cadre de la MC, chose que nous n'avons pas eu le temps d'entreprendre durant ce travail de thèse.

L'objectif principal de l'**étude 5** consistait en l'évaluation des stockages énergétiques musculaires des individus MC. En effet, la revue de la littérature a notamment rapporté que les gènes FITM1 et FITM2, impliqués dans le stockage des triglycérides nouvellement synthétisés en gouttelettes lipidiques (Kadereit et al. 2008), apparaissaient sous-exprimés dans le tissu musculaire des participants MC (Galusca et al. 2018). De façon concordante avec la sous-expression de ces gènes, l'**étude 5** a effectivement montré, pour la première fois, un stockage d'IMTG particulièrement faible chez les participants MC (indice global : -17 %, fibres de types I : -17 %, fibres de type IIA -14 %). Ces résultats, obtenus sur le groupe entier des participants MC (femmes et hommes), diffèrent cependant en fonction du genre. En effet, ce faible stockage d'IMTG se retrouve chez les femmes MC (indice global : -21 %, fibres de type I -20 %) tandis que le seuil de significativité n'est pas atteint chez les hommes MC. En dépit du faible contenu en IMTG, l'activité enzymatique de la ß-HAD n'apparaît pas significativement différente chez les participants MC, malgré des valeurs assez faibles. Cette absence de significativité peut s'interpréter de deux façons : soit la forte variabilité inhérente à ce type d'analyse enzymatique pourrait expliquer la non-significativité, soit l'activité de la ß-HAD non diminuée en dépit d'un stockage d'IMTG très faible pourrait finalement refléter une forte oxydation des IMTG. De rares observations pourraient étayer cette hypothèse : l'**étude 5** a montré des activités enzymatiques non diminuées de la cytochrome *c* oxydase et du complexe IV (CIV) de la chaîne mitochondriale chez l'individu MC, ainsi qu'une activité du complexe II (CII) qui aurait même tendance à être supérieure. Néanmoins, de nombreux arguments sont au contraire en faveur d'une faible capacité oxydative du tissu musculaire du sujet MC. Tout d'abord, les résultats de typologie et de capillarisation obtenus par l'**étude 5** et par l'étude précédente (Galusca et al. 2018) iraient dans le sens d'une faible capacité oxydative. De plus, cette étude précédente a montré une faible capacité de la cytochrome *c* oxydase (Galusca et al. 2018) et l'**étude 5** a mis en évidence une faible activité enzymatique de la citrate synthase du cycle de Krebs (en aval de la ß-oxydation des acides

gras). Par ailleurs, une étude menée sur la même cohorte de sujets que notre **étude 5** a analysé des échantillons de muscles frais par respirométrie et a observé des activités du complexe I (CI) et du CII de la chaîne respiratoire qui auraient tendance à être inférieures chez les sujets MC *vs.* T (Ling et al. 2019). Finalement, l'ensemble de ces observations tendent à soutenir l'hypothèse d'une plus faible capacité oxydative mitochondriale musculaire chez l'individu MC. Cependant, maintenant que nous avons pu objectiver une diminution du stockage lipidique musculaire, il serait tout de même pertinent, dans de futures études, d'explorer certains aspects mécanistiques du métabolisme lipidique musculaire de participants MC afin d'apporter de nouveaux éléments de compréhension et de discussion.

Par ailleurs, l'**étude 5** a également mis en évidence un faible stockage du glycogène musculaire chez les sujets MC *vs.* T (-6 % pour les fibres de types I et -5 % pour les fibres de types IIA). Les résultats enzymatiques montrent une activité de l'énolase similaire entre les sujets MC et T, ce qui témoignerait d'une activité de la glycolyse non diminuée en dépit du plus faible stockage de glycogène. Comme pour le stockage des IMTG, le faible stockage de glycogène semble davantage affecter les femmes MC que les hommes MC. Même si ces différences liées au genre restent, à ce jour, difficiles à interpréter, cela souligne en revanche la pertinence d'inclure des sujets MC masculins dans les études cliniques. Dans la mesure où un faible stockage de glycogène musculaire peut limiter certains efforts physiques, ce résultat amène également à se questionner quant à de potentielles répercussions à l'effort chez l'individu MC.

L'exploration du tissu musculaire de l'ensemble des sujets MC et T a été effectuée à l'état de base mais également à la suite d'une surnutrition de 2 semaines qui consistait en l'ingestion quotidienne d'une bouteille de Renutryl® Booster (+600 kcal/jour – 48.5 % glucides, 31.5 % lipides, 20 % protéines). L'objectif de cette surnutrition était d'en analyser l'effet sur le tissu musculaire des sujets MC, principalement sur le stockage musculaire des substrats énergétiques. Alors que le contenu en IMTG

semble être resté stable chez les sujets T avec la surnutrition, les résultats ont montré une augmentation des IMTG chez les participants MC (indice global : +11 %, fibres de type I : +10 %, fibres de type IIA : +9 %). Les analyses enzymatiques montrent également des tendances à l'augmentation de la CII (+5 %) et de la CIV (+17 %) chez les sujets MC. L'une des hypothèses que nous pourrions avancer est la suivante : en réponse à la surnutrition, les sujets MC pourraient avoir augmenté le stockage lipidique musculaire mais également l'oxydation de ces lipides, contrairement aux sujets T. Néanmoins, l'absence d'augmentation des activités de la ß-HAD, de la citrate synthase, et de la cytochrome *c* oxydase tendrait plutôt à infirmer cette hypothèse.

Finalement, l'**étude 5** a confirmé l'hypotrophie musculaire des sujets MC, leur faible capillarisation musculaire ainsi que leur profil typologique faiblement oxydatif. Bien que cela puisse être discuté, les résultats des activités enzymatiques iraient également dans le sens d'un profil faiblement oxydatif. Si cette **étude 5** a permis de conforter certains résultats de l'étude précédente (Galusca et al. 2018) et d'en discuter d'autres, elle a également rapporté certaines observations pour la première fois, notamment avec l'évaluation de biopsies musculaires provenant d'hommes MC et avec l'analyse du stockage des substrats énergétiques musculaires. L'**étude 5** a souligné la pertinence d'explorer la physiologie de l'homme MC, dont plusieurs résultats diffèrent de ceux des femmes MC. Enfin, le résultat principal de l'**étude 5** a été l'observation d'un stockage d'IMTG et de glycogène affaibli chez le sujet MC. Ce faible stockage énergétique musculaire couplé à une faible capacité oxydative musculaire irait plutôt dans le sens d'un tissu musculaire métaboliquement moins actif chez le sujet MC, ce qui tendrait plutôt à s'opposer aux précédents éléments de discussion en faveur d'une dépense énergétique possiblement importante chez cette population.

Néanmoins, sur la même cohorte de participants que notre **étude 5**, le tissu adipeux est au contraire apparu plus oxydatif que chez les sujets T (Ling et al. 2019). De plus, nos données montrent une corrélation positive significative ($R=0.53$, $p=0.04$) entre la masse grasse totale et le métabolisme de repos chez les femmes MC, qui n'est pas retrouvée chez les femmes T. D'après ces observations, il est possible d'émettre l'hypothèse selon laquelle le tissu musculaire présent en petite quantité chez les sujets MC serait de surcroît peu énergétique, tandis que le tissu adipeux, relativement peu diminué, occasionnerait quant à lui une forte dépense d'énergie. Si cette hypothèse venait à être confirmée, le sous-poids du sujet MC pourrait éventuellement être davantage expliqué par l'activité métabolique de sa masse grasse plutôt que par celle de sa masse musculaire. Bien que cette interprétation ne constitue qu'une simple hypothèse, elle soulève néanmoins l'intérêt d'explorer davantage les aspects métaboliques de ces différents tissus dans l'objectif d'une meilleure compréhension physiologique de la MC.

Limites

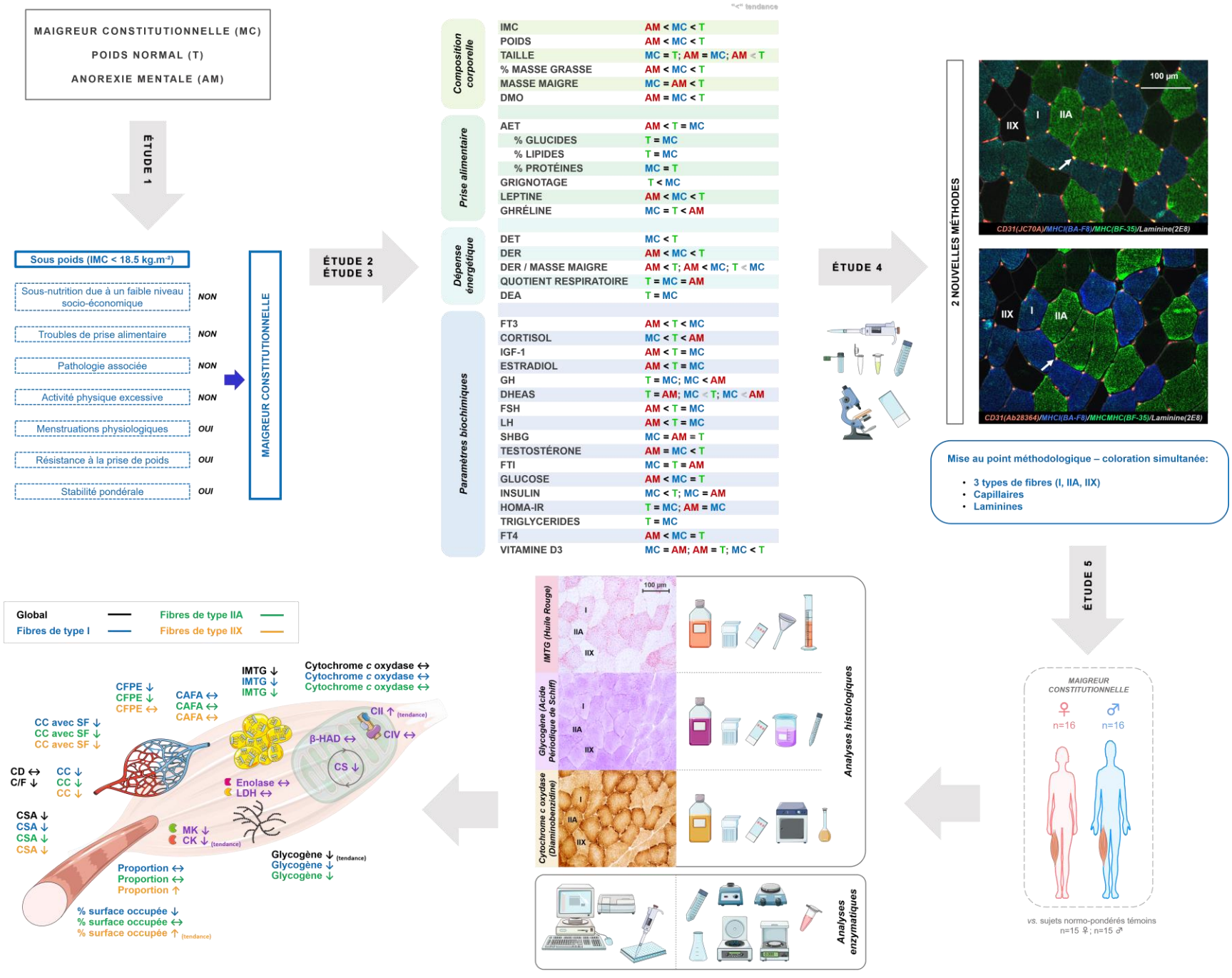
Ce travail de doctorat a tout d'abord mis en exergue le faible nombre d'études portant sur la MC, avec un nombre encore plus faible d'études ayant inclus des hommes présentant une MC. Seuls 39 articles auraient été publiés sur la MC, et seules 12 études cliniques auraient inclus des hommes MC dans leur protocole. Ainsi, les approches systématique et méta-analytique de l'**axe 1** de ce travail de doctorat sont limitées par un nombre d'études relativement faible. Par ailleurs, la forte diversité des variables analysées, mais également des méthodes employées, a pu constituer une limite de cet **axe 1**. La qualité du report des résultats au sein des études analysées peut également être une limite de cet **axe 1**, ainsi que nous l'avons mis en avant dans les tableaux de risques de biais (**études 1 et 3**). D'autre part, l'**étude 1** nous a permis de proposer un arbre décisionnel d'aide au diagnostic de la MC, basé sur la revue systématique des critères de diagnostic utilisés à l'inclusion de participants MC dans les études cliniques. Bien que cet arbre constitue un premier outil d'aide au diagnostic qui nous semble pertinent, il est néanmoins destiné à être étoffé au fur et à mesure de la caractérisation clinique et expérimentale de la MC. Comme nous l'avons discuté, certains dosages hormonaux (FT3, leptine, cortisol) ou questionnaire (DEBQ – échelle de la restriction cognitive alimentaire) pourraient peut-être constituer de nouveaux éléments de diagnostic s'ils venaient à être validés par un nombre suffisant d'études. Concernant l'**axe 2** de ce travail de doctorat, les principales limites méthodologiques concernent l'évaluation de la prise alimentaire et du NAP des participants. Lors de la mise en place des protocoles de surnutrition, la compliance représente l'un des enjeux clés de l'étude. Afin de vérifier la compliance des sujets et d'éviter au maximum les comportements de compensation alimentaire, les participants ont été en contact régulier avec les chercheurs. Néanmoins, le contrôle de la prise alimentaire effective n'a été mené que sur la seconde semaine de surnutrition, et non sur l'ensemble de la période de surnutrition. Une autre limite peut concerner l'évaluation de la prise alimentaire, qui a été effectuée de façon auto-déclarée (SUVIMAX) et

