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Guest Editors: Andrea C. Buchholz, Marta Van Loan, Leah Whigham, and Henry Lukaski

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Editorial Board

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Editorial

Lifestyle Modification to Promote Weight Loss in the Absence of Energy Restriction

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With the obesity epidemic showing no signs of abating, there is ongoing interest in altering energy balance (i.e., decreasing energy intake and/or increasing energy expenditure) to promote weight, specifically fat, loss. However, short- and long-term outcomes of, and adherence to, decreasing energy intake are variable, and reports of weight regain are common. There is thus increasing interest, both at the lay public and scientific levels, in lifestyle modifications to promote weight loss in the absence of energy restriction. In this special issue, we present two review papers and three randomized control trials that address such lifestyle modifications.

The first paper is a review of weight control strategies and the effects of these strategies on the hormonal responses associated with appetite control and regulation. A. Schwarz et al. conclude that consumption of smaller and more frequent meals comprised of lower fat and moderate protein, normal sleep of 8 hours/day, and control of stressors and psychological stress may help to attenuate the hormonal responses associated with appetite control. This is followed by the review of B. N. Wu and A. J. O'Sullivan, in which the authors stress the importance of considering gender differences in energy metabolism. This conclusion arises from the observation that compared to men, women consume fewer calories per kilogram lean mass and burn fat more preferentially during exercise, suggesting that the relationship between energy consumed and energy expended is different between the genders. The authors attribute the greater fat mass observed in women to ovarian hormones, particularly estrogen, which may promote postprandial conversion of dietary energy into body fat. From these two reviews, we appreciate the influence of lifestyle modification and gender on various aspects of energy balance. This understanding may in turn culminate in strategies to control or reverse fat gain beyond those that emphasize only energy restriction.

In line with the dietary recommendations presented in the review of A. Schwarz et al., L. Ferguson-Stegall et al., in the first of three studies presented as part of this special issue, compared the effects of three experimental beverages: low-fat chocolate milk, an isocaloric carbohydrate beverage and a placebo beverage on training adaptation (cycling) of 32 untrained nonobese men and women. After each daily training session, the participants consumed their assigned experimental beverages immediately and again 1 hour after exercise. After 4.5 weeks, the differentials in fat mass loss and lean mass gain at the whole body and trunk levels were greater for the chocolate milk group versus carbohydrate group. The authors attribute this observation to the availability of amino acids in milk for anabolism and muscle mass gain, and the fat-loss-promoting effect of dairy calcium and protein, consistent with Schwarz and colleagues' recommendation for consumption of moderate protein and lower fat foods.

The next study, by S. L. Tey et al., examined the effects of daily isocaloric consumption of hazelnuts, chocolate, or potato chips for 12 weeks, compared to a control group receiving no snacks. Participants were 118 nonobese men and women; they were not provided any dietary counselling nor were they instructed to change their diets in any way. Despite the finding of no main effect of treatment, a subanalysis of adherent participants revealed that a higher baseline

BMI was associated with a lower waist circumference at 12 weeks in the nut group versus control group. Also, dietary quality improved significantly in the nut group. The authors conclude that nuts can be incorporated into the diet without adversely affecting body weight, while improving overall diet quality. We extend this conclusion with the suggestion that if this observation were to hold over a longer study duration, then nuts might help to reverse the fat gain seen with aging. It is also tempting to suggest that regular consumption of nuts may reduce abdominal obesity in people with higher BMIs, although this requires confirmation.

In the final study, M. H. Pedersen et al. randomized 78 overweight adolescent boys to consume bread with fish oil or vegetable oil for 16 weeks. Unlike the previous study, in this study participants were counselled to improve diet and exercise habits. No changes were observed in resting metabolic rate, lipid oxidation, leptin levels, or body composition. However, this does not rule out metabolic benefits of long-chain polyunsaturated fatty acids in adults or adolescents not undergoing their pubertal growth spurt, nor does it rule out the possibility that obese boys with more pronounced metabolic complications may respond more favourably to a fish oil intervention. The lifestyle intervention was successful in decreasing the boys' sugar consumption, but had no impact on physical activity level, which the authors suggest indicates that changing an obviously poor dietary habit is easier than committing to increasing physical activity.

Given that weight loss success via energy restriction is difficult to achieve in the short term, with long-term success being even more elusive, novel approaches to control the obesity epidemic are warranted. Taken together, the five papers presented in this special issue support various lifestyle modifications to promote weight loss in the absence of energy restriction, although as with most diet-disease relationships, not consistently so. These modifications may include, but are not limited to, smaller and more frequent meals comprised of lower fat and moderate protein (e.g., dairy products and nuts, the latter of which are high in total fat that may not be highly bioavailable and are low in saturated fats), normal sleep of 8 hours/day, and control of stressors and levels of psychological stress. Further research is required to confirm these associations at various stages of the lifecycle, particularly in obese people.

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Review Article

A Review of Weight Control Strategies and Their Effects on the Regulation of Hormonal Balance

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The estimated prevalence of obesity in the USA is 72.5 million adults with costs attributed to obesity more than 147 billion dollars per year. Though caloric restriction has been used extensively in weight control studies, short-term success has been difficult to achieve, with long-term success of weight control being even more elusive. Therefore, novel approaches are needed to control the rates of obesity that are occurring globally. The purpose of this paper is to provide a synopsis of how exercise, sleep, psychological stress, and meal frequency and composition affect levels of ghrelin, cortisol, insulin GLP-1, and leptin and weight control. We will provide information regarding how hormones respond to various lifestyle factors which may affect appetite control, hunger, satiety, and weight control.

1. Introduction

Obesity is a multifaceted problem with many contributing factors including but not limited to genetics, hormone levels, overconsumption of food, and sedentary lifestyle. Dietary adherence has been shown to be negatively associated with the degree of caloric restriction [1]. In light of this evidence, development of strategies to promote weight loss in the absence of purposeful caloric restriction could be advantageous in the battle against obesity.

Since obesity is viewed as a multi-faceted problem, this paper proposes several different lifestyle factors that may be modified in order to contribute to weight reduction in the absence of caloric restriction. These lifestyle factors are macronutrient composition of meals, meal frequency, exercise, sleep, and psychological stress. In particular, this paper focuses on how the manipulation of these lifestyle factors may positively or negatively influence ghrelin, glucocorticoids (in particular, cortisol), insulin, and leptin to promote weight loss. These hormones were not chosen for review because they are the only hormones involved in weight regulation, but rather because together they are well-researched and cover a wide range of physiological functions connected with obesity and weight control. For instance, another hormone of interest in the regulation of body weight is glucagon-like peptide-1 (GLP-1). Exenatide, a GLP-1 receptor agonist, was administered to determine its effects on glycemic control and weight over an 82-week period in patients with type 2 diabetes [2]. At week 30 of the study, the amount of weight lost from baseline was 2.1 ± 0.2 kg compared to 0.3 to 0.9 kg for placebo. Reduction in body weight was progressive, resulting in an average weight loss of 4.4 ± 0.3 kg at week 82 [2]. Therefore, considering these results, the hormones in this review are not to be viewed as the only affecters of body weight regulation.

Ghrelin is a 28-amino-acid-residue peptide predominantly secreted by the stomach with substantially lesser amounts being detected in several other tissues [3]. Ghrelin appears in two major forms: acylated and nonacylated ghrelin. Acylated ghrelin is the active form of ghrelin whereas nonacylated ghrelin, which is present in greater quantities than acylated ghrelin, appears to be biologically inactive [3]. Ghrelin is a pleiotropic hormone demonstrating many different roles. Ghrelin displays strong growth hormone releasing

activity through the binding to and activation of growth hormone secretagogue receptor type 1a [3]. In addition to growth hormone release, ghrelin stimulates the release of prolactin and adrenocorticotropic hormone, negatively influences the pituitary-gonadal axis, stimulates appetite, influences sleep, controls gastric motility, and modulates pancreas function [3]. Specifically, in this paper, ghrelin will be investigated in relation to lifestyle factors.

Activation of the hypothalamic-pituitary-adrenal axis results in the eventual production of the glucocorticoid known as cortisol [4]. Corticotropin-releasing hormone from the hypothalamus stimulates the release of adrenocorticotropic hormone released from the pituitary gland [4]. Adrenocorticotropic hormone is responsible for the subsequent release of cortisol from the fascicular zone of the adrenal cortex [4]. Cortisol is an adrenal steroid hormone that regulates adaptive responses to varying types of stress [5]. Specifically, in this paper, cortisol will be investigated in relation to meal frequency, exercise, sleep, and psychological stress.

Leptin is a polypeptide hormone product of the *ob* gene that is produced and secreted by adipocytes [6]. The amount of leptin produced is directly proportionate to the triglyceride content of adipocytes (i.e., the size of the fat cells) [6]. Leptin is a crucial link in the signaling process between changes in body fat and control of energy balance [6]. Specifically, in this paper, leptin will be investigated in relation to sleep.

Insulin is a peptide hormone that is released from the beta cells of the pancreas [7]. Insulin serum concentration increases with feeding and decreases with starvation [7]. Serum insulin levels are most sensitive to changes in blood glucose concentrations. Insulin binds to its receptor to initiate GLUT-4 translocation to the cell membrane in order to allow glucose to enter the cell for energy production and/or storage [7]. Specifically, in this paper, insulin will be investigated in relation to macronutrient composition of meals, meal frequency, exercise, and sleep.

2. Macronutrient Composition of Meals

The macronutrient composition of meals is one variable that may contribute to excessive caloric intake under ad libitum conditions resulting in unwanted weight gain and the onset of obesity. The postprandial endocrine response associated with meals of varying macronutrient proportions may give some insight as to why certain food combinations lead to greater satiety resulting in less caloric ingestion than others. Identifying and exploiting the macronutrient proportions that are associated with satiety and favorable postprandial endocrine responses may be a useful strategy for initiating weight loss through appetite regulation in the absence of purposeful calorie restriction.

A majority of the recent studies involving the ingestion of meals of varying macronutrient proportions have focused on the resulting actions of the postprandial ghrelin and insulin response [8–13]. Both exogenous insulin administration and endogenous postprandial insulin response have resulted in suppression of ghrelin [14, 15]. In addition, faulty regulation of ghrelin suppression is associated with insulin resistance [16]. During a euglycemic-hyperinsulinemic clamp procedure, reduction of total and acylated ghrelin was greater in insulin-sensitive overweight and obese individuals versus insulin-resistant individuals of similar body type [16]. Postprandial ghrelin suppression has also been shown to be proportional to meal calorie content, and this association of meal calorie content with ghrelin suppression is reduced in obese individuals [17]. Generally, obese individuals exhibit lower fasting ghrelin levels than their normal-weight counterparts which suggests that it may not be the initial amount of plasma ghrelin that regulates appetite but the magnitude and duration of postprandial ghrelin suppression and its ensuing rise to fasting levels [13, 17].

The fat, protein, and carbohydrate compositions of meals have differing effects on ghrelin suppression as well as appetite regulation and satiety. In one study, high-fat meals (71% energy from fat) led to less ghrelin suppression at 30 minutes post-ingestion when compared to a high-carbohydrate (88% energy from carbohydrates) meal of equal caloric load [13]. In the same study, both lean and obese subjects reported less satiety 30 minutes after the ingestion of the high-fat meal compared with the high-carbohydrate meal. Moreover, Monteleone and colleagues [18] showed similar results in that ingestion of a high-carbohydrate meal (77% energy) more effectively suppressed ghrelin and appetite compared to ingestion of a high-fat meal (75% energy) in nonobese women. In a separate study, Foster-Schubert et al. [9] also demonstrated the inability of fats to effectively suppress postprandial ghrelin concentrations when compared to carbohydrates. In this study, however, no difference in appetite between the macronutrients was reported. Additionally, Kong et al. [11] reported that obese postmenopausal women with higher insulin concentrations had a positive correlation between saturated fat intake and ghrelin levels. Based on the results of these studies, isocalorically increasing the proportion of fat calories in relation to calories from carbohydrates and proteins is not a viable strategy to decrease appetite and ad libitum calorie ingestion.