qui peut conserver, de fait, une certaine part de subjectivité. Concernant l'estimation du NAP des participants, l'absence de comportements liés à une activité physique excessive a été vérifiée à l'inclusion des sujets par le questionnaire MOSPA (Iqbal et al. 2006), dont la pertinence reste discutable ; ce questionnaire n'ayant été validé que chez 50 femmes pakistanaises enceintes (Iqbal et al. 2006). Si ce questionnaire donne une première approximation visant à vérifier l'absence d'activité physique excessive chez les participants, l'étude plus approfondie du NAP dans le cadre de la MC nécessitera d'utiliser des questionnaires d'activité physique plus adaptés, et même éventuellement de valider un questionnaire spécifique à cette population MC. Si une approche par questionnaire a été effectuée à l'inclusion des sujets, l'analyse du NAP en tant que résultat a en revanche été effectuée par accélérométrie en conditions de vie réelles sur une durée de 5 jours. L'accéléromètre utilisé (ActiHeart®) présente néanmoins certaines limites technologiques concernant la précision d'analyse des profils d'activité et de sédentarité des sujets, et donc la fiabilité des résultats obtenus. Nous suspectons que ce type d'appareil puisse sous-estimer le niveau d'activité, en particulier sur des mouvements de « fidgeting » qui semblent pourtant être particulièrement présents chez le sujet MC (Marra et al. 2007).

Perspectives

Comme l'a détaillé l'étude de Ling concernant la même cohorte que notre **étude 5** (Ling et al. 2020), les sujets MC de ce protocole de surnutrition (+600 kcal/jour – 48.5 % glucides, 31.5 % lipides, 20 % protéines – 2 semaines) sont parvenus à une prise de poids légère mais significative. Pourtant, le protocole de surnutrition lipidique (+630 kcal/jour – 100 % lipides – 4 semaines) avait échoué à faire prendre du poids aux sujets MC (Germain et al. 2014). Ces observations nous amènent ainsi à formuler l'hypothèse qu'une surnutrition équilibrée légèrement hyperprotéinée ((Ling et al. 2020), **étude 5**) constituerait une meilleure stratégie de prise de poids chez l'individu MC qu'une surnutrition exclusivement lipidique. Étant donné que les sujets MC présentent un pourcentage de masse grasse dans les normes et une faible masse maigre (**études 2** et **3**), la proposition d'un régime hyperlipidique semblerait finalement discutable. Les régimes riches en graisse augmentent de surcroît le risque de développer une maladie chronique (Woteki and Thomas 1992). Par ailleurs, nous avons apporté la preuve que les sujets MC présentent une MM extrêmement diminuée (**étude 2**) et des fibres musculaires de très petites tailles (**étude 5**), mais seraient capables de prendre du poids, de façon modérée mais significative, en réponse à une surnutrition équilibrée légèrement hyperprotéinée (Ling et al. 2020). De fait, il nous semble qu'une stratégie de prise de masse musculaire, plutôt qu'une prise de masse grasse, pourrait être plus efficace chez cette population. En conséquence, il nous apparaît pertinent de proposer une surnutrition hyperprotéinée couplée à un entraînement en musculation (Stokes et al. 2018) dans l'objectif d'aider, de façon saine et efficace, cette population à prendre du poids.

Conclusion graphique



AET : apports énergétiques totaux, AM : patients présentant une anorexie mentale, CAFA : capillaires par section transversale de fibres, CC : indice des capillaires au contact, CD : densité capillaire ; C/F : ratio capillaires par fibre ; CFPE : indice d'échange capillaires–périmètre de fibre, CSA : surface de section transversale, DEA : dépense liée à l'activité physique, DER : dépense énergétique de repos, DET : dépense énergétique totale, DHEAS : dehydroepiandrosterone sulfate, DMO : densité minérale osseuse, FSH : follicle-stimulating hormone, FT3 : free triiodothyronine, FT4 : free thyroxine, FTI : indice de testostérone libre (ratio testostérone/SHBG), GH : growth hormone, HOMA-IR : homeostasis model assessment of insuline resistance, IGF-1 : insulin-like growth factor-1, IMC : indice de masse corporelle, LH : luteinizing hormone, MC : sujets maigres constitutionnels, SF : sharing factor, SHBG : sex hormone-binding globulin, T : sujets

FIGURE 29 – CONCLUSION GRAPHIQUE DU TRAVAIL DE DOCTORAT

Conclusion générale

Les travaux de recherche conduits durant ce projet de doctorat ont permis 1) d'éprouver et de mettre en exergue les apports clés de la littérature scientifique relative à la MC grâce à des approches systématiques et méta-analytiques (**axe 1**) ; et 2) d'explorer les caractéristiques morphométriques, fonctionnelles et métaboliques du tissu musculaire de femmes et d'hommes présentant une maigreur constitutionnelle, comparativement à des participants normo-pondérés (**axe 2**).

Ce travail de doctorat s'est ainsi concrétisé par :

- l'établissement de critères de **diagnostic** de la maigreur constitutionnelle, illustré par un arbre décisionnel d'aide au diagnostic et basé sur une revue systématique de la littérature
- l'évaluation de la **physiologie** des personnes présentant une maigreur constitutionnelle, basée sur une analyse systématique et méta-analytique
- la caractérisation du **phénotype musculaire** de **femmes** et **d'hommes** présentant une maigreur constitutionnelle à l'état basal et en réponse à un protocole de surnutrition, basée sur l'analyse histologique de biopsies musculaires.

Alors que la maigreur constitutionnelle demeure aujourd'hui encore peu connue et reconnue, il nous semble que ce travail de doctorat a permis d'apporter une contribution au diagnostic clinique de la maigreur constitutionnelle, mais également à la compréhension de cette physiologie particulière, dans l'objectif, à terme, de proposer un suivi et une prise en charge appropriés à cette population.

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ANNEXES

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Letter to the Editor

Constitutional thinness: body fat metabolism and skeletal muscle are important factors

The excellent review by Bailly *et al.*[1] highlights various abnormal clinical conditions important to help rule out constitutional thinness, including eating disorders, associated pathology and over-exercise, along with a history of weight, physiological menses and weight gain resistance. However, two additional factors that can significantly influence health should also be considered in constitutional thinness: body fat metabolism and skeletal muscle strength. Dysfunction in these tissues may be at least or more common than those noted by the authors (who note that constitutional thinness individuals do not seem to be characterised by a very low body fat percentage despite their low BMI).

Body fat is an important metabolic tissue. Abnormalities include underfat and overfat, which impair health[2]. While overfat, whose global prevalence may exceed 80 %[3], is a common cause of chronic disease, physical impairment and raises the risk of infectious disease, both overfat and underfat are associated with immune impairment, in particular reduced glutathione and increased oxidative stress levels[4]. Both conditions can also influence the appearance of leanness.

Reliance of body weight and BMI to assess leanness can be deceptive as these measures may not accurately reflect body fat content[2]. Forty percent or more of normal-weight, non-obese individuals may be overfat[5]. The waist-to-height ratio is an effective clinical tool to rule out overfat, while dual-energy X-ray absorptiometry can accurately determine percent body fat.

Sarcopenia, whose prevalence in the elderly may be as high as 50 %, is associated with significant loss of muscle mass yet is often concurrent with excess body fat (called sarcopenic overfat) leading to both a reduction of weight and impaired adiposity[2]. Cachexia is also associated with significantly reduced muscle mass and can occur due to unhealthy changes in fatty tissue. Both sarcopenia and cachexia can influence the appearance of thinness.

Muscle weakness is a common clinical condition that raises the risk of adverse health outcomes including physical impairment, morbidity and all-cause mortality[6], with hand-grip strength an indicator of overall body strength and a predictor of health outcomes[7]. While muscle mass contributes significantly to weight and BMI, it is not necessarily associated with muscle strength as thin individuals with lean muscles can be strong due to the increased muscle fibre contraction.

The assessment of body fat and muscular strength is an additional factor important in patients presenting with concerns about excess thinness.

I am the sole author and declare no conflicts of interest.

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Letter to the Editor

Invited Letter to Editor in response to: Constitutional thinness: body fat metabolism and skeletal muscle are important factors

Our research group recently published a systematic review discussing the criteria actually used in the definition and diagnosis of constitutional thinness (CT)[1]. Our main aim was to systematically identify the inclusion criteria used in any available clinical trial that enrolled participants with CT. Despite the heterogeneity of the criteria and thresholds used in the thirty-five reviewed studies, the following points were frequently identified: no eating disorder, no associated disease, no over-exercising, no amenorrhoea, weight gain resistance and stable body weight. As also pointed out by our analysis, most of the included clinical trials defined a threshold of thinness in their inclusion criteria using BMI cut-offs and less frequently the percentage of body fat. In his recent and relevant letter to the Editor, Dr Maffetone[2] highlighted the quality and pertinence of our work, suggesting, however, to reinforce the consideration of both body fat metabolism (pointing moreover the limitation induced by the use of BMI only) and skeletal muscle strength that might be of importance when it comes to individuals with CT.

We would like here to thank Dr Maffetone for his encouraging and constructive comment and collectively agree that body fat is a highly important criterion to consider in CT, especially given the recent study that showed smaller adipocytes but higher mitochondrial respiratory capacities in adipose tissue of CT participants[3]. Our systematic review, which exclusively focused on the inclusion criteria used in available publications, identified only two studies that considered body fat percentage in their inclusion criteria – enrolling participants with a body fat below 20 %. While few studies included participants with CT on the basis of body fat, many have used this criterion as an outcome and showed that individuals with CT present non-blunted values, unlike anorectic people[4,5]. These results therefore suggest that people with CT would be underweight, but not underfat. If this were to be confirmed, CT diagnosis could be supported by a state of underweight not associated with underfat, but rather, on the contrary, with 'non-blunted' fat. In accordance with the comment and publications of Dr Maffetone[6], the non-blunted fat mass percentage in CT could account for the relatively healthy state observed in this population.