A study looking at the differences in ghrelin suppression and appetite sensations after either an egg (22% carbohydrate/55% fat/23% protein) or bagel (72% carbohydrate/12% fat/16% protein) breakfast found an inability of either meal to suppress ghrelin [19]. In fact, ghrelin levels were increased after both meals although the rise was significantly less after the egg breakfast. Despite the unexpected increase in ghrelin, the egg breakfast was associated with significantly less "hunger" and greater feelings of satisfaction than the bagel breakfast at three hours after ingestion. Also, consumption of the egg breakfast resulted in a lower overall energy intake compared to consumption of the bagel breakfast during the subsequent 24-hour period. The researchers credited the feelings of satiety associated with consumption of the egg breakfast to the suppressed ghrelin response and tempered insulin/glucose fluctuation [19]. Examination of other research studies suggests that the increased satiety and subsequent decrease in energy intake might be at least partly

explained by the higher protein content (23% of calories for egg breakfast versus 16% of calories for bagel breakfast) of the egg breakfast [8, 10, 20]. Specifically, evidence from Leidy et al. [21] supports the idea that a high-protein intake (25% of meal calories from protein) at breakfast may be important for sustained fullness during periods of energy restriction. Conversely, when a high-protein breakfast (58.1% protein, 14.1% carbohydrate) was compared to a high-carbohydrate breakfast (19.3% protein, 47.3% carbohydrate), no difference in subsequent appetite or ad libitum caloric intake during lunch was observed [22]. However, a larger suppression of postprandial ghrelin was observed for the high-protein breakfast [22]. Numerous studies provide evidence that supports the use of a high-protein diet to induce appetite suppression and decreased ad libitum energy intake. Weigle et al. [20] reported that an increase of protein from 15% to 30% of dietary intake resulted in a decrease of ad libitum caloric intake and weight loss. These effects were evident in the absence of ghrelin suppression. Beasley et al. [8] also reported that a meal with 25% protein suppressed appetite (compared to a 15% protein, high-fat group and a 15% protein, high-carbohydrate group). Similar to the results presented by Weigle et al. [20], reduction of appetite could not be explained by a suppression of postprandial ghrelin [8]. The likely mechanism of action of high-protein diets on appetite suppression and decreased caloric intake may be outside of the control of ghrelin. This is evidenced by the mixed results of studies reporting protein's suppression of postprandial ghrelin with some researchers reporting large differences in postprandial suppression of ghrelin [9] and others reporting no differences in postprandial suppression of ghrelin [8, 10, 20] when high-protein meals are compared to meals of lower-protein composition.

In addition to a relatively high protein intake (~30% of total calorie intake), moderate carbohydrate intake may also be important in regulating sensations of hunger and calorie ingestion. As mentioned earlier, endogenous insulin secretion may result in the suppression of ghrelin [15]. In a recent study of healthy Pima Indians, higher plasma insulin responses were associated with a decrease in subsequent carbohydrate consumption and less weight gain [23]. These results further point to insulin as a regulator of calorie consumption. While, in one study [24], a short-term isocaloric high-carbohydrate diet was not associated with a lower overall energy intake when compared to a high-fat diet, another study [25] demonstrated that a high-protein meal (35% of total calories) with moderate carbohydrate intake (45% of total calories) was able to persistently suppress ghrelin at levels significantly lower than baseline when compared to either a mixed-diet (50% carbohydrate, 20% protein) or high-fat (45% carbohydrate, 45% fat) meal. It is unclear whether the glycemic index or glycemic load plays a direct role in the regulation of appetite and subsequent energy intake through tempered insulin responses as results from past studies have been conflicting [26, 27]. Furthermore, insoluble fiber at high doses (33–41 grams) may help to regulate appetite as well as energy intake [28, 29].

In conclusion, evidence tends to suggest that for the regulation of ad libitum caloric ingestion, a high-protein diet (~30% of total calories) may be beneficial whereas high-fat diets should most likely be avoided. It would appear to be most beneficial if the additional protein is ingested from solid food sources as food form also appears to play a role in feelings of satiety [30, 31]. Future research should investigate the effects of large doses of insoluble fiber (33 to 41 grams) in addition to a high-protein diet to determine if this combination could further attenuate appetite and energy intake.

3. Meal Frequency

Another factor associated with weight control that may not necessarily have a concomitant decrease in caloric intake is meal frequency. Caloric intake can be affected by caloric density in food, total energy consumption as well as meal frequency, but Solomon et al. [32] suggests that feeding frequency has received the least amount of empirical research. Additionally Stote et al. [33] most recently reported that nutrient-dense and low-calorie diets have received significant attention with regards to weight control and health outcomes, but the influence of meal frequency is yet to be firmly established. Though the general public would suggest that three meals a day are important [33] there have been few well-controlled studies that have compared meal frequency, with the same caloric consumption, and its effect on health outcomes and weight control. To date evidence suggests that less frequent meal consumption with a large bolus of calories at each meal can lead to increases in adipose tissue [34]. Conversely consuming the same amount of calories with more frequent and smaller meals does not seem to impact the deposition of fat [34]. Additionally Solomon et al. [32] report an increase in snacking has positive associations with BMI, but also with caloric intake, suggesting the need for studies involving meal frequency and weight control.

There have been some published findings regarding meal frequency and its effects on preprandial and postprandial satiety and gastric sensations of "fullness" or "emptiness" with some studies suggesting hormones may play a direct or indirect role [35]. These hormonal effects may primarily be related to ghrelin and insulin and possibly cortisol [33, 35].

Much of the research regarding weight control and hormones has focused principally on ghrelin, a hormone produced primarily in the gastrointestinal tract with larger amounts in the stomach [35]. Recent findings have suggested that ghrelin may play a role in the control of food intake and meal frequency as well as energy intake [36]. Specifically ghrelin in the plasma increases preprandially in fasting conditions and before meal initiation and will rapidly decrease nadir postprandially [37] and therefore is reported to play a role in appetite regulation, meal frequency, and hunger [36]. Solomon et al. [32] have suggested that a larger preprandial surge in ghrelin may occur with larger and less frequent meals that could cause more meal initiation and more between-meal snacks. Other study authors have reported that ghrelin may play an important role in metabolic balance by decreasing fat utilization and increasing appetite and meal frequency [38]. The same authors report that this metabolic

role has occurred in both healthy populations and in cancer patients suffering anorexia.

Another hormone thought to regulate appetite is insulin. Postprandial insulin levels have been more controlled with more frequent meals, with meal skipping (primarily skipping breakfast) associated with higher levels of insulin [39], but most of the research regarding insulin and ghrelin has focused on the interaction of the two hormones and how each may affect each other in appetite control and meal initiation and frequency.

Postprandial suppression of ghrelin has been demonstrated to be partially dependent on the release of insulin [32, 40] and can be subject to insulin sensitivity. Previous reports [41] have suggested that the administration of ghrelin in apparently healthy participants has an inverse relationship with insulin levels and causes an increase in plasma glucose concentrations. Other study findings [32] also suggest that insulin and ghrelin have an inverse relationship in fasting conditions as well as with low-frequency meal ingestion, causing poor control of insulin and decrease in glucose consumption [41], yet when meals are consumed more frequently with the same caloric consumption, the insulin-ghrelin relationship is less apparent. The lack of a relationship with high frequency meals may be due to less insulin-related fluctuations in ghrelin and therefore may cause there to be less of a preprandial increase in ghrelin and consequently less gastric sensations or "hunger" [32, 42, 43]. Conversely, an increase in feeding frequency that is associated with an increase in caloric consumption may cause the insulin to exert less control on ghrelin causing an increase in preprandial ghrelin [42]. This can be especially true in Type II diabetics [32]. Postprandial ghrelin suppression and downregulation in insulin-resistant disease states such as diabetes and obesity and in syndromes such as metabolic syndrome is lesser than in apparently healthy populations [32, 34, 44].

A third hormone that may have an effect on appetite control, meal frequency, and regulation is cortisol. One study [36] reported that cortisol levels correlated negatively with ghrelin suggesting that cortisol may peak after ingestion of a meal and return to nadir some time after meal completion. Hypercortisolism in another study [45] suppressed ghrelin levels.

Therefore a novel approach to weight control in the absence of direct caloric restriction could be in the control of ghrelin levels. One study [46] concluded that a ghrelin mimetic increased appetite transiently and that the infusion of ghrelin countered the effect of anorexia in elderly persons. Similarly, other studies suggested a ghrelin mimetic helped to increase appetite and caloric consumption in both end-stage renal disease [47] and cancer patients [38]. Since it has been established that both insulin [36, 46, 48] and cortisol [33, 36] have negative and regulatory effects on the levels of ghrelin, a novel approach for weight control and weight loss would be to attempt to control ghrelin levels by attempting to moderate the preprandial increase. Such control could cause a concomitant decrease in appetite with the possible implication of caloric restriction due to lower levels of hunger. Control of ghrelin, behaviorally, could occur through the increase of meal frequency and smaller meals. The same amount of calories in smaller more frequent meals can help to regulate glucose and therefore insulin and cortisol and indirectly affect the levels of ghrelin in plasma and ghrelin secretion. Soule et al. [48] demonstrated that an injection of ghrelin was associated with an increase in calorie intake in one meal by 30%. Conversely, any attempts to control ghrelin endogenously may have the opposite effect and help reduce the caloric consumption in each meal. Combining both an increased meal frequency with endogenous control of ghrelin could help in the decrease of caloric consumption. More research is needed to confirm these findings with longitudinal outcomes measured.

4. Exercise

The response of total plasma ghrelin to bouts of exercise has been investigated in humans [49–52], horses [53, 54], and rats [55, 56]. In humans, studies reporting the observed levels of ghrelin following acute bouts of exercise are conflicting. More specifically, ghrelin concentrations remained constant during a single bout of treadmill running for one hour at 73.5% VO_{2max} in healthy, physically fit individuals [57] and during rigorous running at increasing intensities in endurance athletes [58] and healthy volunteers [59]. Moderate-intensity resistance exercises in which both eccentric and concentric contractions were performed [60] and acute incremental exercise in healthy men [61] have shown decreases in ghrelin concentrations [62].

While it is inconclusive how ghrelin concentration alone changes in response to an acute bout of exercise, ghrelin can potentially stimulate the release of growth hormone (GH) during exercise [50]. Since the process of releasing GH occurs through the activation of the GH secretagogue receptor [63], and GH increases during acute bouts of exercise, the relationship between exercise, plasma ghrelin levels, and GH has been investigated [50]. In most studies, GH levels increased while plasma ghrelin levels remained unchanged regardless of the duration or intensities of the exercise protocols [49, 58–60]. It is suggested that under these conditions, peripheral circulating ghrelin does not mediate pituitary GH secretion [50]. However, there are studies that contradict the results of the aforementioned studies. For example, a study by Borer et al. [64] reported an increase in the plasma concentration of both ghrelin and GH following exercise energy expenditure in overweight, postmenopausal women [64]. Christ et al. [65] also observed increases in ghrelin and GH concentrations in athletes following a three-hour bout of aerobic exercise at an intensity of 50% VO_{2max} [65].

Ghrelin concentrations have also been investigated concurrently with exercise, hunger, and food intake responses. Recently, Erdmann et al. [50] examined the effect of a continuous bout of exercise (for up to two hours), performed at low to moderate intensity, on food intake. One group of subjects exercised with a bicycle and performed one control protocol and two uninterrupted exercise protocols: cycling for 30 min at a low intensity (50 W) and cycling for 30 min

at a moderate intensity (100 W). Though there was an initial increase in ghrelin concentration observed during the first protocol, the concentration remained unchanged during the second protocol. Hunger and food intake responses were not significantly different from the controls. Another group of subjects exercised with a bicycle and performed one control protocol and three uninterrupted exercise protocols: cycling for 30, 60, and 120 min, all at low intensity (50 W). Ghrelin levels increased by 50–70 pg/mL above baseline for the respective bouts of exercise. Moreover, food intake response after 120 min of bicycle exercise was significantly greater than the controls and compared to the first two exercise protocols [50]. This study suggests that duration has more of an impact than intensity with respect to an increased ghrelin response after a continuous bout of exercise.