Similarly, we definitely agree that skeletal muscle is an important factor that can influence an appearance of thinness – for instance in the case of cachexia or sarcopenia, as rightly pointed out by Dr Maffetone. CT is not pathology induced and does not specifically concern elderly people (the mean ages of CT participants ranged from 19·4 to 42·4 years in the reviewed articles) but may still be linked to skeletal muscle issues. This hypothesis might be further supported by the high resting metabolic rate to fat-free mass ratio of CT participants observed in some studies[4,7]. Our group recently performed histochemical analyses from muscle biopsies collected in CT volunteers, in order to characterise their muscle phenotype and assess potential adaptations[8,9]. According to our results, individuals with CT, in agreement with their lower muscle mass[4,7–9], showed smaller fibre cross-sectional areas of all muscle fibre types compared with normal-weight participants[8,9]. They also have a lower oxidative profile with a lower capillary supply, a lower proportion of type I slow oxidative fibres in favour of a high proportion of type IIX fast glycolytic fibres, a lower citrate synthase enzyme activity and a down-regulation of genes involved in the metabolism of TAG – fat storage-inducing transmembrane 1 (FITM1) and 2 (FITM2)[8,9]. Muscle fibres of CT individuals also presented lower intramuscular TAG and lower glycogen content[9]. CT individuals seem to present an untypical muscle phenotype, and these recent results reinforce the need for further explorations of muscle physiology but also functionality in such individuals. This is, once more, absolutely in line with the comment from Dr Maffetone and definitely raises the need for the evaluation of physical capacities in this population. It might be of particular interest to assess parameters such as strength, aerobic capacity or metabolic flexibility, in the light of our histological observations. Despite the importance of considering muscle tissue in the context of CT, this was, to the best of our knowledge, only explored in our two previous studies so far[8,9]. It therefore seems essential to achieve greater scientific and statistical power before integrating some criteria relative to muscle function in the diagnosis of CT.

While our review aimed at systematically reporting the criteria used so far in the inclusion of participants with CT[1], it also pointed out that body fat and skeletal muscle were generally not considered in these criteria and were evaluated in few studies only. Our conclusions and the constructive comment from Dr Maffetone call for further physiological and functional investigations of both adipose and muscle tissues in individuals with CT, to better understand and diagnose this condition and hopefully propose appropriate and effective intervention strategies favouring weight gain in this population.

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The authors declare that there are no conflicts of interest.

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POSTER – JOURNÉES DES ÉCOLES DOCTORALES (JED)

CARACTÉRISATION DU PHÉNOTYPE MUSCULAIRE CHEZ DES SUJETS PRÉSENTANT UNE MAIGREUR CONSTITUTIONNELLE

Mélina BAILLY, Natacha GERMAIN, Bogdan GALUSCA, Léonard FÉASSON, Daniel COURTEIX, Julien VERNEY

CONTEXTE

Les personnes maigres constitutionnelles (MC) présentent :

- un état naturel et non pathologique de sous-poids stable (indice de masse corporelle <17.5 $kg.m^{-2}$)
- une réelle volonté de gain de poids mais sans y parvenir, en absence de tout désordre de prise alimentaire

Objectif : étude des caractéristiques structurelles et métaboliques du tissu musculaire de sujets MC

MÉTHODE

Des biopsies musculaires du *vastus lateralis* ont été prélevées chez 30 sujets MC (15 femmes, 15 hommes) et 31 sujets normo-pondérés contrôles (C) (16 femmes, 15 hommes).

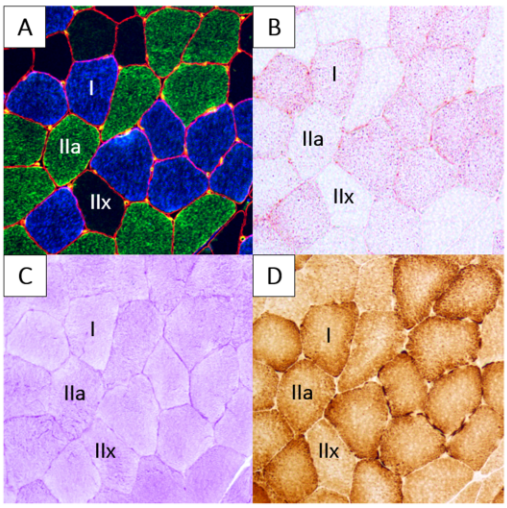

Fig 1: Typologie musculaire (A), triglycérides intramusculaires (IMTG) (B), glycogène musculaire (C), activité de la Cytochrome c Oxydase (COx) (D)

RÉSULTATS

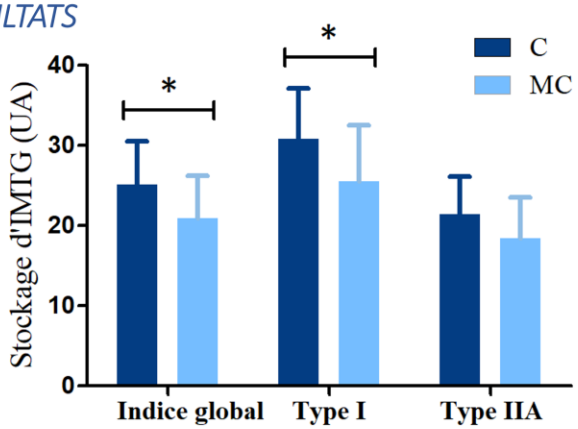

*Fig 2: Stockage de triglycérides intramusculaires (IMTG) chez des sujets maigres constitutionnels (MC) (n=30) et contrôles (C) (n=31), * p<0.05*

Les sujets MC présentent des valeurs inférieures aux sujets C concernant : le stockage global d'IMTG (p=0.029, -17%), la surface de section transversale des fibres de type I (p<0.001, -21%), IIA (p=0.0012, -17%) et IIX (p=0.03, -16%), les indices de capillarisation et la proportion de fibres lentes de type I (p=0.038).

La proportion de fibres rapides glycolytiques de type IIX est supérieure chez les sujets MC *vs* C (p=0.043). L'activité de la citrate synthase est inférieure chez les sujets MC *vs* C (p=0.014, -18%). Le stockage de glycogène et l'activité de la COx ne diffèrent pas des sujets contrôles.

CONCLUSION

MC : phénotype musculaire

TYPOLOGIE
- % fibres type I : MC < C
- % fibres type IIX : MC > C

CAPILLARISATION
- Indices capillaires globaux et locaux : MC < C

TAILLE DES FIBRES
- Surface de section (fibres de types I, IIA et IIX) : MC < C

STOCKAGE DES SUBSTRATS
- Stockage IMTG : MC < C
- Stockage glycogène : MC = C

La maigreur constitutionnelle se caractérise par un phénotype musculaire faiblement oxydatif

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ORIGINAL RESEARCH

Pediatric OBESITY WILEY

Delayed meal timing after exercise is associated with reduced appetite and energy intake in adolescents with obesity

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Summary

Background: While the beneficial effects of exercise on appetite might depend on its timing during the day or relative to a meal, this remains poorly explored in youth.

Objectives: To examine the importance of meal timing (+30 vs +90 minutes) after performing exercise on energy intake, appetite and food reward in adolescents with obesity.

Methods: Eighteen adolescents with obesity randomly completed three conditions: (a) lunch (12:00 PM) set 30 minutes after a rest session (11:00 AM); (b) lunch (12:00 PM) set 30 minutes after an exercise session (11:00 AM)(MEAL-30); (c) lunch (01:00 PM) set 90 minutes after an exercise session (11:00 AM)(MEAL-90). Lunch and dinner ad libitum energy intake was assessed, food reward (LFPQ) assessed before and after lunch, and before dinner, appetite sensations were assessed at regular intervals.

Results: Energy intake was lower at MEAL-90 than MEAL-30 and CON at lunch ($P < .05$ and $P < .01$, respectively) and lunch + dinner combined ($P < .001$). A decrease in intake (g) of protein, fat and carbohydrate was observed. Post-exercise hunger was lower on MEAL-90 compared with CON. No condition effects were found at lunch for food reward.

Conclusions: Delaying the timing of the meal after exercise might help affect energy balance by decreasing ad libitum energy intake without increasing hunger and by improving satiety in adolescents with obesity.

KEYWORDS

adolescent, appetite, energy intake, exercise timing, food reward, obesity

1 | INTRODUCTION

While practitioners and clinicians constantly work on the improvement of their weight loss interventions, trying to identify the best exercise characteristics (modality, intensity, duration, etc.) to prescribe, the need to also consider the timing of exercise has been recently suggested.[1] Recent studies effectively show that the beneficial effects of exercise might also depend on its timing during the day or its delay/position regarding a meal.[1] Some studies for instance showed that performing acute exercise one to 3 hours after a meal could enhance the glycemic response in patients with type II diabetes[2-5] while others showed a better postprandial lipemia response when exercise was performed immediately before the meal.[6-8]

Looking at the alarming progression of overweight and obesity among children and adolescents, it seems necessary to deepen our understanding on the effects of exercise on overall energy balance, in order to optimize our weight loss strategies. It is now clear that physical exercise does not only impact energy expenditure, it also affects

energy intake and appetite control in youth and adolescents with obesity.[9] The current literature mainly investigated the effect of exercise duration,[10,11] intensity[12-14] or modality[15] on subsequent food intake, appetite sensations or food reward, while the potential role played by the timing of exercise remains poorly explored.[16]

In 2017, Mathieu et al assessed the effects of exercising immediately before or after a lunch meal in primary school children on overall energy balance.[17] Although they did not observe any difference on energy intake between conditions (before or after the meal), their results highlight the beneficial effect of performing pre-meal moderate-to-vigorous over low-intensity exercise on subsequent energy intake.[17] More recently, similar results were obtained among adolescents with obesity whose energy intake and food reward remained unchanged whether the adolescents performed 30 minutes of cycling exercise (65% VO_{2peak}) immediately before or after their lunch meal.[18] Interestingly, others investigated the potential effect of the delay between an acute exercise bout and the following meal on energy intake and appetite. In their work, Albert et al compared the effects of exercising (treadmill running at 70% VO_{2max}) 45 or 180 minutes before lunch, in normal weight adolescents.[19] The authors observed an 11% reduction of the adolescents' ad libitum energy intake and a 23% decrease in fat intake when the exercise was performed 45 minutes before lunch, compared to 180 minutes. Moreover, there were no difference in terms of appetite sensations and no energy compensation at the following snack or dinner. Our research group recently examined the effect of the exercise-meal delay on energy intake, appetite and food reward among adolescents with obesity.[20] According to our results, a 30-minute cycling exercise bout (65% VO_{2max}) performed 60 minutes before lunch favoured a 14% reduction of ad libitum energy intake while the same exercise performed 180 minutes before lunch did not affect the adolescents' energy intake. While appetite sensations (hunger, fullness, prospective food consumption and desire to eat) did not differ between conditions, our results also showed a significantly lower pre-meal explicit liking for high-fat relative to low-fat foods when the exercise was set close to the meal, suggesting the implication of the food reward system.[20] Altogether, these results seem to show a beneficial effect of exercising close to a meal on overall energy balance in adolescents.

Although these studies compared exercises of similar characteristics (eg, duration, modality and intensity), their metabolic demand might have been different due to their divergent delay from breakfast, which might have important implications when it comes to subsequent energy intake. Indeed, it has been shown that the metabolic activity during exercise, particularly the contribution of the energy substrates, is different depending on the delay between a breakfast and this exercise.[21] The substrate oxidation during exercise, especially the rate of carbohydrate oxidation has been associated with subsequent energy intake,[22] particularly in adults with obesity.[23,24] Investigating the effect of the timing of exercise on appetite and energy intake needs to consider not only its delay with the following meal but also the time interval between exercise and the previous food intake.

In that context, the aim was to examine the importance of meal timing (+30 or +90 minutes) after performing exercise on energy intake, appetite and food reward in adolescents with obesity.