Studies that utilize long-term exercise protocols have shown ghrelin concentrations to increase in response to the subsequent exercise-induced decrease in body weight that acts via a negative feedback loop that controls body weight [51, 52, 66]. Circulating ghrelin levels have demonstrated an increase over time in healthy women who lost weight during a three month, energy-deficit-imposing diet and exercise regimen [52]. Also, it has been reported that total plasma ghrelin increases by 18% in sedentary, overweight, postmenopausal women who lost more than 3 kg of body weight after a 12-month aerobic exercise protocol [51]. However, Morpurgo et al. [67] conducted a three-week exercise training program that reduced body weight in obese patients and found that fasting and nonfasting ghrelin levels were unchanged at the conclusion of the program compared to levels observed before the program began [67].

Much like the ghrelin response, the insulin response is also sensitive to acute bouts of both endurance and resistance exercise. Endurance exercise promotes oxidation in the skeletal muscle, which is a major component in the mediation of insulin action [68–71]. Although an acute bout of endurance exercise enhances insulin sensitivity [72–74], it has been reported that an acute bout of sprint interval exercise has no effect on insulin sensitivity in healthy subjects [75].

Since insulin controls the metabolism and uptake of glucose in muscle cells and mediates blood glucose levels, serum insulin concentrations correlate with fluctuations in blood glucose [76]. This response is increased when proteins and carbohydrates are consumed at any stage of a workout, including pre-, mid-, and postworkout [77–80]. However, if a protein/carbohydrate supplement is not ingested at any stage during resistance exercise, it has been demonstrated that serum insulin levels slightly decrease [81]. For example, Raastad et al. [81] observed a 3% decrease in insulin concentrations compared to baseline values 30 min after a moderate-intensity resistance exercise protocol in male athletes. The same study also observed a 4% decrease in insulin concentrations compared to baseline values 30 min after a high-intensity resistance exercise protocol in the same subjects, revealing that there was no significant difference in the levels of insulin when the two protocols were compared [81].

Resistance exercise protocols that utilize hypertrophy lifting strategies also show that the acute insulin response remains unchanged [79, 82–84]. However, if a protein/carbohydrate supplement is ingested prior to the workout, there is a significant increase (up to a 5-fold increase, on average) in the insulin response following exercise [85]. Williams et al. [84] observed that insulin levels only rose significantly in resistance-trained men after ingestion of a carbohydrate-protein supplement compared to when the same subjects ingested a placebo following bouts of resistance exercise using a load equal to each subject's 10-RM [84]. Dietary intake that includes consumption of any combination of amino acids and carbohydrates during a stage of resistance exercise maximizes insulin's influence on tissue anabolism and protein synthesis by decreasing protein catabolism [77, 80] and utilizing enhanced blood flow in the skeletal muscles [76].

Hypertrophy schemes and other resistance exercise protocols are known to produce acute increases in other hormones, without supplementation, including the catabolic hormone cortisol [85]. The antagonistic effects of catabolic hormones and anabolic hormones, such as testosterone, promote muscle growth and protein metabolism during rest in between workouts [86–89]. These processes are accomplished through the inhibitory actions of cortisol in the synthesis of contractile muscle proteins [90], as opposed to testosterone, which has the opposite effect [91, 92], resulting in increased muscle mass [93, 94]. Furthermore, cortisol promotes triglycerides to be hydrolyzed into free fatty acids and glycerol [95–97], thereby allowing for increases in exercise performance and recovery [98]. Circulating cortisol can also be a source for energy production, as high levels of the hormone can initiate gluconeogenesis in the liver [97]. Increases in cortisol concentration may also lead to an increase in appetite and energy intake. This was demonstrated by Tataranni et al. [99] who showed that a therapeutic dose of glucocorticoids increased appetite and energy intake of healthy male subjects. Conversely, low cortisol concentration results in hypophagia and possibly decreased energy intake [100].

An acute bout of resistance exercise has been shown to cause significant increases in cortisol in men although the changes observed in the hormone levels are influenced by intensity, duration, muscle mass and loading schemes, and the degree to which the subject has been trained for this type of exercise [94, 101]. Increases in circulating cortisol concentrations are typically proportional to the intensity of the exercise performed, but reach a maximum threshold value that is dependent upon the duration of the exercise protocol [96, 98].

Increases in circulating cortisol levels have also been observed in women after an acute bout of resistance exercise [94] and have similar responses to men performing the same protocol [102]. However, one study by Häkkinen and Pakarinen [103] showed no statistical change in cortisol levels among women in three different age groups (30 years, 50 years, and 70 years) who performed the same heavy resistance exercise protocol while a significant increase in cortisol was observed in the middle-aged men compared to the younger and older men, who performed the same heavy resistance exercise protocol and were classified with respect

to age, in the same manner as the women who participated in this study [103].

With regards to an acute bout of endurance training, cortisol normally increases with respect to exercise intensity [104–106], but the correlation is not necessarily linear [107]. Circulating cortisol concentrations have been demonstrated to increase in trained men after repeated 100m sprints [108] and increase in type 1 diabetics during and after sprinting exercises [109]. Moreover, cortisol levels have been reported to increase in short (7 min) and longer (40 min) bouts of rowing, an exercise that combines both aerobic and anaerobic actions [110]. Although no change or even a reduced change in cortisol concentrations at low exercise intensities has been reported [98], this observation has been refuted [107]. Similarly, the effects of acute bouts of high-intensity endurance exercise in athletes are conflicting. One study reported no change in cortisol concentration in endurance-trained athletes following 10 min of moderate- to high-intensity running [111], but another study reported an increase in cortisol concentration in male athletes following a ramp incremental cycle ergometry exercise to exhaustion [112].

The cortisol response to exercise is also influenced by subject training status. In untrained men, postexercise cortisol concentrations, measured after one, six and eight weeks from starting a heavy resistance exercise protocol, increased compared to preexercise values while resting cortisol concentrations decreased at eight weeks from starting the program [102]. In moderately trained men, cycling at moderate to high intensities provokes increases in circulating cortisol concentrations, while cycling at low intensities reduces circulating levels of the hormone [98]. When comparing long-term trained to untrained middle-aged men, cortisol levels increased only in the untrained group after performing multiple sets of a superset strength training protocol at 75% of 1 repetition maximum, which suggests that long-term, strength-trained men need a higher-volume exercise protocol in order to counteract the lower responsiveness in cortisol values observed in this study [113]. No changes have been observed in cortisol concentrations among untrained females who trained for eight weeks and were divided into four groups: control, endurance exercise (up to 80% MHR by the 8th week), resistance exercise (up to 80% 1RM in three sets and six repetitions), and concurrent exercise [114].

Cortisol concentrations also differ among trained subjects who have different training histories. For example, cortisol levels are less pronounced in endurance-trained subjects compared to resistance-trained subjects who performed the same exercise program, which included both endurance and resistance exercises [115]. Other factors that can influence the observed elevations in circulating cortisol concentrations during resistance exercise include plasma volume reductions (when corrected still result in elevated cortisol levels) [116] and anabolic steroid use [117].

Exercise can help to control weight through an increase in the number of calories expended. The amount of calories expended during exercise has an equivalent effect on body composition as the same amount of calories being restricted from the diet with the added benefit of cardiovascular and muscular conditioning [118]. In fact, substantially increasing the amount of calories expended by exercise during weight loss may lead to better long-term weight control and markers of health [119]. However, one negative result of such high exercise volume may be the accompanying rise in cortisol. Such an increase in cortisol could possibly be blunted through nutritional supplementation of fish oil and phosphatidylserine. Noreen et al. [120] were able to demonstrate that supplementation with 4 grams of fish oil daily (1,600 mg/d eicosapentaenoic acid and 800 mg/d docosahexaenoic acid) led to increased fat free mass and decreased fat mass in men and women after 6 weeks compared with 4 grams daily of safflower oil. The fish oil supplementation did not result in a statistically significant decrease in salivary cortisol compared with the placebo group, but there was an existing trend. The confidence intervals for pre- and postmeasurements of salivary cortisol after fish oil supplementation ($-0.127\,\mu$g/dL, $-0.002\,\mu$g/dL) support the trend that fish oil supplementation may lower cortisol concentration [120]. The proposed mechanism of lowering cortisol is through a decrease of IL-6 which has been shown to be a stimulator of the hypothalamic-pituitary-adrenocortical (HPA) axis independent of corticotropin-releasing hormone (CRH) activity [121]. In another study, short-term phosphatidylserine supplementation of 600 mg daily was able to blunt increases in cortisol induced by training in athletes [122]. Ten male subjects were administered phosphatidylserine or placebo for 10 days and then subjected to a moderate-intensity treadmill exercise protocol. Before the postsupplementation testing session, basal cortisol levels were lower than presupplementation levels for the phosphatidylserine group compared with placebo. Exercise-induced cortisol response was also reduced by 39% in the supplement group compared to placebo. Testosterone levels in the phosphatidylserine group also increased compared to the placebo group, but these results were not statistically significant. The resulting testosterone to cortisol ratio for the phosphatidylserine group was improved which may lead to further positive physiological consequences [122]. The findings of this study suggest that phosphatidylserine is effective in attenuating the cortisol response to exercise and may potentially prevent the physical deterioration that can result from increased training volume [122].

In conclusion, exercise, especially at higher volumes, is an important part of any weight loss regimen. Exercise may exert its weight control benefits through its positive effects on insulin and ghrelin. Exercise can also help one adapt to the physiological effects of cortisol, but, exercising at very high volumes in an attempt to maximize energy expenditure may lead to an excessive cortisol response. Therefore, blunting the cortisol response associated with very high volumes of exercise through the use of fish oil and phosphatidylserine supplementation may be a viable option.

5. Sleep

An often-overlooked aspect of weight control is that of sleep duration. An inverse relationship between sleep duration

and body mass has been reported in both adult [123] and adolescent [124] populations as well as in different cultures [125–129]. Since metabolism, endocrinology, and circadian rhythms are tightly linked, this finding should be intuitive.

While there are several hormones that affect weight control, leptin, ghrelin, cortisol, and insulin are a few affected by sleep. Given the roles each hormone plays in metabolism or appetite control, learning how to manipulate the hormonal response behaviorally could be of benefit in managing weight control with or without caloric restriction. The effects of these hormones are interconnected, but each hormone will be briefly papered independently to elucidate the effects sleep has on each.

Well-controlled studies have demonstrated the effect of sleep duration on leptin. Under normal, healthy circumstances, leptin levels peak between midnight and early morning with a nadir between midday to midafternoon [130]. Thus the peak occurs during the dark phase of the 24-hour cycle and the nadir during the bright phase. Sinha et al. [130] suggested that this nocturnal elevation in leptin was to suppress appetite during normal sleeping hours. Interestingly, changes in the light/dark cycle and meal timing have been shown to alter plasma leptin levels [131]. When subjects underwent a 12-hour time zone shift, analogous to inverting the light/dark cycle, peak and nadir leptin levels were shifted by about 12 hours [131]. Furthermore, leptin peaks and nadirs were shifted, yet again, when meal consumption was altered by 6.5 hours [131]. Additionally, chronic sleep deprivation has been shown to reduce the amplitude of diurnal variation with leptin [132]. Similar reductions occur in leptin levels and amplitude of diurnal variation when sleep duration is reduced from 8 hours to 4 hours [133]. This phenomenon has also been reported in a large population-based study where short sleep duration was associated with low leptin levels [134]. The same population-based study reported shortened sleep duration to be associated with high ghrelin levels and increased BMI [134].

While ghrelin levels increase in the fasting state and are known to decrease shortly after food consumption [135], levels are also known to peak during the night and decrease before waking hours [135–137]. Relative to sleep, ghrelin has been shown to promote slow-wave sleep (SWS), which is important for recovery of metabolic function [138]. In sleep-deprived states, the nocturnal rise in ghrelin was blunted [136]. Furthermore, Dzaja et al. [136] reported that although the peak response was blunted in the absence of sleep, ghrelin levels steadily increased until early morning hours and remained elevated until after breakfast hours. Given ghrelin's pleiotropic nature (i.e., its effects on appetite, stimulation of growth hormone, and promotion of SWS in normal situations), the loss of sleep seems to have multiple effects on ghrelin levels. If sleep duration is shortened and peak levels of ghrelin are blunted but those levels do not fall to nadir until after normal breakfast hours, the result could be an increase in appetite leading to increased caloric consumption. Additionally, it has also been demonstrated that growth hormone secretion would be blunted [136]. It is believed that ghrelin may act in a synergistic fashion with growth-hormone-releasing hormone [139] as well as act as an interface between the hypothalo-pituitary-adrenocortical system and the hypothalamo-pituitary-somatotrophic system [138]. In accordance with this role as an interface between these two systems, ghrelin not only increased the duration of SWS sleep, but it also increased cortisol levels [138].

As has been reported for leptin and ghrelin, a relationship between sleep and cortisol levels exists [133, 140, 141]. In normal, healthy conditions, cortisol levels decline prior to sleep onset and rise again during late night hours reaching a peak during early morning [142, 143]. During acute sleep restriction cortisol levels were reduced in early morning hours [141, 144], but increased during evening hours when leptin levels were blunted [133]. This relationship points to the interaction between cortisol and leptin and a possible role for sleep on weight control via hormonal regulation. While cortisol increases leptin production in a dose-dependent manner [145], the regulation of leptin by glucocorticoids is not absolute [146]. Conversely, leptin has a suppressive effect on the HPA axis [147]. Thus, a feedback loop exists between the two. Additionally, cortisol increases food consumption [99]. The cortisol and leptin response to reduced sleep duration would indicate that in a sleep deprived state, as leptin levels fall, which has a decreased effect on appetite suppression, concomitant increases in cortisol may cause an increase in food consumption [133]. The cortisol response to acute sleep restriction appears to be similar in a chronic situation. In a large study using self-reported sleep duration, sleep restriction and high sleep disturbance resulted in elevated cortisol levels during evening hours [140] supporting the results observed in the acute studies.

Finally, sleep loss has been proposed as a risk factor for insulin resistance and type 2 diabetes [148]. After six days of sleep restricted to 4 hours, peak glucose responses after breakfast were elevated indicating decreased glucose tolerance [149]. The decrease in glucose tolerance was also reported in a follow-up study where subjects underwent two days of restricted sleep where glucose levels were elevated while insulin levels were lower [148, 150]. Recent epidemiological studies support the acute findings as decreased sleep duration has been reported as an increased risk factor for impaired fasting glucose [151] and impaired glucose tolerance [152].

Based on these aforementioned studies, it would be apparent that increasing sleep duration could be a valid approach to weight control with or without caloric restriction as well as metabolic complications associated with obesity. However, research on the effects of increased sleep on weight management is limited. Recently a feasibility study on this very topic was reported and is underway, but final data has not been reported [153]. Thus, more studies are needed to ascertain whether increasing sleep duration is an effective tool in controlling obesity.

6. Psychological Stress

Another one of the factors that may contribute to weight gain and obesity is psychological stress [154]. Increased levels

of perceived stress are thought to influence eating behavior [155]. In fact, in a recent paper it was reported that during stressful conditions approximately 40% of people eat more food, 40% consume less food, and 20% do not alter their eating patterns [155]. Tataranni et al. [99] demonstrated that individuals who were given an exogenous glucocorticoid ate significantly more food throughout the day as compared to participants who were only given a placebo. Moreover, during periods of increased stress, certain individuals may choose foods that are higher in fat and sugar (i.e., comfort foods). The ingestion of these comfort foods is thought to improve mood and decrease stress via opioidergic and dopaminergic neurotransmission [156]. Thus, consuming these foods can be construed as a type of coping mechanism to mitigate stress in certain populations. However, if these energy-dense food choices are consumed chronically in response to stress, it could theoretically lead to increased food consumption and ultimately weight gain [156]. The influence of psychological stress on various hormone levels and their effect on hunger and weight management are areas of interest in obesity research. In particular, cortisol and ghrelin are two hormones that are possibly thought to influence eating following a stressful event [157].

Chronic-stress-induced secretion of cortisol and/or increased HPA axis activation may specifically contribute to central/abdominal obesity [158, 159]. Cortisol appears to affect visceral fat to a greater degree than subcutaneous fat due to abdominal adipose tissue having increased cell density and more glucocorticoid receptors [158]. Central fat, also known as visceral obesity, is particularly undesirable because it is associated with hypertension, cardiovascular disease, and diabetes [158]. Interestingly, the mental stress from tracking calorie intake alone can induce a stress response and increase the secretion of cortisol [160].

Research suggests that 24-hour urinary and/or plasma cortisol have been positively associated with central obesity [161, 162]. Cortisol levels are believed to peak ~20–30 minutes after waking [159]. The degree of the cortisol response to waking may be positively related to chronic perceived stress [159, 163]. Wallerius et al. [163] reported that in 53-year-old men, morning cortisol values were positively associated with body mass index, waist to hip ratio (WHR), and abdominal sagittal diameter. Similarly, another study conducted by Steptoe and colleagues [159] demonstrated that in 47 to 59 year-old men there was a significant association between waist to hip ratios and elevated cortisol response to waking (i.e., 30 minutes after waking). Interestingly, this association was not observed in women [159]. A more recent study by Larsson et al. [164] utilizing a large sample of participants (1671 men and women between 30–75 years old) measured both morning and delta cortisol (morning minus evening cortisol). It was demonstrated that salivary cortisol levels were generally higher in women as compared to men [164]. This finding is contrary to other studies that have reported that men were found to have higher cortisol levels than women [165, 166]. Furthermore, Larsson and cohort [164] reported that in women abdominal obesity was significantly associated with low morning and delta cortisol, but this was not observed in men [164]. It was hypothesized by the authors that an inverse relationship between morning cortisol and WHR in women may be due to enhanced clearance rate of cortisol in visceral fat secondary to the high density of glucocorticoid receptors [164]. Interestingly, a study by Ljung et al. [167] reported a similar inverse relationship between morning cortisol and WHR in men as well. It should be noted that some of the equivocal findings in cortisol research may, in part, be due to the differences in number of subjects in the studies, the time of day cortisol is measured, and whether cortisol was measured in the plasma, urine, or saliva [164]. Thus, it is difficult to make definitive statements regarding the differences of cortisol levels between sexes; however, it does appear that cortisol plays a role in development of central adiposity.

Knowing that perceived stress may increase cortisol levels, strategies to reduce psychological stress should be considered as a means to combat weight gain. Although regular exercise and a healthy diet are effective ways to mitigate psychological stress, the focus of this section is to describe research that has demonstrated alternative ways (i.e., nonexercise or nutrition interventions) to decrease stress and/or cortisol. Mindfulness-Based Stress Reduction (MBSR), developed at the University of Massachusetts about 30 years ago, is a program that attempts to teach people to cope more effectively with stressors [168]. The MBSR model incorporates the practice of mindfulness, meditation in a group setting, group dialogue, daily home practice, and providing theoretical material related to stress management and the mind-body connection [168]. Several studies have reported that participating in the MBSR program appears to positively affect the stress response and the HPA axis [168]. Specifically, MBSR reduced cortisol levels in certain diseased states such as cancer patients [169, 170]. However, other studies that utilized females with heart disease [171] and HIV-infected males and females [172] did not find a significant difference in cortisol levels with MSBR when compared to a control group. Although the aforementioned studies were in diseased populations and did not specifically look at weight reduction, it could be theorized that if cortisol levels could be reduced via MBSR or another similar modality, then decreases in weight may possibly result. However, more research is needed before definitive recommendations can be made.

Ghrelin may also be affected by psychological stress, and, like cortisol, it may influence eating [157]. To date, however, less has been published regarding the effects of perceived stress on ghrelin in humans. Plasma ghrelin levels have been shown to increase approximately twofold immediately prior to a meal and decrease within an hour after the cessation of eating [135]. Similarly, in other studies, ghrelin tends to increase linearly with hunger scores [173]. Interestingly, ghrelin appears to be inversely related to BMI [174, 175]. To test the effect of stress on ghrelin, Raspopow [157] utilized the Trier Social Stress Test (TSST), which includes public speaking and an arithmetic problem in front of a panel of judges and administered it to college-aged women. The women were categorized as "emotional" or "nonemotional" eaters via a questionnaire. Blood was taken throughout the TSST. The investigators reported that regardless of eating status (i.e., emotional versus nonemotional), ghrelin levels

increased moderately in response to stress [157]. However, when the participants were given food, ghrelin levels decreased significantly in the nonemotional eaters, but remained stable in the emotional eaters [157]. The authors concluded that the lack of decline of ghrelin among emotional eaters may provide a continued signal to prolong eating [157]. One could hypothesize that if this occurred chronically, it could lead to weight gain.

Rouach et al. [174] examined the effects of psychologically induced stress on plasma ghrelin levels in patients with binge-eating disorder (BED) and in healthy subjects of normal or increased body mass index (BMI). As in the previous study by Raspopow [157], all participants were subjected to the TSST. In addition to other variables, plasma ghrelin levels were measured throughout the test. Equally important, subjects were requested to rate their urge to eat uncontrollably, subjective feelings of stress, and desire to eat sweets via a visual analog scale both prior to and after the TSST [174]. The investigators reported that after correcting for gender, age, and BMI there were no differences in ghrelin levels throughout the test among the groups or over time in each individual group [174]. However, a significant difference in ghrelin was observed after the three groups were reanalyzed according to their cortisol response to stress [174]. Specifically, ghrelin levels increased in cortisol responders, but either no change or a decrease in ghrelin levels was observed in cortisol nonresponders [174]. The authors ultimately concluded that ghrelin was independent of BMI and that psychological stress may indeed increase ghrelin levels, but it does not appear to influence the drive to eat uncontrollably or eat sweets after a stressful event [174].

In conclusion, due to the limited number of human studies, it is difficult to make conclusive statements regarding the effects of ghrelin on appetite and weight management. It does appear from scant evidence that perceived stress may increase ghrelin, but it is unclear whether that would translate into eating more food following a stressful event. Given exogenously via an infusion, it has been reported to increase appetite and subsequent feedings. At this point in time, however, it is not fully known whether or not stress-reducing techniques, such as the aforementioned MBSR, can significantly alter ghrelin levels, nor is it known whether chronic perturbations in ghrelin levels can appreciably affect long-term weight maintenance. More research is needed in these areas to draw concrete conclusions.

7. Conclusion

Although changes in the lifestyle factors discussed in this paper may result in weight control through hormone regulation and non-purposeful calorie restriction, the ultimate goal of any weight loss strategy is to expend more calories than one ingests. As is well-known, this may be accomplished by increasing exercise, by decreasing caloric intake, or by both simultaneously. More than likely negative caloric balance will have to be reached through some sort of dietary restriction considering that 30 minutes of running for a 180 pound individual results in an expenditure of only about 440 calories [176] which is just about equivalent to the calories contained in only 6 ounces of 75% fat ground beef.