2 | MATERIALS AND METHODS

2.1 | Participants

Eighteen adolescents with obesity (according to[25]) aged 12-15 years (Tanner stage 3-4) were enrolled in this study (12 boys [12.6 ± 1.2 years] and 6 girls [13.0 ± 1.6 years]). They were recruited through the local Pediatric Obesity Center (Tza Nou, La Bourboule, France), based on the following main inclusion criteria: (a) to be free of any medication known to influence appetite or metabolism; (b) to be free of any contraindication to physical activity; (c) to be classified as physically inactive (taking part in less than 2 hours of physical activity per week as assessed using the International Physical Activity Questionnaire -IPAQ[26]). This study was conducted in accordance with the Helsinki declaration and all the adolescents and their legal representative received information sheets and signed consent forms as requested by the local ethical authorities (Human Ethical Committee authorization reference: 2019-A00530-57; Clinical Trial reference: NCT03968458).

2.2 | Design

After a preliminary medical inclusion visit performed by a paediatrician to control for the ability of the adolescents to complete the study, they were asked to perform a maximal aerobic test and their body composition was assessed by dual-energy X-ray absorptiometry (DXA). The adolescents thereafter completed the three following experimental sessions (1 week apart) in randomized order: (a) lunch (at 12:00 PM) set 30 minutes after a rest session (at 11:00 AM); (b) lunch (at 12:00 PM) set 30 minutes after an exercise session (at 11:00 AM; MEAL-30); (c) lunch (at 1:00 PM) set 90 minutes after an exercise session (at 11:00 AM; MEAL-90). On the three occasions, participants received a standardized breakfast (08:00 AM) and were asked to remain at rest (CON) or to cycle for 30 minutes at 11:00 AM and eat either 30 minutes (on MEAL-30; lunch at 12:00 PM) or 90 minutes (on MEAL-90; lunch at 1:00 PM) after exercise. Dinner was provided to the adolescents at 6:30 PM They were asked to complete the Leeds Food Preference Questionnaire (LFPQ)[27] before and after the lunch meal and before dinner. Lunch and dinner energy intake were assessed via ad libitum buffet-style meals. Appetite sensations were measured at regular intervals throughout the day. Outside the experimental conditions and between the two ad libitum test meals, the adolescents stayed in the laboratory, devoid of any food cues, and were requested not to engage in any moderate-to-vigorous physical activity and mainly completed sedentary activities such as reading, homework or board games. Figure 1 details the whole design of the study.

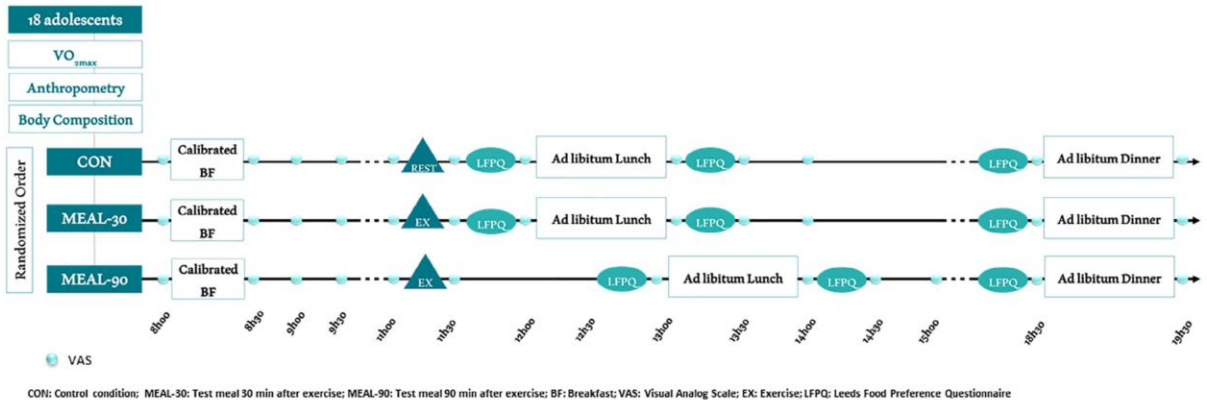

FIGURE 1 Study design

2.3 | Anthropometric characteristics and body composition

Body mass and height were measured wearing light clothing while bare-footed, using a digital scale and a standard wall-mounted stadiometer, respectively. Body mass index (BMI) was calculated as body mass (kg) divided by height squared (m^2) and the sex and age dependent French reference curves were used to obtain the BMI percentile.[28] Fat mass (FM) and fat-free mass (FFM) were assessed by dual-energy X-ray absorptiometry (DXA) following standardized procedures (QDR4500A scanner, Hologic, Waltham, MA, USA). These measurements were obtained during the preliminary visit by a trained technician.

2.4 | Peak oxygen uptake test ($\dot{V}O_{2peak}$)

Each adolescent performed a $\dot{V}O_{2peak}$ test on a traditional ergometer.[29] The initial power was set at 30 W during 3 minutes, followed by a 15 W increment every minute until exhaustion. The adolescents were strongly encouraged by the experimenters throughout the test to perform their maximal effort. Maximal criteria were: heart rate >90% of the theoretical maximum heart rate (210-0.65 × age), respiratory exchange ratio (RER = $\dot{V}CO_2/\dot{V}O_2$) > 1.1 and/or $\dot{V}O_2$ plateau. Cardiac electrical activity (Ultima SeriesTM, Saint Paul, MN) and heart rate (Polar V800) were monitored and the test was coupled with a measurement of breath-by-breath gas exchanges (BreezeSuite Software, Saint Paul, MN), that determined $\dot{V}O_2$ and $\dot{V}CO_2$. Volumes and gases were calibrated before each test. $\dot{V}O_{2peak}$ was defined as the average of the last 30 seconds of exercise before exhaustion.

2.5 | Experimental conditions

Rest condition (CON). During this condition, the adolescents were asked to remain quiet and were not allowed to engage in any physical activity. They were asked to stay seated on a comfortable chair (30 minutes) between 11:00 and 11:30 AM, not being allowed to talk, read, watch TV or to complete any intellectual tasks. Energy expenditure was assessed during the 30-minutes rest period using portable indirect calorimetry (K4b^2, COSMED Inc., Rome, Italy).

Lunch condition 30 minutes after exercise (MEAL-30). Between 11:00 AM and 11:30 AM, the participants performed a 30-minutes moderate-intensity exercise bout (65% VO_{2peak}) on a cycle ergometer. The intensity was controlled by heart rate records (Polar V800) using the results from the maximal aerobic capacity testing. Exercise-induced energy expenditure was calculated based on the results obtained during the maximal oxygen uptake test.

Lunch condition 90 minutes after exercise (MEAL-90). The adolescents performed the same exercise bout as MEAL-30 and at the same time, but the ad libitum lunch meal was served at 1:00 PM (90 minutes after the end of the exercise).

2.6 | Energy intake

At 08:00 AM, the adolescents consumed a standardized calibrated breakfast (500 kcal) respecting the recommendations for their age (composition: bread (50 g), butter (10 g), marmalade (15 g), yoghurt (125 g) or semi-skimmed milk (20 cL), fruit or fruit juice (20 cL)). Lunch and dinner meals were served ad libitum using a buffet-type meal. The content of the buffets was determined using a food preference and habits questionnaire filled in by the adolescents during the inclusion visit, as previously described.[30] Top rated items as well as disliked items and items liked but not usually consumed were excluded to avoid over-, under- and occasional consumption. The lunch menu was beef steak, pasta, mustard, cheese, yoghurt, compote, fruits and bread. The dinner menu was ham/turkey, beans, mashed potato, cheese, yoghurt, compote, fruits and bread. Food items were presented in abundance and the adolescents were told to eat until comfortably full. Adolescents made their choices and composed their trays individually before joining their habitual table (5 adolescents per table). Lunch and dinner were served in a quiet environment free of music, cellphones or television. Food items were weighed by the

experimenters before and after each meal. Energy intake and macronutrient composition (proportion of fat, carbohydrate and protein) were calculated using the software Bilnut 4.0. This methodology has been previously validated and published.[30] Lunch and total relative energy intake (REI) were calculated such as: energy intake - exercise-induced energy expenditure.

2.7 | Subjective appetite sensations

Appetite sensations were collected at regular intervals throughout the day using visual analogue scales (150-mm scales).[31] Adolescents had to report their hunger, fullness, desire to eat (DTE) and prospective food consumption (PFC) before and immediately after breakfast, prior and after rest (CON) or exercise (MEAL-30 and MEAL-90), before and immediately after lunch, 30 minutes and 60 minutes after lunch, before and immediately after dinner.

2.8 | Food liking and wanting

The Leeds Food Preference Questionnaire, described in greater methodological detail by Dalton and Finlayson,[32] provided measures of food preference and food reward. The adolescents were presented with a culturally (food items and language) adapted version of the LFPQ following the recent recommendations from Oustric et al.[33] Participants were presented with an array of pictures of individual food items common in the diet. Foods were chosen by the local research team from a validated database to be either predominantly high (>50% energy) or low (<20% energy) in fat but similar in familiarity, protein content, palatability and suitable for the study population. The LFPQ has been deployed in a range of research[32] including a recent exercise/appetite trial in young French males[34] and adolescents.[20,35,36]

Explicit liking was measured by participants rating the extent to which they like each food ("How pleasant would it be to taste this food now?"). The food images were presented individually, in a randomized order and participants made their ratings using a 100-mm VAS. Implicit wanting was assessed using a forced choice methodology in which the food images were paired so that every image from each of the four food types was compared to every other type over 96 trials (food pairs). Participants were instructed to respond as quickly and accurately as they could to indicate the food they want to eat the most at that time ("Which food do you most want to eat now?"). Reaction times for all responses were covertly recorded and used to compute mean response times for each food type after adjusting for frequency of selection.

Responses on the LFPQ were used to compute mean scores for high-fat, low-fat, sweet or savoury food types (and different fat-taste combinations). Fat bias scores were calculated as the difference between the high-fat scores and the low-fat scores, with positive values indicating greater liking or wanting for high-fat relative to low-fat foods and negative values indicating greater liking or wanting for low-fat relative to high-fat foods. Sweet bias scores were calculated as the difference between the sweet and savoury scores, with positive values indicating greater liking or wanting for sweet relative to savoury foods and negative values indicating greater liking or wanting for savoury relative to sweet foods.

2.9 | Statistical analysis

Statistical analyses were performed using Stata software, Version 13 (StataCorp, College Station, TX, US). The sample size estimation was determined according to (a) CONSORT 2010 statement, extension to randomized pilot and feasibility trials (Eldridge et al. 2016[37] and (b) Cohen's recommendations[38] who has defined effect-size bounds as: small (ES: 0.2), medium (ES: 0.5) and large (ES: 0.8, "grossly perceptible and therefore large"). So, with 15 patients by condition, an effect-size around 1 can be highlighted for a two-sided type I error at 1.7% (correction due to multiple comparisons), a statistical power greater than 80% and an intra-class correlation coefficient at 0.5 to take into account between and within participant variability. All tests were two-sided, with a Type I error set at 0.05. Continuous data was expressed as mean ± SD (SD) or median [interquartile range] according to statistical distribution. The assumption of normality was assessed by using the Shapiro-Wilk test. Daily (total) area under the curve (AUC) were calculated using the trapezoidal method. Random-effects models for repeated data were performed to compare three conditions (a) considering the following fixed effects: time, condition and time x condition interaction, and (b) taking into account between and within participant variability (subject as random-effect). A Sidak's type I error correction was applied to perform multiple comparisons. As proposed by some statisticians[39,40] a particular focus will be also given to the magnitude of differences, in addition to inferential statistical tests expressed using *P*-values. The normality of residuals from these models was studied using the Shapiro-Wilk test. When appropriate, a logarithmic transformation was proposed to achieve the normality of dependent outcome.