The need for novel approaches to control rates of obesity is warranted. Our paper suggests that various lifestyle factors can help attenuate the hormonal responses normally associated with appetite control and regulation. Specifically, eating more frequent and smaller meals comprised of moderate protein levels and lower fat, obtaining normal sleep of 8 hours a day, and controlling stressors and levels of psychological stress could more readily control the levels of "appetite hormones." Additionally, it is recommended that exercise, both endurance and resistance training, should be incorporated into any lifestyle or behavior enhancement program. In closing, development of a weight loss program requires an integrative approach of many professionals including physicians, psychologists, nutritionists, and exercise physiologists who can as a team propose an optimal personalized strategy taking into account all aspects of obesity.

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Review Article

Sex Differences in Energy Metabolism Need to Be Considered with Lifestyle Modifications in Humans

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Women have a higher proportion of body fat compared to men. However, women consume fewer kilojoules per kilogram lean mass and burn fat more preferentially during exercise compared with men. During gestation, women store even greater amounts of fat that cannot be solely attributed to increased energy intake. These observations suggest that the relationship between kilojoules consumed and kilojoules utilised is different in men and women. The reason for these sex differences in energy metabolism is not known; however, it may relate to sex steroids, differences in insulin resistance, or metabolic effects of other hormones such as leptin. When considering lifestyle modifications, sex differences in energy metabolism should be considered. Moreover, elucidating the regulatory role of hormones in energy homeostasis is important for understanding the pathogenesis of obesity and perhaps in the future may lead to ways to reduce body fat with less energy restriction.

1. Introduction

Fat gain is always considered to be a result of long-term positive energy balance, whereby daily energy intake exceeds expenditure. From the onset of puberty to menopause, women maintain a greater percentage body fat mass (FM) than men despite smaller energy intake per kg lean mass [1] and preferential use of fat as a fuel during exercise compared to men [2]. A potential reason for these findings is that the greater FM in women relates to more efficient fat storage during nonexercising periods, especially postprandial periods [3]. During pregnancy, women deposit between 2.4 to 5.9 kg of body fat, even when undernourished [4]. In well-nourished women, the energy cost of gestation is approximately 370 MJ [5]. How this energy requirement is met is not explained purely by an increase in energy consumption, as past studies failed to demonstrate this in the first half of pregnancy [6, 7]. The reproductive years and gestation are characterised by elevated levels of ovarian hormones. Evidence indicates that oestrogens contribute to the gender differences in FM and the gestational changes in body composition [3]. Human and animal studies have explored possible mechanisms of action by these hormones [8, 9]. When considering lifestyle modifications, the sex difference in energy metabolism needs to be considered.

The first half of this paper focuses on differences between men and women: the gender differences in FM are outlined, aspects of energy metabolism that may account for these differences are discussed, and the key metabolic roles of ovarian hormones are discussed. Against this backdrop, the second half of this paper focuses on body composition and energy balance during pregnancy.

2. Gender Differences in Body Composition Throughout Life

Like many mammals, humans show significant differences in fat-free mass (FFM) and FM between the sexes. The *National Health and Nutrition Examination Survey* III (NHANES III) of 15,912 subjects, showed that non-Hispanic white females aged between 12 and 80 years have a higher percentage of FM than males, starting from puberty and varying from 6% to 11% higher for every decade studied (see Table 1

Table 1: Percentage FM in healthy non-Hispanic white men and women. Adapted from NHANES III [10].

Age range (years)	Males (%)	Females (%)	Difference (%)
12–13.9	18.4	24.8	6.4
20–29.9	21.8	31.0	8.2
30–39.9	23.6	33.0	9.4
40–49.9	24.2	35.4	11.2
50–59.9	25.1	37.3	12.2
60–69.9	26.2	36.9	10.7
70–79.9	25.1	35.9	10.8

and Figure 1) [10]. Other studies also support the notion that the significant sexual divergence in body composition commences with puberty [3]. This sex difference holds across all ethnic groups and has been observed in all populations although its magnitude is influenced by ethnic, genetic, and environmental factors [11]. Not only is there a difference in percent FM between the sexes, there is also a well-recognised difference in body fat distribution.

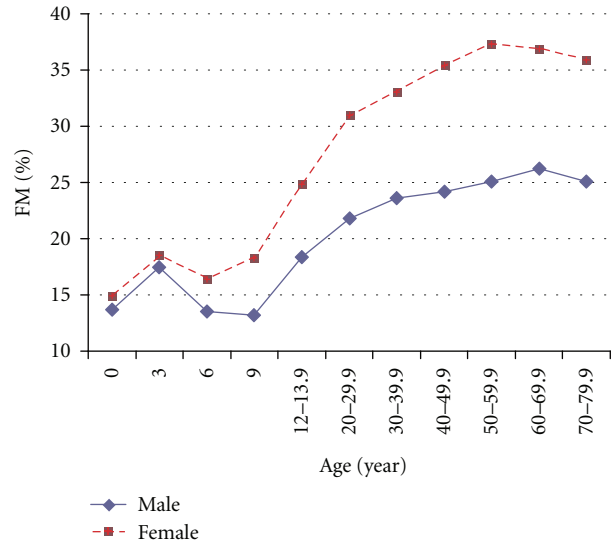

Figure 1: Percentage fat mass (FM) in males and females showing the divergence that occurs at puberty and persists through the pre-menopausal years. Combined data from Chumlea et al. [10] and Fomon et al. [20].

3. Gender Difference in Energy Metabolism

It may be postulated that women store more fat because they consume more energy than they expend or that they store the consumed fat more efficiently. However, when daily energy intake is compared in the cohort of subjects from NHANES III, men consumed more energy, even after adjusting for fat-free mass (187 $kJkg^{-1}$ versus 170 $kJkg^{-1}$) [1, 10]. One possible explanation is that women are more efficient at conserving energy and storing it as fat. Supporting this notion is the recognition that women must reduce their dietary intake by a greater proportion to achieve the same degree of weight loss as men [12]. Another observation is that in the first half of pregnancy, women increase their FM without evident increases in energy intake or decreases in expenditure. This ability to increase FM without substantial increases in energy intake points to the existence of metabolic adaptations that may contribute to the gender difference in FM.

3.1. Exercise Metabolism. Differences in rates of glucose and fat oxidation during exercise do not seem to explain the gender difference in FM. Women preferentially burn a higher fat-to-glucose fuel mixture during exercise [13]. Despite this, women lose less fat than men when faced with a similar energy deficit [14–17]. This may be related to more efficient fat storage during non-exercising periods [18, 19], considering less than 5% of the day is spent exercising in most healthy people. The higher fat mass in women may allow them to preferentially use this energy source as a fuel while exercising, whereas during the non-exercising times, women are storing fat more efficiently compared with men.

3.2. Postprandial Metabolism. Since women do not consume more energy compared to men, yet preferentially oxidise fat during exercise, it seems logical to propose that their higher

Table 2: Expression of sex hormone receptors in adipose tissues. Adapted from Mayes, 2004 [26].

Receptor	Visceral fat		Subcutaneous fat	
	Female	Male	Female	Male
OR-α	+	+	++	+
OR-β	+	+	+++	+++
PR-A	–	–	+	–
PR-B	+	–	++	–
AR	++	++	+	+

OR oestrogen receptor; PR progesterone receptor; AR androgen receptor.

FM is due to increased fat storage during non-exercising periods. Indeed, women were found to revert to a state of reduced fatty acid oxidation immediately after exercise, which persists for hours [21]. In addition, postprandial free fatty acid release from adipose tissue was reported to be lower in women than men [22, 23]. Several cross-sectional studies comparing men and women demonstrated that men oxidised a greater percentages of ingested fat [24, 25]. Using radiotracers, these authors also showed that postprandial fatty acid uptake by upper body subcutaneous and lower body adipose tissues were higher in women than men. Since the amount of energy expended in postabsorptive and postprandial states is greater than during exercise, this will have a great bearing on overall fat storage and FM.

Oestrogen is believed to be partly responsible for this reduction in postprandial fatty acid oxidation. Prospective studies using oral oestrogen therapy reported reductions in postprandial fatty acid oxidation. One study found significant reductions in postprandial fatty acid oxidation associated with a small increase in FM [27]. Similar changes were found in growth-hormone-deficient women on oral

oestrogen therapy [28]. Another study reported larger reductions in postprandial fatty acid oxidation with oral oestrogen compared with transdermal therapy associated with a significant increase in FM [29]. This route-dependent observation raises the possibility that oral oestrogen therapy exerts its influence on the liver during first-pass metabolism. Therefore, studies using exogenous oestrogens have demonstrated that efficient fat storage in women was mediated through reduced postprandial fatty acid oxidation most likely because of an oestrogenic influence on hepatic processing of dietary fats.

However, the metabolic effect of exogenous oestrogen treatment may differ from endogenous oestrogens for several reasons. Exogenous synthetic oestrogens are generally more potent [30], and they have different pharmacokinetic attributes [31]. Several types of endogenous oestrogens exist, and each may have slightly different or synergistic actions [30]. Prospective studies during the hyperoestrogenic state of pregnancy are ideal for investigating the effects of endogenous oestrogens on postprandial fatty acid oxidation. However, the logistical difficulties of studying pregnant women prepregnancy has meant that to date, there has been limited prospective studies, of sufficient sample size, on energy metabolism during pregnancy. Spaaij et al. [32] studied 27 women from pre-pregnancy to delivery. They found that postprandial fat oxidation did not differ from prepregnant values during the first 13 weeks and actually increased afterwards. In cross-sectional studies, Nagy and King [33] detected no difference in postprandial fatty acid oxidation between 6 nonpregnant and 10 pregnant subjects, while a larger study ($n = 23$) detected a significant reduction in fatty acid oxidation in the pregnant group [34]. However, due to significant intersubject variations, findings from cross-sectional studies should be interpreted with caution.

In conclusion, a reduction in postprandial fatty acid oxidation has been shown to promote FM gain. Exogenous oestrogen treatment appears to induce this reduction, possibly by suppressing hepatic processing of dietary fats during first-pass metabolism. However, due to the complex actions of endogenous oestrogens, the small number of studies, and inconsistencies in study design, the effects of endogenous oestrogens on metabolism requires further research.

4. Regulation of Metabolism and Body Composition by Sex Hormones

Women's higher proportion of FM and the increase in FM during the first half of pregnancy may be due to the influence of sex hormones on metabolic processes such as lipolysis and fatty acid storage. In the literature reviewed, there is evidence to suggest that these effects can be mediated through hepatic targets, adipocyte targets, and adipokines such as leptin. However, how these pathways interplay is complex and generally poorly understood.

4.1. Hepatic Targets. Oestrogen may have an inhibitory effect on fatty acid oxidation on the liver, a major site on fatty acid metabolism. Several *in vitro* studies in murine hepatocytes showed that pharmacological concentrations of oestrogen reduced ketogenesis (a product of fatty acid oxidation) and increased fatty acid incorporation into triglycerides [9, 35]. Similar findings were reported in human subjects, where oral oestrogen therapy administered to hypogonadal and postmenopausal women reduced postprandial fatty oxidation and increased triglyceride levels [29, 36–38]. This indicates that exogenous oestrogen directs intrahepatic fatty acids away from oxidative pathways and into lipogenic pathways.

In contrast, the effects of endogenous oestrogens are much harder to elucidate. Studies comparing women in follicular and luteal phases of the menstrual cycle detected no difference in energy metabolism, possibly because the change in oestrogen levels varies and there is also the influence of progesterone [24, 39]. Studies correlating oestrogen concentration and postprandial fatty acid oxidation in pregnant and non-pregnant subjects have looked at whole body metabolism of fat rather than isolating the effects on the liver. In addition, progesterone, which has been shown to have a synergistic and antagonistic effect, depending on the organ system, with oestrogen has not been studied in this context [8].