3 | RESULTS

Eighteen adolescents with obesity participated in this study. Their mean age was 12.7 ± 1.3 years, body weight was 88.9 ± 23.6 kg (with a BMI of 33.3 ± 6.5 kg/m^2 [z-BMI 2.2 ± 0.4]), with a percentage of body fat mass of 37.6 ± 5.0% and a FFM of 53.1 ± 12.5 kg.

The adolescents had a $\dot{V}O_{2peak}$ of 21.8 ± 4.6 mL/min/kg. Energy expenditure induced by the exercise (total duration 30 minutes) was significantly higher compared to the 30-minutes resting energy expenditure (168.8 ± 43.6 and 46.9 ± 14.9 kcal, respectively; $P < .001$).

Table 1 details the results related to absolute and relative energy intake. At lunch, absolute ad libitum energy intake was significantly lower in MEAL-90 than MEAL-30 and CON ($P < .05$ and $P < .01$, respectively) and in MEAL-30 than CON ($P < .05$). Dinner ad libitum

TABLE 1 Absolute and relative energy intake in response the three conditions

		CON	MEAL-30	MEAL-90		ES		
		Mean (SD)	Mean (SD)	Mean (SD)	*P*	CON vs MEAL-30	CON vs MEAL-90	MEAL-30 vs MEAL-90
Energy intake (kcal)	Lunch	1380 (185)	1347 (313)*	1168 (234)**[a]	.0143	−0.12 [−0.60, 0.35]	−0.71 [−1.19, −0.24]	0.59 [0.11, 1.06]
	Dinner	796 (294)	931 (260)	748 (245)[b]	.0363	0.48 [0.00, 0.96]	−0.20 [−0.67, 0.28]	0.68 [0.20, 1.15]
	Total	2175 (330)	2277 (476)	1925 (360)***[c]	.0001	0.27 [−0.21, 0.74]	−0.80 [−1.28, −0.33]	1.07 [0.59, 1.54]
Relative energy intake (kcal)	Lunch	1337 (188)	1172 (313)*	1006 (246)***[a]	.0003	−0.56 [−1.03, −0.08]	−1.08 [−1.56, −0.61]	0.52 [0.04, 1.00]
	Total	2119 (332)	2110 (489)	1755 (366)***[c]	<.0001	−0.11 [−0.58, 0.37]	−1.16 [−1.63, −0.68]	1.06 [0.59, 1.54]

Abbreviations: CON, control condition; ES, effect size; ES: post hoc effect size; MEAL-30, test meal 30 min after exercise; MEAL-90, test meal 90 min after exercise; SD, standard deviation.
*P < .05 vs CON; **P < .01 vs CON; ***P < .001 vs CON; [a]P < .05 MEAL-30 vs MEAL-90; [b]P < .01 MEAL-30 vs MEAL-90; [c]P < .001 MEAL-30 vs MEAL-90.

energy intake was significantly lower in MEAL-90 compared with MEAL-30 (P < .01) with no difference between the exercise conditions and CON. Total daily absolute ad libitum energy intake was significantly lower in MEAL-90 compared with both CON and MEAL-30 (P < .001).

REI at lunch was significantly higher in CON compared with MEAL-30 and MEAL-90 (P < .05 and P < .001, respectively) and total REI was significantly higher in CON compared with MEAL-90 (P < .001). Both lunch (P < .05) and total REI (P < .001) were significantly lower in MEAL-90 than MEAL-30.

The lunch and total absolute intake of protein, fat were significantly lower in MEAL-90 compared with both CON (P < .01 and P < .05, respectively) and MEAL-30 (P < .01 and P < .05, respectively) while their intake at dinner was significantly lower in MEAL-90 compared with MEAL-30 (P < .05). The absolute intake of CHO was significantly lower in MEAL-90 compared with CON at lunch (P < .05) and significantly higher in MEAL-30 compared with CON at dinner (P < .05). Total absolute CHO intake was only significantly lower in MEAL-90 compared with CON (P < .05). No significant difference was observed between conditions regarding the relative intake of each macronutrient. Table 2 details these results.

Figure 2 presents the results related to appetite sensations. Fasting hunger, fullness, PFC and DTE did not differ between conditions. After the standardized breakfast, significant differences between conditions were found: hunger and DTE were higher in MEAL-30 than MEAL-90 (P = .003 and P = .02, respectively) and CON (P = .010 and P = .016, respectively), while PFC was greater in MEAL-30 than MEAL-90 only (P = .021). Before exercise, hunger was significantly lower during both exercise conditions than during CON (P < .001 for both). After exercise, this difference remained significant only between CON and MEAL-90 (P = .004). Immediately before lunch, hunger and PFC were significantly lower in MEAL-30 compared with CON (P = .036 and P = .041, respectively). Post-lunch sensations were similar between conditions. Pre-dinner hunger was lower during both exercise conditions compared with CON (P = .006 for MEAL-30 and P = .003 for MEAL-90). Pre-dinner fullness was greater in MEAL-30 and MEAL-90 compared with CON (P = .006 and P = .003, respectively). Regarding pre-dinner DTE and PFC, only MEAL-90 was significantly lower than CON (P = .006 and P = .005, respectively). Concerning the daily AUC (Figure 2), relative to CON, hunger and DTE were significantly lower in MEAL-30 (P = .019 and P = .05, respectively) and MEAL-90 (P = .034 and P = .031, respectively).

As detailed in Table 3, there was a significant condition effect for pre-dinner explicit liking fat bias (P = .004), with explicit liking for high-fat foods being lower in MEAL-90 compared with both CON (P = .001) and MEAL-30 (P = .004). While explicit liking taste bias significantly decreased in response to the lunch meal during the CON condition (P < .001), this significant meal effect disappeared during both exercise conditions, without a meal x condition interaction. Implicit wanting taste bias significantly increased in response to the lunch test meal during MEAL-90 (P = .04), and no meal effect was observed in CON and MEAL-30.

TABLE 2 Macronutrient intake in response the three conditions

		CON	MEAL-30	MEAL-90		ES		
		Mean (SD)	Mean (SD)	Mean (SD)	*P*	CON vs MEAL-30	CON vs MEAL-90	MEAL-30 vs MEAL-90
Proteins (g)	Lunch	73.8 (11.5)	71.9 (17.2)	60.7 (13.9)**[b]	.0059	−0.13 [−0.61, 0.34]	−0.76 [−1.24, −0.29]	0.63 [0.15, 1.10]
	Dinner	42.0 (18.4)	46.8 (14.4)	37.2 (13.2)[a]	.1811	0.25 [−0.22, 0.73]	−0.30 [−0.78, 0.17]	0.56 [0.08, 1.03]
	Total	115.9 (22.6)	118.7 (23.8)	98.8 (19.4)***[c]	.0007	0.08 [−0.40, 0.55]	−0.85 [−1.32, −0.37]	0.93 [0.45, 1.40]
Proteins (%)	Lunch	21.5 (2.3)	21.4 (3.0)	20.8 (2.3)	.5108	0.05 [−0.42, 0.53]	−0.07 [−0.55, 0.40]	0.23 [−0.25, 0.70]
	Dinner	20.8 (5.2)	19.9 (3.1)	20.1 (3.6)	.8811	0.17 [−0.31, 0.64]	0.01 [−0.46, 0.49]	−0.06 [−0.53, 0.42]
	Total	21.3 (2.5)	21.0 (2.0)	20.6 (2.3)	.6248	0.10 [−0.38, 0.58]	−0.05 [−0.53, 0.42]	0.14 [−0.33, 0.62]
Lipids (g)	Lunch	45.4 (9.6)	45.0 (14.2)	38.1 (12.5)*[a]	.0146	−0.06 [−0.53, 0.42]	−0.54 [−1.01, −0.06]	0.48 [0.06, 1.01]
	Dinner	28.8 (19.0)	33.8 (15.1)	26.1 (14.3)[a]	.0642	0.33 [−0.15, 0.80]	−0.18 [−0.66, 0.30]	0.51 [0.03, 0.98]
	Total	74.3 (18.0)	78.8 (19.9)	65.8 (19.1)*[b]	.0123	0.25 [−0.23, 0.72]	−0.54 [−1.01, −0.06]	0.79 [0.31, 1.26]
Lipids (%)	Lunch	29.8 (5.8)	30.3 (8.0)	29.2 (7.3)	.1910	0.05 [−0.42, 0.53]	−0.07 [−0.55, 0.40]	0.13 [−0.35, 0.60]
	Dinner	30.0 (12.9)	31.3 (10.6)	29.7 (9.8)	.0277	0.17 [−0.31 0.64]	0.01 [−0.46, 0.49]	0.15 [−0.32, 0.63]
	Total	30.7 (5.8)	31.2 (4.8)	30.5 (5.7)	.9655	0.10 [−0.38, 0.58]	−0.05 [−0.53, 0.42]	0.15 [−0.32, 0.63]
CHO (g)	Lunch	166.7 (39.4)	160.8 (52.8)	144.2 (34.6)*	.1649	−0.14 [−0.62, 0.33]	−0.52 [−0.99, −0.04]	0.37 [−0.10, 0.85]
	Dinner	92.8 (31.5)	109.9 (31.5)*	91.9 (29.4)[a]	.0269	0.52 [0.04, 0.99]	−0.036 [−0.54, 0.41]	0.58 [0.11, 1.06]
	Total	259.5 (56.1)	270.7 (70.0)	233.9 (49.7)[a]	.0751	0.17 [−0.31, 0.64]	−0.45 [−0.92, 0.03]	0.61 [0.14, 1.09]
CHO (%)	Lunch	48.0 (7.6)	47.5 (10.5)	49.5 (9.1)	.2149	0.06 [−0.53, 0.42]	0.15 [−0.33, 0.62]	−0.20 [−0.68, 0.27]
	Dinner	49.7 (15.6)	48.9 (12.4)	50.7 (10.7)	.0840	−0.01 [−0.48, 0.47]	0.13 [−0.34, 0.61]	−0.14 [−0.61, 0.34]
	Total	47.8 (7.4)	47.4 (6.1)	48.7 (7.3)	.9547	−0.05 [−0.53, 0.42]	0.14 [−0.34, 0.61]	−0.19 [−0.67, 0.28]

Abbreviations: CHO, carbohydrates; CON, control condition; ES, effect size; ES: post hoc effect size; MEAL-30, test meal 30 min after exercise; MEAL-90, test meal 90 min after exercise; SD, standard deviation.
*P < .05 vs CON; **P < .01 vs CON; ***P < .001 vs CON; [a]P < .05 MEAL-30 vs MEAL-90; [b]P < .01 MEAL-30 vs MEAL-90; [c]P < .001 MEAL-30 vs MEAL-90.