4.2. Adipocyte Targets. Oestrogen, progesterone and androgen receptors are present in adipose tissues [26]. As Table 2 shows, the expression of these receptors varies by depot and gender [40, 41]. Oestrogen receptors are higher in subcutaneous deposits in women, which may explain why women have greater subcutaneous gluteal and femoral deposits of fat [40, 41]. Genetic males with androgen insensitivity have a female body habitus [42], while women given exogenous androgens or suffering from virilising disorders will develop a male body habitus [40, 43–45]. Postmenopausal women experience an increase in waist to hip ratio and the amount of the visceral adipose tissue depot [32, 46, 47], which is partially reversed by oestrogen administration [48]. All this evidence indicates that the binding of sex hormones to their adipose tissue receptorspossibly promotes adipogenesis in some regions of the body. Although, it is known that many genes inadipocytes are transcriptionally regulated by sex hormones [26], the precise cellular mechanisms have not been fully elucidated.

4.3. Leptin. Leptin is an adipose tissue-derived hormone that inhibits fat gain by promoting hypophagia and hypermetabolism [49]. Thus, leptin has an important role in helping FM to remain relatively constant during adulthood. There is a gender difference in leptin levels which develops at puberty and is believed to be induced by sex hormones.

Leptin concentrations are higher per kilogram body weight in women than men. This difference is eliminated after adjusting for circulating concentrations of sex hormones [50]. Studies have found that leptin production was inhibited by androgens and promoted by oestrogens [26, 50]. Oestrogen has direct effects on FM as it upregulates leptin expression in adipocytes [51]. Central effects may also be

present as oestrogen receptors have been detected in the hypothalamic nuclei controlling energy homeostasis. Circulating oestrogens are proposed to bind to these receptors and alter hypothalamic sensitivity to leptin-mediated signals, thus influencing secretion of leptin and possibly influencing metabolism and even fertility [52–54].

However, the relationship between leptin, oestrogen, and body composition is complex, as there is no change in leptin with menopause or with oestrogen replacement therapy [26]. Weight loss is associated with reduced leptin levels and hypogonadism [55]. In addition, the hyperandrogenism and diminished oestrogen surge in polycystic ovaries disease do not affect leptin levels [56, 57]. Therefore, the role of leptin in regulating FM is potentially influenced by oestrogen however, the mechanism of action is not completely clear.

5. Energy Balance in Pregnancy

Female reproduction requires increased amounts of energy. Yet, throughout history, women have carried their conceptus to term under a wide range of nutritional conditions. This suggests the presence of powerful metabolic adaptations [3]. During gestation, energy is required to grow the tissues of conception and reproduction, to maintain these tissues, and to prepare for lactation. Butte and King [58] found that an average weight gain of 13.8 kg, which includes 4.3 kg of fat, represents gestational energy needs. Based on this model, the Food and Agriculture Organization, World Health Organization, and United Nations University have calculated the energy requirement of pregnancy to be 360–370 MJ. This equates to an extra 1300 kJ/day, which is 15% above non-pregnant needs. However, gestational requirements have been shown to range from 30 MJ to 520 MJ in undernourished to overnourished women [59]. This variability points to the presence of metabolic adaptations for sustaining pregnancy under different nutritional conditions.

In theory, this additional demand can be met by either increasing energy intake, decreasing expenditure, and/or mobilising fat stores. In contrast to expectations, numerous prospective and cross-sectional studies found that the first half of pregnancy is associated with little or no increase in energy intake [6, 60–62]. One study followed women prospectively through pregnancy and reported that energy intake in the first trimester of pregnancy is identical to pre-pregnancy [63]. Instead of reducing energy expenditure increased progressively during gestation [4, 64, 65]. Similarly, changes in diet-induced thermogenesis during pregnancy have not been consistently reported and therefore a reduction in diet-induced thermogenesis may not account for the positive energy balance [4, 33, 66–70].

These above observations raise the possibility that the energy costs of pregnancy are met by reductions in total energy expenditure. However, conclusive evidence fails to show that significant increases in energy intake or decreases in energy expenditure are the major contributors to the increase in FM in the first half of pregnancy. It is also important to note that pregnancy is a very plastic metabolic state, because even undernourished women can maintain FM [59]. Therefore, the cause of gestational fat gain may be mainly due to changes in metabolic pathways regulating the oxidation or storage of specific fuels especially fat.

6. Conclusion

Throughout their reproductive life, women maintain a higher proportion of body fat compared to men, and this difference is accentuated during the hyperoestrogenic state of pregnancy. However, studies have failed to demonstrate an energy surplus on all accounts. It is possible that women underestimate their food intake; however, some studies have reported that men underestimate their food intake compared with women [71]. The differences in physical activity between the sexes also need to be considered. Women do have a greater percent body fat, and it is possible that ovarian hormones, particularly oestrogen, may account for these observations by promoting postprandial conversion of dietary energy into fat. This theory needs to be supported by larger prospective studies and studies during natural hyperoestrogenic states such as pregnancy. Oestrogens' actions may be mediated through hepatocyte and adipocyte targets and through regulation of hormones such as leptin. Further studies are needed to elucidate how these hormonal pathways interact and influence their targets.

When considering lifestyle modifications, the sex difference in energy metabolism needs to be considered. Goals that take into account gender rather than just body weight or energy intake need to be utilised. Considering the high prevalence of obesity in modern society, it is important to understand the factors that regulate energy homeostasis and subsequently contribute to excess body fat. In the future, this understanding may culminate in strategies to control or reverse fat gain that do not only emphasise energy restriction.

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Research Article

Aerobic Exercise Training Adaptations Are Increased by Postexercise Carbohydrate-Protein Supplementation

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Carbohydrate-protein supplementation has been found to increase the rate of training adaptation when provided postresistance exercise. The present study compared the effects of a carbohydrate and protein supplement in the form of chocolate milk (CM), isocaloric carbohydrate (CHO), and placebo on training adaptations occurring over 4.5 weeks of aerobic exercise training. Thirty-two untrained subjects cycled 60 min/d, 5 d/wk for 4.5 wks at 75–80% of maximal oxygen consumption (VO_2 max). Supplements were ingested immediately and 1 h after each exercise session. VO_2 max and body composition were assessed before the start and end of training. VO_2 max improvements were significantly greater in CM than CHO and placebo. Greater improvements in body composition, represented by a calculated lean and fat mass differential for whole body and trunk, were found in the CM group compared to CHO. We conclude supplementing with CM postexercise improves aerobic power and body composition more effectively than CHO alone.

1. Introduction

It is well established that aerobic exercise training leads to cardiovascular, skeletal muscle, and metabolic adaptations. Cardiovascular adaptations include increased stroke volume and cardiac output, which contributes greatly to increased maximal oxygen consumption (VO_2 max) [1, 2]. Skeletal muscle adaptations include increases in activators of mitochondrial biogenesis such as peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), and increased activity of oxidative enzymes such as citrate synthase and succinate dehydrogenase [3–10]. While many investigations have addressed the effects of endurance exercise training on such adaptations, few have examined the role of postexercise nutritional supplementation in facilitating the adaptive process.

The beneficial effects of postexercise supplementation in the form of carbohydrate (CHO) or carbohydrate-protein (CHO+PRO) supplements following an acute exercise bout have been the focus of many investigations. Several studies performed by our laboratory, and others have demonstrated a greater improvement in acute exercise recovery with CHO+PRO supplementation compared to CHO alone [11–14] or to placebo [15]. Okazaki and colleagues [16] recently compared the effects of a CHO+PRO supplement to a placebo supplement in older male subjects who cycled for 60 min/d, 3 d/wk for 8 wk at 60–75% VO_2 peak. They reported a twofold increase in VO_2 max in the CHO+PRO group compared to the placebo group [16]. Thus, nutritional supplementation may increase the magnitude of training adaptations compared to the exercise stimulus alone. However, it was not possible to determine from their results if the increase in VO_2 max was due to cellular or systemic adaptations. Moreover, their experimental design did not allow for macronutrient specific comparisons, as they did not include a CHO-only supplement.

Recently, chocolate milk (CM) has been investigated as a practical and effective CHO+PRO postexercise recovery supplement after aerobic exercise [17–19]. In addition, several investigations have reported the efficacy of milk-based supplements in increasing protein synthesis [20] and lean mass accrual [19, 21, 22] in response to resistance exercise. However, the effects of aerobic endurance exercise training and nutritional supplementation on body composition changes have not been investigated.

Therefore, the purpose of the present study was to investigate training adaptations that occurred after a 4.5 wk aerobic endurance exercise (cycling) training program when supplementing after each daily exercise session with a CHO+PRO supplement in the form of CM, CHO or placebo. We aimed to determine if nutritional supplementation resulted in a greater increase in VO_2 max and skeletal muscle oxidative enzyme activity. We also sought to determine if supplementation resulted in a greater increase in lean mass and a greater decrease in fat mass. Although the exercise training was expected to induce positive adaptations in VO_2 max and muscle oxidative capacity, we hypothesized that postexercise CM supplementation would induce a greater extent of adaptations than would occur with CHO or PLA supplementation. We further hypothesized that the CM group would demonstrate greater lean body mass increases and fat mass decreases compared to the CHO and PLA groups.

2. Materials and Methods

2.1. Subjects. Thirty-two healthy, recreationally active but untrained males and females (16 males and 16 females) between 18 and 35 years old completed the study. Subject characteristics are listed in Table 1. In order to be classified as recreationally active but not endurance trained, subjects could not have exercised regularly more than 3 h/wk over the last 2 years, and had VO_2 max values of <40 mL/kg/min for females and <45 mL/kg/min for males. Potential subjects who did not meet these criteria were screened out of the study. A total of 36 subjects were admitted to the study. Subjects were separated into three groups matched for age, gender, and body composition and then were randomized into one of the 3 treatment groups. Four subjects voluntarily withdrew due to illness or work scheduling conflicts. Written informed consent was obtained from all subjects, and the study was approved by The University of Texas at Austin Institutional Review Board.

2.2. Research Design. This study followed a randomized, double-blinded, placebo-controlled design. The protocol for the training period is shown in Table 2. The entire protocol period was 7 wk long and consisted of the following: a baseline testing week, the first and second weeks of training, a midpoint testing week in which subject's VO_2 max was reassessed, followed by 2 days of training, training weeks 3 and 4, and a partial week during which the end testing was performed. Subjects reported to the laboratory before the start of their training period on two occasions, once for a baseline biopsy and dual energy X-ray absorbency (DEXA) scan for body composition determination (described below), and again the following day for determination of lactate threshold (LT), maximal oxygen consumption (VO_2 max) and maximal workload (W_{max}). This same test battery and schedule was repeated at the end of the training period (Table 2).

The LT test was performed first, followed by the VO_2 max test after a 5-min cool-down between the two tests. These tests were performed on a VeloTron DynaFit Pro cycle ergometer (RacerMate, Seattle, Wash, USA). LT was determined using 5-min stages beginning at 70 Watts (W) for males and 50 W for females. The Watts were increased by 25 W (males) or 20 W (females) each stage for the first 3-4 stages, followed by increases of 15 W (males) or 10 W (females) for the last 2-3 stages. A drop of blood was collected onto a lactate test strip after a finger stick during the last minute of each stage, and lactate levels were measured using a Lactate Pro LT-1710 lactate analyzer (Arkray, Inc., Minamiku, Kyoto, Japan). LT was defined as the breakpoint at which lactate levels begin to rise above baseline levels. After the 5-min cool-down in which the subjects pedaled easily and drank water *ad libitum*, the VO_2 max test began. VO_2 max was measured using a True One 2400 system (ParvoMedics, Sandy, UT). Subjects breathed through a Hans Rudolph valve, with expired gases directed to a mixing chamber for analysis of oxygen (O_2) and carbon dioxide (CO_2). Outputs were directed to a computer for calculation of ventilation, O_2 consumption (VO_2), CO_2 production (VCO_2), and respiratory exchange ratio (RER) every 15 s.

The protocol for establishing VO_2 max consisted of 2 min stages beginning at 125 W for males or 75 W for females. The workload was increased by 50 W (males) or 30 W (females) every 2 min until 275 W and 200 W, respectively. After this point, the workload increased 25 W (males) or 20 W (females) every minute until the subject could not continue to pedal despite constant verbal encouragement. The criteria used to establish VO_2 max was a plateau in VO_2 with increasing exercise intensity and RER > 1.10.

Maximum power output in Watts was calculated from the VO_2 max test data using the formula adapted from Åstrand and Rodahl [23]:

$$W_{max} = (VO_2\ \text{max mL} - 300\ \text{mL}\ O_2)/12.5\text{W/mL}\ O_2. \quad (1)$$

The workload for the desired intensity level of the training rides (75% of VO_2 max for the first 3.5 weeks and 80% for week 4) was then set as percentages of the W_{max} as follows:

$$W = [(VO_2\ \text{max mL} \times \%VO_2\ \text{max desired}) - 300\,\text{mL}\ O_2] / 12.5\ \text{W/mL}\ O_2. \quad (2)$$

With the exception of determining W_{max} for the purposes of setting ride intensity levels, the baseline and end testing consisted of the same tests in the same order.

During the training weeks, subjects reported to the training laboratory each morning after fasting overnight. All subjects began the rides as a group at the same time each day

Table 1: Subject characteristics at baseline.

	All subjects (32)	CM (11)	CHO (11)	PLA (10)
Age (y)	22.0 ± 0.5	22.1 ± 0.7	21.3 ± 0.9	22.6 ± 1.0
Weight (kg)	71.7 ± 2.4	70.9 ± 5.1	71.2 ± 3.1	73.2 ± 4.5
Height (cm)	168.6 ± 1.5	169.1 ± 2.3	168.0 ± 2.7	168.8 ± 3.1
VO_2 max ($L \cdot min^{-1}$)	2.6 ± 0.2	2.7 ± 0.3	2.6 ± 0.2	2.6 ± 0.2
VO_2 max ($mL \cdot kg \cdot min^{-1}$)	35.9 ± 1.9	36.8 ± 1.4	35.7 ± 2.2	35.2 ± 2.1

Values are mean ± SE. No significant differences existed between the groups at baseline.
Numbers in parentheses indicates subject numbers.

Table 2: Protocol for training period.

	Mon	Tue	Wed	Thurs	Fri	Sat	Sun
Baseline	LT and VO_2 max testing; biopsy; DEXA scan						
Week 1 (75% VO_2 max)	30 min	40 min	50 min	60 min	60 min	Rest	Rest
Week 2 (75% VO_2 max)	60 min	60 min	60 min	60 min	60 min	Rest	Rest
Midpoint		VO_2 max testing		60 min	60 min	Rest	Rest
Week 3 (75% VO_2 max)	60 min	60 min	60 min	60 min	60 min	Rest	Rest
Week 4 (80% VO_2 max)	60 min	60 min	60 min	60 min	60 min	Rest	Rest
End	LT and VO_2 max testing; biopsy; DEXA scan						

(6:00 AM or 7:30 AM), Monday–Friday. After each session, subjects were provided one dose of supplement immediately postexercise and were required to drink it in the laboratory. Subjects were then provided a second dose in an opaque to-go cup with a lid and straw and instructed to drink it 1 h later. They were also instructed to not ingest anything other than water until 1 h after ingesting the second dose.

The daily training rides were performed on Kona Dew bicycles (Kona, Ferndale, Wash, USA) mounted on CompuTrainer stationary trainers (RacerMate, Seattle, Wash, USA) interfaced with MultiRider III software (RacerMate, Seattle, Wash, USA). Six bicycles and CompuTrainers were interfaced with the system to allow for training groups of 6 subjects at one time. The bikes were set up based on each subject's physical measurements. The CompuTrainers were calibrated each morning. To minimize thermal stress, air was circulated over the subjects with standing floor fans, and water was provided ad libitum. Investigators encouraged the subjects to drink as needed.

The first week of training served to get the subjects accustomed to cycling for prolonged periods. The first ride was 30 min in duration, the second was 40 min, the third ride, 50 min, and the fourth, 60 min. With the exception of 3 rides the first week, all rides on Monday–Friday were 1 h in duration throughout the training period.

Each training ride began with a 10-min warm up at 60% VO_2 max, after which the work rate was increased to elicit ~75% VO_2 max for duration of each training ride. At the midpoint, VO_2 max was reassessed, and the workloads were adjusted accordingly to keep the subjects exercising at 75% VO_2 max for the third week. For the fourth week, the intensity was increased to 80% VO_2 max. A 5-min VO_2 measurement was performed at the beginning of each week to verify that the workload corresponded to the calculated intensity (%VO_2 max) for each subject. The Wattage calculated for each subject was set by the investigators, and subjects were asked to maintain a cadence of ~70 rpms in order to maintain the Wattage. Subjects were not allowed to shift gears or vary their cadence during the rides. The duration of the training period (4.5 weeks) was chosen, because 4 weeks or less has been shown to be an adequate amount of time to demonstrate VO_2 max and oxidative enzyme activity changes [13, 16].

Table 3: Energy and macronutrient content of the supplements.

	CM	CHO	PLA
CHO, g/100 mL	11.48	15.15	0
PRO, g/100 mL	3.67	0	0
Fat, g/100 mL	2.05	2.05	0
Kcals/100 mL	79.05	79.05	0
Ratio of CHO : PRO	3.12 : 1	—	—

Per 100 mL, CM, chocolate milk; CHO, carbohydrate + fat; PLA, placebo.

2.3. Experimental Beverages. After each daily session, subjects ingested the experimental beverages (CM, CHO, or PLA) immediately and 1 h postexercise. The CM (Kirkland Organic Low-Fat Chocolate Milk, Costco Inc.) and CHO beverages were isocaloric and contained the same amount of fat. The placebo was an artificially flavored and artificially sweetened supplement that resembled the CHO beverage in taste and appearance but contained no calories. Grape-flavored Kool-Aid was selected for the CHO and PLA treatments, because it best matched the dark coloring of the CM treatment visible only through a semiopaque lid on the drink containers. The energy and macronutrient composition of the beverages is shown in Table 3.

The amounts of supplement provided were stratified according to body weight ranges. Subjects weighing less than 63.6 kg (140 lbs) received 250 mL per supplement (197.5 kcals each), totaling 500 mL and 395 kcals. Subjects weighing between 63.6 kg (140 lbs) and 77.2 kg (170 lbs) received 300 mL per supplement (237 kcals), totaling 600 mL and 474 kcals. Subjects weighing between 77.2 kg (170 lbs) and 90.9 kg (200 lbs) received 350 mL per supplement (277 kcals), totaling 700 mL and 554 kcals. Subjects weighing over 90.9 kg (200 lbs) received 375 mL per supplement (296.5 kcals), totaling 750 mL and 593 kcals. For the CHO treatment, the amount of carbohydrate (dextrose) and fat (canola oil) matched that provided in the CM as measured for the individual's weight range. The CM supplement provided an average of 0.94 g carbohydrate, 0.31 g protein, and 0.17 g fat per kg body weight. The CHO supplement provided an average of 1.25 g carbohydrate and 0.17 g fat per kg body weight.

2.4. Diet and Exercise. Subjects were asked to keep their diets and activity levels consistent for the duration of the study (i.e., no significant changes in caloric intake, dietary habits, or activity levels outside of the study's training sessions). The subjects were instructed to maintain a dietary and activity log for the 2 days prior to their baseline and end biopsies and testing. The subjects were also asked to replicate their diet and activity on the days the logs are kept such that the diet and activity was the same on the 2 days prior to each biopsy session. The self-reported activity was compared for consistency in duration and intensity. The diet logs were analyzed for macronutrient composition and total caloric intake using Nutritionist V Dietary Analysis Software (First Data Bank, Inc, San Bruno, Calif, USA). All subjects complied with the diet and activity requirements. Subjects were instructed to arrive at the laboratory having fasted overnight for 12 h for every exercise session and laboratory visit except for the LT and VO_2 max testing sessions.

2.5. Lactate Threshold and VO_2 max. These measures were determined at baseline and at the end of the 4.5 wk training period, as shown in Table 2. The protocol for these tests is detailed above.

2.6. Muscle Biopsy Procedure. Muscle biopsies were taken at baseline and the end of the training period, as shown in Table 2. Prior to each biopsy, the subject's thigh was cleansed with 10% betadine solution and 1.4 mL of 1% Lidocaine Hydrochloride (Elkins-Sinn, Inc., Cherry Hill, NJ) was injected to prepare the leg for the muscle biopsy. Approximately ~45–60 mg wet wt of tissue was taken from the vastus lateralis through a 5–8 mm incision made through the skin and fascia, 6 inches from the midline of the thigh on the lateral side and 2.5 inches above the patella. The tissue samples were trimmed of adipose and connective tissue and immediately frozen in liquid nitrogen at −80°C for subsequent analysis.

2.7. Muscle Tissue Processing. The muscle samples were weighed and cut in half. One half of the tissue sample was used for the determination of citrate synthase and succinate dehydrogenase activity, and the other half for measurement of total PGC-1α content. For the enzymatic analyses, samples were homogenized in ice-cold buffer containing 20 mM Hepes, 2 mM EGTA, 50 mM sodium fluoride, 100 mM potassium chloride, 0.2 mM EDTA, 50 mM glycerophosphate, 1 mM DTT, 0.1 mM PMST, 1 mM benzamidine, and 0.5 mM sodium vanadate (pH 7.4) at a dilution of 1 : 10. Homogenization was performed on ice using 3×5 s bursts with a Caframo RZRl Stirrer (Caframo Limited, Warton, Ontario, Canada). The homogenate was immediately centrifuged at 14,000 g for 10 min at 4°C, the supernatant aliquoted to storage tubes for each assay and stored at −80°C. For determination of total PGC-1α content, the tissue samples were homogenized at a dilution of 1 : 10 in a modified RIPA buffer based on a previously described protocol [24] containing: 50 mM Tris-HCL (pH 7.4); 150 mM NaCl (pH 7.4); 1% each Igepal CA-630 and sodium deoxycholate; 1 mM each EDTA (pH 7.4), Na_3VO_4 (pH 10), NaF, and phenylmethylsulfonyl fluoride; 1 μg/mL each aprotinin, leupeptin, and pepstatin. Homogenization was performed on ice using 4×5 s bursts with a Caframo RZRl Stirrer (Caframo Limited, Warton, Ontario, Canada). The homogenates were sonicated on ice for 10 s and then centrifuged at 5,000 g for 20 min at 4°C. The supernatant was aliquoted to storage tubes and stored at −80°C. Protein concentration was determined from the supernatant using a modified version of the Lowry assay [25] for each sample and was measured before each of the assays were performed.

PGC-1α Content. Total PGC-1α content was determined by Western blotting. Total α-tubulin content was also determined as a housekeeping protein. Aliquots of homogenized muscle sample supernatants and standards were slowly thawed over ice and diluted 1 : 1 with sample buffer containing 1.25 M Tris, pH 6.8, glycerol, 20% SDS, 2-mercaptoethanol, 0.25% bromophenol blue solution, and deionized water. Samples containing 70 μg of total protein were separated on 10% polyacrylamide gels by SDS-PAGE for 75 min at 200 V (Bio-Rad Laboratories, Hercules, Calif, USA) After electrophoresis, the gels were electrotransferred using a semi-dry transfer cell (Bio-Rad Laboratories, Hercules, Calif, USA) using 25 V for 18 min to 0.4 μm polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, Mass, USA). The membranes were blocked in TTBS (TBS, 50 mM Tris, 150 mM NaCl, containing 0.1% Tween-20), and 10% nonfat dry milk for 2 h at room temperature on a rocking platform at medium speed. The membranes were then washed in 1x TTBS 3 times for 5 min each wash. Using the molecular weight markers visible on the membranes as a guide, the membranes were cut at the 75 kD marker. The upper section was used for detection of PGC-1α, and the lower section was used to detect α-tubulin. Each membrane section was incubated overnight at 4°C on a rocking platform at low speed with antibodies directed against PGC-1α (no. 515667, EMD Calbiotech/Merck KGaA, Darmstadt, Germany), and α-tubulin (no. 2144, Cell Signaling, Danvers, Mass, USA).