4 | DISCUSSION

The timing of exercise relative to a meal has been recently highlighted for its influence on energy intake and appetite control,[1,16] with some recent studies suggesting a better effect of acute exercise performed close to a meal on energy intake and appetite in both adolescents who are lean[19] and adolescents with obesity.[20] However, these studies did not consider the potential impact of the delay between the exercise and the previous breakfast intake. It has been shown that this delay will impact the metabolic nature of exercise such as the substrates used,[21] which might, in turn, differently affect subsequent energy intake.[22-24] In that context, the aim of the present study was

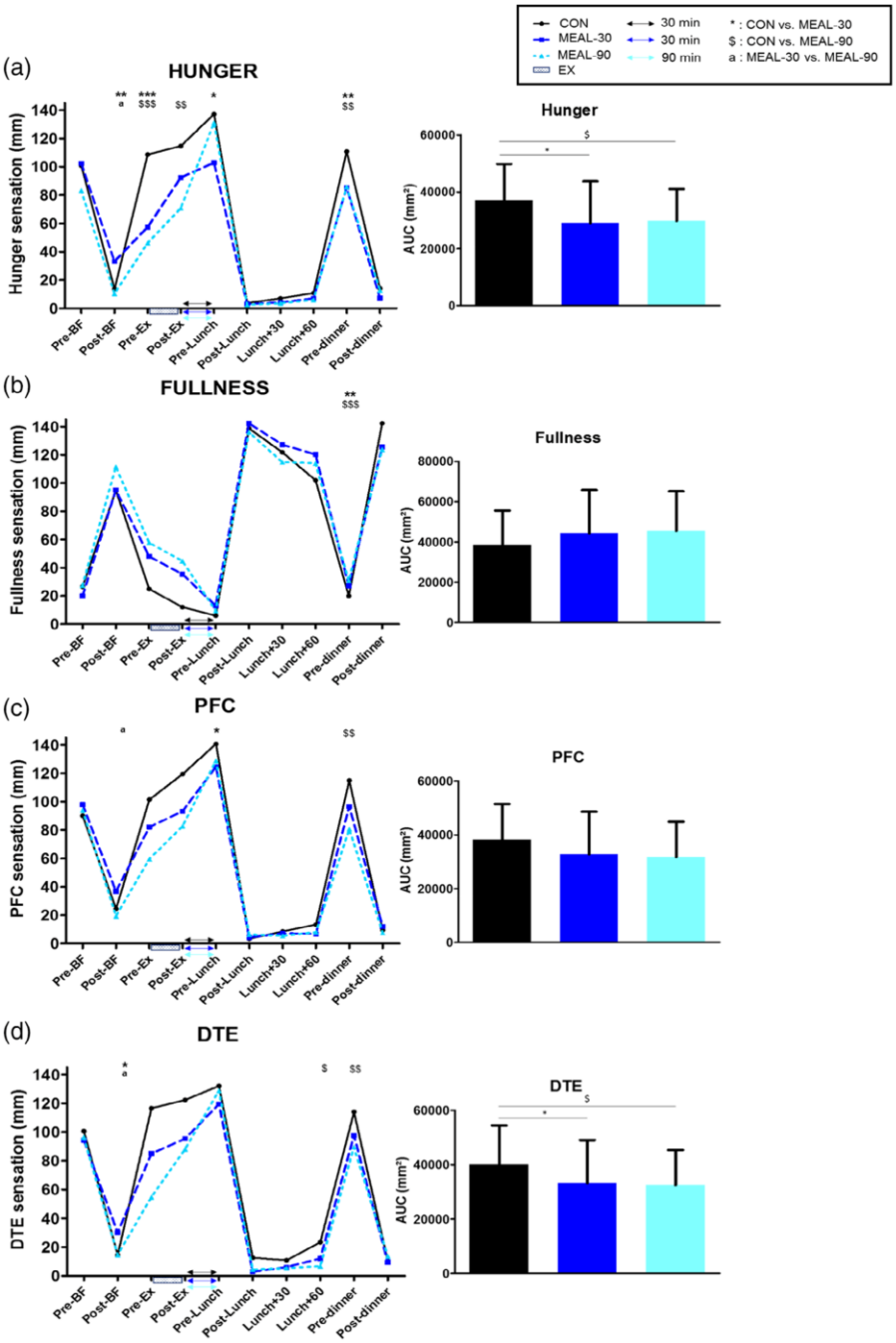

FIGURE 2 Daily appetite sensations and AUC for hunger, fullness, prospective food consumption and desire to eat

CON: Control Condition; MEAL-30: Test meal 30 min after exercise; MEAL-90: Test meal 90 min after exercise; BF: Breakfast; VAS: Visual Analog Scale; EX: Exercise; AUC : Area Under the Curve

TABLE 3 Pre- and post-test meal food reward on the three experimental conditions

	CON	MEAL-30	MEAL-90		Interaction time × condition		
	Mean (SD)	Mean (SD)	Mean (SD)	*P*	CON vs MEAL-30	CON vs MEAL-90	MEAL-30 vs MEAL-90
Implicit wanting							
Fat bias							
Before lunch	22.32 (31.15)	19.96 (33.15)	22.80 (31.68)	.78	0.99	0.58	0.56
After lunch	20.21 (45.58)	17.63 (48.49)	12.61 (29.50)	.46			
P before vs after lunch	*0.88*	*0.80*	*0.90*		0.00 [−0.48-0.48]	−0.13 [−0.61-0.34]	−0.14 [−0.62-0.33]
Before dinner	4.37 (64.45)	20.74 (19.89)	14.99 (26.63)	.49			
Taste bias							
Before lunch	31.60 (33.67)	34.17 (41.81)	24.90 (32.49)	.76	0.93	0.14	0.26
After lunch	25.60 (54.02)	27.00 (67.00)	43.59 (30.79)	.59			
P before vs after lunch	*0.69*	*0.85*	*0.04*		0.02 [−0.45-0.50]	0.36 [−0.11-0.84]	0.27 [−0.20-0.75]
Before dinner	38.24 (37.81)	40.40 (40.11)	42.30 (28.12)	.98			
Explicit liking							
Fat bias							
Before lunch	10.02 (19.71)	12.52 (16.35)	10.53 (19.64)	.34	0.57	0.77	0.86
After lunch	5.29 (9.39)	5.14 (10.66)	4.08 (9.25)	.94			
P before vs after lunch	*0.27*	*0.03*	*0.11*		−0.14 [−0.61–0.34]	−0.07 [−0.55-0.40]	0.04 [−0.43-0.52]
Before dinner	11.35 (19.83)	9.04 (16.34)	2.44 (13.00)***[b]	<.001			
Taste bias							
Before lunch	26.18 (20.37)	21.95 (23.03)	20.31 (22.89)	.82	0.10	0.25	0.74
After lunch	12.78 (19.10)	18.08 (25.78)	14.47 (27.62)	.73			
P before vs after lunch	*<0.001*	*0.38*	*0.19*		0.40 [−0.07-0.88]	0.28 [−0.19-0.76]	−0.08 [−0.56-0.40]
Before dinner	24.00 (24.58)	21.40 (26.08)	20.76 (28.74)	.99			

Abbreviations: CON, control condition; MEAL-30, test meal 30 min after exercise; MEAL-90, test meal 90 min after exercise; SD, standard deviation.
***$P < .001$ vs CON; [b]$P < .01$ MEAL-30 vs MEAL-90; *P* values and effect size are presented for interactions.

to investigate the effect of exercise performed at the same delay from breakfast on energy intake, appetite sensations and food reward at the following lunch set either 30 or 90 minutes after exercise in adolescents with obesity.

According to our results, both exercise conditions (MEAL-30 and MEAL-90) led to significantly lower absolute energy intake at lunch compare to CON. This is in line with previous studies in similar populations showing reduced subsequent intake in response to acute exercise set at the same time of the morning.[12,14,20,41] Interestingly, absolute energy intake was also significantly lower in MEAL-90 compared with MEAL-30, suggesting a greater anorexigenic effect when exercise does not immediately precede the meal. Additionally, total and dinner absolute energy intake were lower during MEAL-90 only, with total daily energy intake reduced by 12% (250 kcal/d) and 16% (352 kcal/d) compared with CON and MEAL-30, respectively. These results are reinforced by a lower lunch relative energy intake after MEAL-30 compared with CON and lower lunch and total REI during MEAL-90 compared with both MEAL-30 and CON. Importantly, while most of the available evidence supports the anorexigenic effect of intensive exercise,[13,36,42,43] our results reinforce more recent work observing reduced food intake in response to moderate-to-vigorous exercise in adolescents and children with obesity.[41]

While available evidence indicates the beneficial effect of exercising close to a meal on subsequent energy intake,[19,20] our results seem to suggest that more than the exercise-meal delay itself, the interval between the exercise and the following eating episode is of importance.

A balanced buffet meal offering several items selected to avoid any over-, under- or occasional-consumption (as previously validated[30]) was offered to adolescents which provided the opportunity to also assess their macronutrient intake. While none of the relative intake of fat, protein and carbohydrate were found different between conditions, their absolute consumption at lunch was reduced only in MEAL-90 compared with CON, and compared with MEAL-30 for protein and lipid. Interestingly, the absolute intake of carbohydrate at dinner increased in MEAL-30 compared with the two other conditions. The macronutrient responses observed in MEAL-90 seem in line with Albert et al in lean adolescents[19] and with our previous study in adolescents with obesity,[20] showing reduced absolute macronutrient intake after moderate exercise set at the end of the morning. The current study however missed to find similar results in MEAL-30, suggesting here the potential importance of the delay between the exercise and the previous eating episode (breakfast). Indeed, in these previous studies, the appetitive responses to exercise set at different times of the morning, and then at different delays from breakfast, were compared, meaning that despite similar duration, modality and intensity, the exercise was not of similar metabolic and energetic load,[21] which might explain our results. Unfortunately, it was not possible in the present study to measure the substrate oxidation during exercise and at rest. Furthermore, it remains difficult to reach a consensus regarding the effect of acute exercise on macronutrient intake in lean adolescents and in adolescents with obesity based on the available evidence.[43]

Regarding the adolescents' subjective appetite sensations, our results show a lower daily (AUC) hunger and desire to eat in both exercise conditions compared with CON. Although pre-lunch hunger and PFC were significantly lower in MEAL-30 compared with CON, which could have contributed to the lower observed ad libitum energy intake, they remained unchanged in MEAL-90 while the decreased food consumption was even more pronounced. This inconsistency between appetite sensations and energy intake reinforce the previously described uncoupling effect of exercise between these sensations and food consumption.[44] Interestingly however, post-lunch sensations were identical between exercise conditions, suggesting a similar satiating effect of lunch meals despite lower intakes in MEAL-30 and particularly in MEAL-90, limiting any potential subsequent compensatory responses. This is even reinforced by the significantly reduced food intake observed at dinner in MEAL-90. This is of particular importance since energy deficits, especially when induced by reduced energy intake, have been shown to generate a subsequent compensatory rise in food intake, with physical exercise limiting or avoiding such a compensation.[34,45]

Some recent studies have highlighted the importance of considering the effect of exercise on food reward to better understand its impact on subsequent energy intake in adolescents with obesity.[35] We also assessed whether the liking and wanting for food could be impacted by the delay between eating episodes and exercise in this population. In 2018, Miguet et al observed reduced relative preference for fat and sweet taste, and implicit wanting for high-fat foods (also using the LFPQ) in response to an ad libitum meal set 30 minutes after a 16-minute cycling high intensity interval exercise in a similar population.[35] According to the present results, none of the pre or post lunch components of liking and wanting were different between conditions. These results are contradictive with those from Miguet et al,[35] especially regarding our MEAL-30 condition that had the same delay between the exercise and the meal. However, the exercise intensities were different (high intensity intermittent exercise vs moderate intensity continuous exercise), reinforcing once more the importance of the exercise intensity in the subsequent control of energy intake. Interestingly, we can see here a significantly lower explicit liking for high-fat food immediately before dinner in MEAL-90 compared with the two others, which might contribute to the observed reduced dinner ad libitum food intake. Our results are however also in contradiction with some recently published from our group, showing different food reward responses depending on exercise-meal timing in adolescents with obesity.[20] A lower pre-meal explicit liking for high-fat relative to low-fat foods was observed when the adolescents performed 30 minutes of moderate intensity cycling 60 minutes before lunch compared with the same exercise performed 180 minutes before lunch.[20] The different LFPQ timing between MEAL-90 and the two other conditions must be considered when interpreting our results. Indeed, food reward was assessed pre- and post-lunch meaning that its delay from exercise was different, which might have affected the results. Although there is a growing interest in the effect of exercise on food reward in this population, evidence remains too limited to

draw any conclusion and further studies using standardized designs are needed.

The present results must be interpreted in light of some limitations. First, as for the other published studies examining the timing of exercise relative to a meal,[16,17,19,20] the lack of direct evaluation of the adolescents' oxygen consumption and substrate oxidation using indirect calorimeters, as well as the lack of a lean control group to examine the potential weight status effect, are the two main limitations. Although the laboratory-based nature of this work constitutes a strength as it allows a better control of the adolescents' activity and intake, it might also not be representative of their habitual daily free-living setting, such as the school setting for instance, as previously underlined by Mathieu et al in healthy adolescents.[17] Finally, the lack of tracking of the adolescents' food intake over 24 to 48 hours for practical reasons also limits the interpretation of our results.[12]

In line with the present work, another potential important factor, while not addressed in the current study, is the timing of exercise (and food intake) with regards to circadian/diurnal rhythms. Emerging evidence suggests that the timing of exercise[46,47] (and food intake[48,49]) impact body weight regulation. Any effects observed from exercise-meal delays may be a result of an interaction with circadian/diurnal oscillations occurring relative to sleep/wake times. Future studies should propose a more complete and integrative exploration of the chronobiologic regulations of energy intake and overall energy metabolism in such adolescents with obesity. Indeed, not only the timings of exercise and/or energy intake should be considered, but also their interactions with the adolescents' sleep, to better understand and potentially regulate their 24-hours circadian rhythm.[50,51] Some key physiological actors of this circadian clock, such as ghrelin and leptin for instance, who are particularly involved in the control of appetite and respondents to sleep and exercise should be mainly considered.[52]

5 | CONCLUSION

To conclude, the present study reinforces the interest in the timing of exercise relative to a meal to affect overall energy balance in youth with obesity; highlighting the importance of the time interval between both the exercise and the previous eating episode, and the exercise and the following meal. According to these results, delaying the timing of the meal after exercise might help reduce energy balance by decreasing ad libitum energy intake without increasing hunger and by improving satiety in adolescents with obesity. Future studies should question the importance of the exercise-meal timing on the longer term. While further acute and chronic studies are needed, these results contribute to the current limited body of evidence in the area and seem important in order to optimize weight loss strategies.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

A.F. and D.T. conceived experiments. A.F., M.M. and M.B. carried out experiments, A.F. and D.T. analysed data. K.B. was involved in writing the paper and all authors had final approval of the submitted and published versions.

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SHORT COMMUNICATION

Does exercising before or after a meal affect energy balance in adolescents with obesity?

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KEYWORDS
Exercise-meal timing;
Appetite;
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Obesity;
Adolescent

Abstract *Background and aim:* Exercise timing has been suggested to affect appetite and energy intake (EI). The aim of this study was to examine the impact of exercising immediately before or after a meal on EI, appetite sensations and food reward (FR) in adolescents with obesity.
Methods and results: Seventeen adolescents with obesity completed 3 experimental sessions (randomized controlled trial): rest + lunch (CON); exercise + lunch (EX-MEAL); lunch + exercise (MEAL-EX). The exercise consisted of cycling 30 min at 65%VO_{2peak}. Outcomes included *ad libitum* EI (weighed lunch and dinner), FR (Leeds Food Preference Questionnaire at pre- and post-combination of exercise/rest and lunch, and pre-dinner) and appetite sensations (visual analogue scales). EI was not different between conditions. Compared with CON, relative EI at lunch was lower in EX-MEAL and MEAL-EX ($p \leq 0.05$) and daily only in MEAL-EX ($p < 0.01$). Postprandial fullness was higher in EX-MEAL compared to CON. Compared with CON, both EX-MEAL and MEAL-EX attenuated the increase in wanting for sweet food and reduced explicit liking for fat.
Conclusions: These preliminary results suggest that exercising immediately before or after a meal produce few differences in appetite and have small beneficial effects on overall energy balance in adolescents with obesity, as well as on FR.
Clinical trials: NCT03967782.

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Introduction

It is now recognized that physical exercise not only increases energy expenditure but it can also affect appetite and energy intake (EI) in adolescents with obesity, depending on its duration [1,2], intensity [3,4], induced-energy expenditure [5] or as more recently suggested, its timing during the day [6–8].

Albert and collaborators showed in lean adolescents that EI could be reduced by 11% (with a 23% decrease in fat intake) in response to acute exercise (30 min at 65–70% $\dot{V}O_{2peak}$) performed immediately before lunch compared with the same exercise set 3 h before lunch [9]. Similar results have been recently observed in adolescents with obesity who decreased their EI by 115 kcal (10%) 60min but not 180min after similar acute exercise [6,7].

While these studies assessed the effect of the delay between exercise and the following meal on EI and appetite, Mathieu et al. recently investigated whether different meal-exercise patterns (exercise then meal or meal then exercise) could differently affect overall energy balance in normal-weight children [10]. Although the authors did not find any differences between conditions, this remains unexplored among children and adolescents with obesity, who have shown different appetitive responses to exercise [11].

The aim of the present study was to compare the effect of exercising immediately before or after a meal on EI, appetite sensations and food reward (FR) in adolescents with obesity.

Methods

Eighteen adolescents with obesity [12] from the local Pediatric Obesity Center (La Bourboule, France) were recruited for this randomized controlled trial. To be included the adolescents had to: be aged 12–16 years; have a BMI ≥97th percentile [13]; be inactive (IPAQ [14]; be free of any contraindication to physical activity; be free of medication that could influence their nutritional response and metabolism; sign the information notice and consent form as well as their legal representatives; have no medical or surgical history and/or pathology/treatment judged incompatible with the study; not be undergoing an energy restriction or weight-loss program through physical activity at the time of inclusion or within the last 6 months; not be a smoker or regular alcohol consumer. Anthropometric measurements, body composition (Dual-energy X-ray absorptiometry, QDR4500A Hologic, Waltham, MA, USA) and maximal aerobic capacity ($\dot{V}O_{2peak}$) [15] were assessed as previously described [16]. Adolescents randomly completed three experimental sessions (one week apart): i) CON: no exercise and 30-min rest before lunch; ii) EX-MEAL: 30-min cycling exercise (65% $\dot{V}O_{2peak}$) between 12:00–12:30pm followed by lunch between 12:30–1:30pm; iii) MEAL-EX: lunch between 12:30–1:30pm followed by 30-min cycling exercise (65% $\dot{V}O_{2peak}$) between 1:30–2:00pm. Exercise intensity was controlled by the mechanical load imposed to the cycle ergometer and verified using heart rate recording (Polar V800). Energy expenditure was estimated based on the maximal oxygen uptake evaluation. The experimenters weighed the food items before and after each meal. The lunch buffet was composed of beef steak, pasta, mustard, cheese, yogurt, compote, fruits and bread and the dinner of ham/turkey, beans, mashed potato, cheese, yogurt, compote, fruits and bread. The adolescents were allowed to drink water only. *Ad libitum* EI in kcal and macronutrient composition (proportion of fat, carbohydrate and protein) were calculated using the software Bilnut4.0. Adolescents did not have access to food outside the test meals. Relative energy intake (REI) was obtained by subtracting exercise-induced energy expenditure from lunch and total (=lunch + dinner) EI. Hunger, fullness, desire to eat (DTE) and prospective food consumption (PFC) were assessed throughout the day (pre-breakfast, post-breakfast, post-breakfast+30min, post-breakfast+60min, pre-ex/rest, post-ex/rest, pre-lunch, post-lunch, post-lunch+30min, post-lunch+60min, pre-dinner, post-dinner) using visual analogue scales [17]. Pre- and post-combination of exercise/rest and lunch, as well as pre-dinner FR (liking and wanting for high-fat relative to low-fat food (fat bias) and sweet relative to savoury food (taste bias)) was assessed using the Leeds Food Preference Questionnaire [18] as previously described [16]. This study was approved by the appropriate ethical institutions (2019-A00507-50) and registered as a clinical trial (NCT03967782). Of the 18 participants, one did not complete all the sessions for personal reasons (not related to the study) leaving the final sample at 17 adolescents.

Sample size was determined according to previous works reported in literature [6,7] and to an estimation based on effect-size difference greater than 0.6, for a two-sided type I error at 1.8%, a statistical power at 80% and an intra-class correlation coefficient at 0.5 (three conditions for a same subject). Area under the curve (AUC) was calculated using the trapezoidal method. Random-effects models for repeated data were performed. A particular focus was also given to the magnitude of differences, in addition to inferential statistical tests expressed using p-values (two-sided Type I error set at 0.05 and Sidak's type I error correction applied to multiple comparisons).

Results

Seventeen adolescents (9 boys) with obesity participated in this study. Their mean age was 12.8 ± 1.4 years, body weight was 88.0 ± 15.4 kg, with a body mass index of 33.4 ± 5.7 kg/m^2 (z-BMI 2.2 ± 0.4), body fat mass of 38.0 ± 4.2%, fat-free mass of 52.5 ± 9.2 kg and $\dot{V}O_{2peak}$ of 21.8 ± 5.9 ml/min/kg.

Lunch, dinner and daily EI were not different between conditions (Table 1). Lunch REI was lower in EX-MEAL ($p < 0.05$) and MEAL-EX ($p < 0.01$) compared to CON. Daily REI was lower in MEAL-EX compared with CON ($p < 0.01$). Macronutrient intake at lunch, dinner and daily was not different between conditions.

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Table 1 Absolute and Relative Energy Intake in response the three conditions.

	CON	EX-MEAL	MEAL-EX	p	ES [95% CI]		
	Mean (SD)	Mean (SD)	Mean (SD)		*CON vs. EX-MEAL*	*CON vs. MEAL-EX*	*EX-MEAL vs. MEAL-EX*
Energy Intake (kcal)							
Lunch	1245 (372)	1163 (288.9)	1150 (314)	0.49	−0.14 [-0.62,0.33]	0.26 [-0.73,0.22]	0.12 [-0.36,0.59]
Dinner	752 (279)	776 (302)	732 (262)	0.69	0.15 [-0.33,0.62]	−0.07 [-0.54,0.41]	0.21 [-0.27,0.68]
Total	1997 (514)	1939 (501)	1882 (488)	0.36	−0.04 [-0.51,0.44]	−0.31 [-0.79,0.16]	0.27 [-0.21,0.74]
Relative Energy Intake (kcal)							
Lunch	1206 (383)	989 (286)*	989 (300)**	0.03	−0.76 [-1.24,-0.29]	−0.86 [-1.34,-0.39]	0.10 [-0.38,0.57]
Total	1929 (520)	1786 (511)	1721 (477)*	0.08	−0.42 [-0.90,0.05]	−0.69 [-1.17,-0.22]	0.27 [-0.21,0.74]

CON: control condition; EX-MEAL: Exercise before test meal; MEAL-EX: Exercise after test meal; SD: Standard Deviation; *: *vs.* CON ($p < 0.05$); **: *vs.* CON ($p < 0.01$).

Hunger was lower at 12:00pm in EX-MEAL compared with CON ($p = 0.003$) and MEAL-EX ($p = 0.0003$). Fullness was higher post-lunch ($p = 0.01$) and post-lunch+30 mins ($p = 0.04$) in EX-MEAL compared with CON (Fig. 1). No differences in daily AUC were found between conditions.

Pre- and post-combination of exercise/rest and lunch, and pre-dinner implicit wanting and explicit liking for fat and savory foods did not differ between conditions (Table 2). In response to the combination of rest + lunch (CON), implicit wanting for sweet foods increased ($p = 0.04$). Explicit liking for fat foods decreased only after the

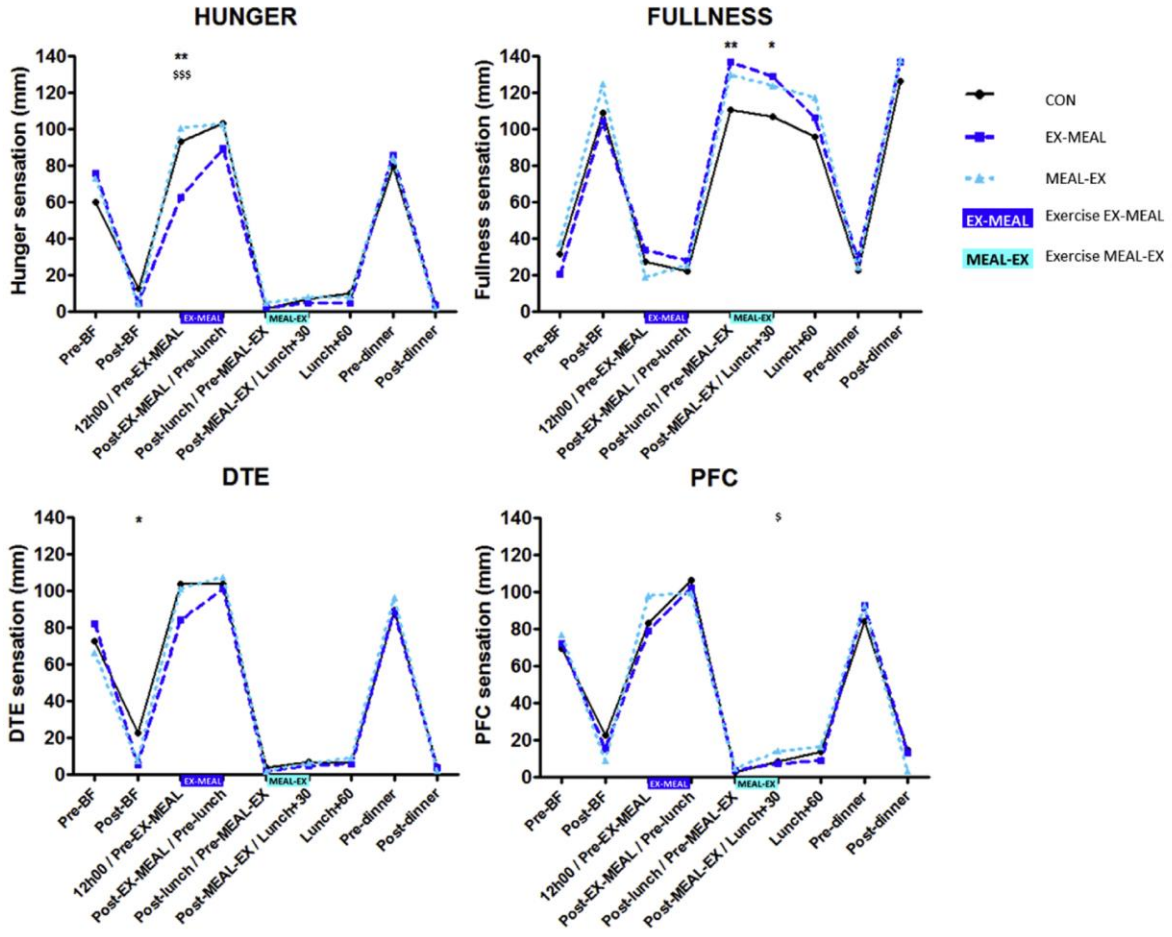

Figure 1 Daily Hunger(A); Fullness(B); Desire to Eat(DTE; C) and Prospective Food Consumption(PFC; D); BF: Breakfast; CON: rest condition; EX-MEAL: Exercise before test meal; MEAL-EX: Exercise after test meal; *: CON *vs.* EX-MEAL $p < 0.05$; **: CON *vs.* EX-MEAL $p < 0.01$; $: EX-MEAL *vs.* MEAL-EX $p < 0.05$; $$$: EX-MEAL *vs.* MEAL-EX $p < 0.001$.

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Table 2 Pre- and post-combination (exercise/rest and lunch) food reward.

	CON	EX-MEAL	MEAL-EX	p	Interaction time x condition		
	Mean (SD)	Mean (SD)	Mean (SD)		*CON vs. EX-MEAL*	*CON vs. MEAL-EX*	*EX-MEAL vs. MEAL-EX*
Implicit Wanting							
Fat Bias							
Before comb.	20.7 (31.3)	24.6 (32.6)	26.3 (38.5)	0.24	0.97	0.89	0.95
After comb.	18.8 (34.9)	21.1 (42.8)	15.5 (34.0)	0.38			
p before vs. after	*0.32*	*0.72*	*0.33*				
Before dinner	14.0 (33.4)	2.3 (27.8)	25.5 (50.2)	0.40			
Taste Bias							
Before comb.	13.3 (21.6)	4.5 (33.9)	−0.1 (48.2)	0.38	0.54	0.79	0.54
After comb.	30.0 (30.5)	12.8 (41.0)	22.2 (55.9)	0.57			
p before vs. after	***0.04***	*0.48*	*0.25*				
Before dinner	12.1 (44.8)	11.9 (44.8)	20.2 (36.0)	0.78			
Explicit Liking							
Fat Bias							
Before comb.	3.3 (17.8)	2.6 (18.5)	5.5 (14.0)	0.33	0.50	0.93	0.53
After comb.	3.6 (15.4)	−0.4 (13.0)	3.4 (15.9)	0.61			
p before vs. after	*0.94*	***p < 0.001***	***0.03***				
Before dinner	5.7 (14.0)	4.1 (16.5)	5.0 (20.5)	0.23			
Taste Bias							
Before comb.	9.2 (12.8)	7.6 (23.6)	9.3 (25.6)	0.85	0.80	0.96	0.81
After comb.	15.6 (23.1)	15.9 (16.5)	5.4 (17.3)	0.31			
p before vs. after	*0.23*	***0.05***	*0.50*				
Before dinner	7.6 (22.8)	13.7 (30.0)	7.1 (26.4)	0.32			

CON: rest condition; EX-MEAL: Exercise before lunch; MEAL-EX: Exercise after lunch; SD: Standard Deviation; comb.: combination of rest/exercise and lunch. In bold are represented the significant p ($p < 0.05$).

exercise conditions (EX-MEAL $p < 0.001$, MEAL-EX $p = 0.03$). Explicit liking for sweet foods increased only in EX-MEAL ($p = 0.05$).

Discussion

This study investigated the effect of exercising immediately before or after lunch on EI, appetite sensations, FR and overall energy balance in adolescents with obesity. While lunch and daily absolute EI did not differ between conditions, daily EI was reduced by 58 kcal (3%) and 115 kcal (6%) in EX-MEAL and MEAL-EX, respectively. Furthermore, both exercise conditions favorably affected overall energy balance. In fact, this reduction of the adolescents' EI in EX-MEAL and MEAL-EX, combined with the observed increased energy expenditure during the exercise (on average 135 kcal in EX-MEAL and 122 kcal in MEAL-EX), can favor a reduction of their daily energy balance of 193 kcal in EX-MEAL and 237 kcal in MEAL-EX, which could favor weight loss if repeated and sustained over time (the chronic effect remaining to be further studied), as previously suggested [6,7].

This is in line with Mathieu et al. who also did not observe any differences in EI but a reduced REI in lean children who performed acute moderate-to-vigorous exercise in two different meal-exercise patterns (exercise then meal or meal then exercise) in a school setting [10]. Their results suggest that further studies should be conducted to assess whether exercising at high-intensity immediately before or after a meal can differently affect EI in youth.

In terms of appetite sensations, moderate-intensity 30-min cycling exercise before lunch seems to favor a higher postprandial fullness compared with rest, suggesting a potential effect of pre-meal exercise not only on EI but also on satiety signaling. Indeed, exercise before a meal appears to increase postprandial fat oxidation [19] and may improve glucose tolerance [20] which offer potential mechanisms to explore in the impact of meal-exercise timing on appetite control. Although Mathieu and colleagues did not assess appetite sensations in their study, our finding is in line with another study in adolescents with obesity showing increased satiety quotient when acute exercise is performed before eating [6,7]. Furthermore, an anticipatory effect on subjective appetite may have occurred as differences in hunger and fullness were observed prior to the exercise in EX-MEAL and MEAL-EX, respectively. Importantly, our results suggest that exercising immediately after a meal does not lead to any perceived-discomfort that could discourage adolescents to exercise or decrease their compliance to physical activity.

Regarding FR, the results suggest that performing exercise, regardless of its timing around a meal, may attenuate the increase in wanting for sweet foods observed after rest then lunch (CON). Moreover, liking for fat decreased after both exercise conditions and only EX-MEAL led to an increase in liking for sweet. This increase in liking for sweet (in parallel with a decrease in fat) may reflect an increase in preference for low-fat sweet foods such as fruits, etc., but remains to be explored further. As recently highlighted by Beaulieu et al. [21], it appears that exercise has beneficial effects on food reward and preferences.

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While similar results are observed when the same exercise is performed after the meal (MEAL-EX), it must be noted that both the pre- and post-combination LFPQ have were performed with a 30-min delay compared with the two other conditions, in order to keep the lunch meal at the same time of the day, which might have impacted the results. Implementing a fourth condition with Meal-Rest that would have followed the same timings and architecture as MEAL-EX may have provided a better comparison. Similarly, although it could have been great to have a larger sample size and gender repartition to question a potential sex effect, it has been previously shown that adolescent boys and girls with obesity experience the same nutritional responses to acute exercises [22].

To conclude, these preliminary results suggest that exercising at moderate-intensity both immediately before or after a meal have small beneficial effects on overall energy balance in adolescents with obesity, as well as on food reward. Pre-meal exercise resulted in increased postprandial sensations of fullness. These findings have implications for practitioners who are constrained by adolescents' daily schedules either in the school or clinical setting.

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Declaration of Competing Interest

None of the authors have a conflict of interest.

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RÉSUMÉ

Scepticisme ou désintérêt : la maigreur constitutionnelle reste aujourd'hui très peu explorée, avec seulement une quarantaine d'études publiées à ce jour. La littérature apparaît non seulement équivoque vis-à-vis de la caractérisation physiologique de la maigreur constitutionnelle, mais également à propos de sa définition et de son diagnostic. Le premier axe avait pour objectif de définir le diagnostic et de caractériser la physiologie de la maigreur constitutionnelle et le second objectif visait à explorer le tissu musculaire de cette population. Les travaux de thèse ont permis de proposer un arbre décisionnel d'aide au diagnostic de la maigreur constitutionnelle basé sur une revue systématique de la littérature. Par ailleurs, l'approche méta-analytique a montré que l'individu maigre constitutionnel présente une physiologie particulière en termes de composition corporelle, métabolisme énergétique et régulation hormonale ; néanmoins très différente de celle du patient présentant une anorexie mentale. De façon préliminaire à l'exploration cellulaire du muscle, ce travail de doctorat a permis de valider de nouvelles méthodes de colorations immunohistochimiques musculaires. Finalement, l'exploration du muscle squelettique du sujet maigre constitutionnel a mis en évidence une forte hypotrophie musculaire, une faible capillarisation, une altération de certaines activités enzymatiques mais également un faible stockage en triglycérides et en glycogène musculaires. Ces résultats ont ainsi permis d'apporter de nouvelles perspectives dans la compréhension de cette physiologie atypique qui caractérise la maigreur constitutionnelle.

ABSTRACT

Scepticism or disinterest: constitutional thinness remains little explored today, with only about forty studies published to date. The literature appears not only equivocal regarding the physiological characterisation of constitutional thinness, but also with regard to its definition and diagnosis. The first axis aimed to define the diagnosis and characterise the physiology of constitutional thinness, and the second objective was to explore the muscle tissue of this population. The present thesis work allowed to propose a decision tree to help in the diagnosis of constitutional thinness, based on a systematic review of the literature. Moreover, the meta-analytical approach showed that the constitutionally thin individuals present a specific physiology regarding body composition, energy metabolism and hormonal regulation; which is however very different from that of patients with anorexia nervosa. Preliminarily to muscle cell exploration, this doctoral work validated new methods of immunohistochemical muscle staining. Finally, the exploration of the skeletal muscle of constitutionally thin subjects revealed a strong muscular hypotrophy, a low capillarisation, an alteration of some enzymatic activities but also a low storage of muscle triglycerides and glycogen. These results have brought new insights in the understanding of this atypical physiology which characterizes constitutional thinness.