The antibodies were diluted 1 : 1000 for PGC-1α, and 1 : 900 for α-tubulin in TTBS containing 2% nonfat dry milk. Following the overnight incubation, membranes were washed three times with TTBS for 5 min each wash and incubated for 1.5 h with a secondary antibody (goat antirabbit, HRP-linked IgG, no. 7074, Cell Signaling, Danvers, Mass, USA). Dilutions were 1 : 7500 for PGC-1α and 1 : 1000 for α-tubulin. The immunoblots were visualized by enhanced chemiluminescence (Perkin Elmer, Boston, Mass, USA) using a Bio-Rad ChemiDoc detection system, and the mean density of each band was quantified using Quantity One 1-D Analysis software (Bio-Rad Laboratories, Hercules, Calif, USA). A molecular weight ladder (Precision Plus Protein Standard, Bio-Rad) and a rodent internal control standard prepared from insulin-stimulated mixed skeletal muscle were also included on each gel. All blots were compared with the rodent control standard and the values of each sample were represented as a percent of standard for each blot.

2.8. Oxidative Enzymes. Citrate synthase (CS) activity was determined according to the protocol of Srere [26] on the homogenates after further dilution of 1 : 10 (wt/vol) with 0.1 M Tris-HCI and 0.4% Triton X-100 buffer (pH 8.1). The rate of appearance of DTNBwas determined spectrophotometrically over 5 min at 412 nm and 37°C using a Beckman DU 640 spectrophotometer (Fullerton, Calif, USA), and was proportional to CS activity. Succinate dehydrogenase (SDH) activity was measured according to the method of Lowry and Passonneau [27]. The amount of NADH produced during a 5 min incubation time was read on a Varian Cary Eclipse fluorometer with an excitation wavelength of 340 nm and emission wavelength of 450 nm (Varian, Inc., Palo Alto, Calif, USA) and corresponded to SDH activity in the sample. CS and SDH activities were expressed as μmol/g/min protein.

2.9. Body Composition. DEXA (Medical Systems Prodigy, General Electric, Madison, Wis, USA) was used to determine both whole body and regional (trunk and legs) changes in fat mass, and lean mass, as well as bone mineral density (BMD). A three-compartment model design for assessing body composition was used, dividing the body into bone, fat mass, and fat-free mass. The total region percentage of fat mass and lean mass were used to assess the subjects' body fat and lean mass levels. The trunk region and legs region were used to assess fat and lean mass changes in the trunk and legs independently. The DEXA machine was calibrated each morning prior to subject measurement. Measurements were performed at baseline and at the end of the training period. The same trained technician performed all of the DEXA scans for the entire study.

The body composition differentials (Figures 4(a), 4(b), and 4(c)) were calculated according to the formula

$$\left(\mathrm{LMkg}_{\mathrm{End}} - \mathrm{LMkg}_{\mathrm{Baseline}}\right) - \left(\mathrm{FMkg}_{\mathrm{End}} - \mathrm{FMkg}_{\mathrm{Baseline}}\right) = \text{Differential (kg)}. \quad (3)$$

Using this formula, a gain in lean mass, and a loss of fat mass would result in a higher differential value than a loss in lean mass and gain in fat mass, or no change in lean and fat mass. This differential was calculated for whole body as well as regional (trunk and legs) changes (Figures 4(a), 4(b), and 4(c)). Therefore, the whole body differential was calculated as follows, using the CM treatment group values as an example:

$$1.408\,\mathrm{kg} - (-1.363\,\mathrm{kg}) = 2.771\,\mathrm{kg}. \quad (4)$$

The regional differentials were calculated by the same formula using the values from those specific regions.

2.10. Statistical Analyses. Using data in the literature similar to the type of study we proposed, a power analysis was performed using G-Power 3.0.10 software (Buchner, Erdfelder and Faul, Dusseldorf University, Germany) for an effect size of 0.3, $P < .05$, and desired power value of 0.8, using 3 treatment groups. A total sample population of 24 subjects was calculated for an actual power of 0.86 although we collected data on 32 subjects total.

VO_2 max, LT, muscle enzyme activity, PGC-1α content and body composition measures (lean mass, fat mass, and weight) taken at baseline and end were analyzed using two-way (treatment x time) analysis of variance (ANOVA) for repeated measures. Differences in the baseline and end measurements for VO_2 max, as well as forthe body composition differentials were analyzed using a one-way ANOVA. For all measures, post hoc analysis was performed when significance was found using least significant difference (LSD). Differences were considered significant at $P < .05$. Effect sizes were calculated for VO_2 max changes and body composition differentials using the value of Cohen's d and the effect-size correlation. Data were expressed as mean ± SE. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, Ill, USA).

3. Results

3.1. VO_2 max and Lactate Threshold. Absolute and relative changes in VO_2 max are shown in Figure 1. No significant differences existed between the groups at baseline. All treatment groups experienced significant increases in absolute and relative VO_2 max over the 4.5 wk training period. The change in both absolute and relative VO_2 max was significantly greater in the CM group compared to CHO ($P < .05$; absolute effect size, 0.86; relative effect size, 0.89) and PLA ($P < .05$; absolute effect size, 0.89; relative effect size, 0.90). The increases in the CHO and PLA groups were not statistically different from each other (Figure 1). LT increased significantly over time in all 3 treatment groups. However, there were no significant differences among the treatments (Table 4).

3.2. Oxidative Enzymes and PGC-1α. No significant treatment or treatment by time effects were found for CS, SDH (Figures 2(a) and 2(b)) or PGC-1α (Figure 3). Significant time effects existed for both enzymes in all treatment groups ($P < .05$). A similar response was found for PGC-1α ($P < .05$).

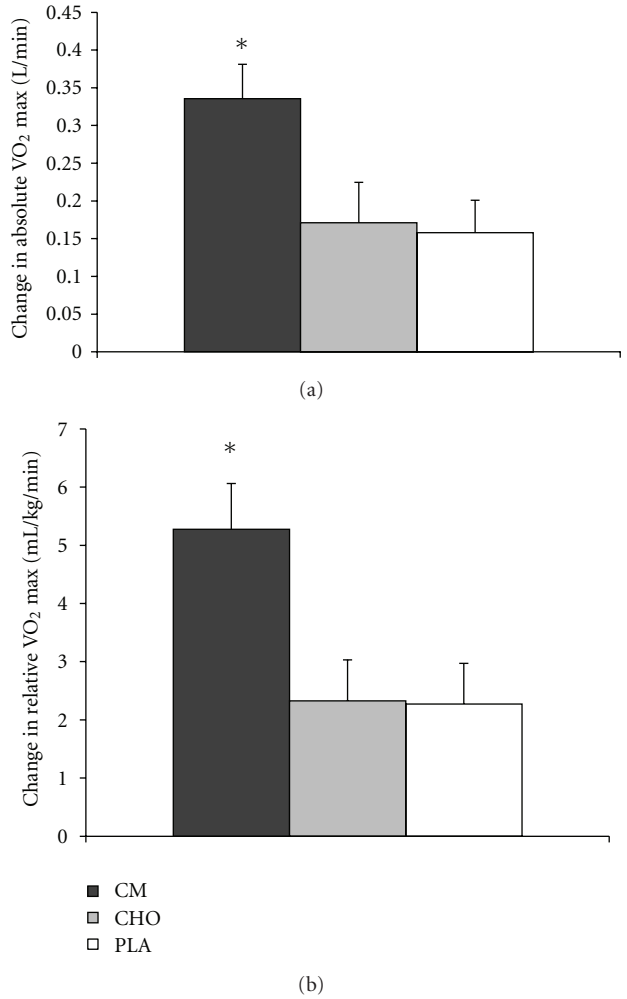

Figure 1: VO_2 max changes after 4.5 wks of aerobic endurance training. (a) Change from baseline in absolute VO_2 max. (b) Change from baseline in relative VO_2 max. Values are mean ± SE. Significant treatment differences: *, CM versus PLA and CHO ($P < .05$).

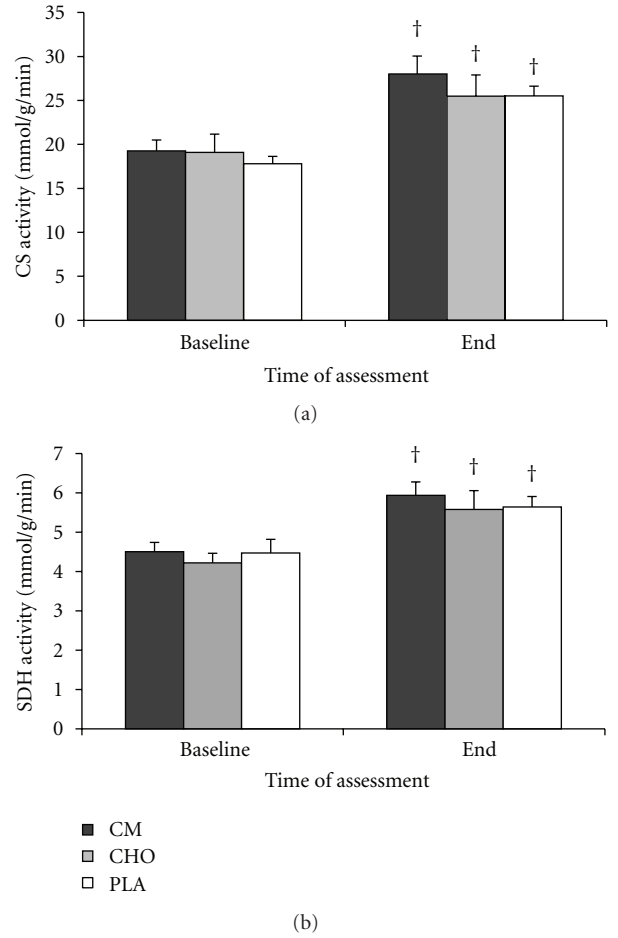

Figure 2: Oxidative enzyme activity. (a) Citrate synthase activity. (b) Succinate dehydrogenase activity. Biopsies were taken at baseline (before starting the training period) and at the end of the 4.5 wk training period. No significant treatment differences were found. †, significant time effect ($P < .05$).

Table 4: Lactate threshold.

	Baseline	End
LT (VO_2, L/min)		
CM	1.61 ± 0.16	1.83 ± 0.16†
CHO	1.47 ± 0.10	1.67 ± 0.11†
PLA	1.53 ± 0.11	1.70 ± 0.13†

Values are mean±SE. Significant differences: †, time only ($P < .05$).

3.3. Body Composition. Changes in body weight, lean mass and fat mass (assessed for whole body, trunk, and legs) are shown in Table 5. Whole-body lean mass increased in all treatment groups, with no treatment differences detected ($P < .05$). Although whole-body fat mass decreased in all groups, the change was not significant for treatment or time. In the trunk region, a significantly greater gain in lean mass was found in the CM group compared to PLA ($P < .05$). Trunk region fat mass differences were not significantly different between treatments although a significant time effect was found for all groups ($P < .05$). In the legs region, significant time effects were found for lean mass increases and fat mass decreases in all groups ($P < .05$).

The whole body and regional differentials are shown in Figures 4(a), 4(b), and 4(c). The whole body differential and the trunk differential were significantly greater in the CM group compared to CHO ($P < .05$; effect size for trunk, 0.81; effect size for whole body, 0.82). Whole body and trunk differentials for PLA were not significantly different than those for CM or CHO. The differential for the legs region was not significantly different among the three treatments. No significant treatment or time differences existed for BMD (Table 5).

4. Discussion

The most significant finding of the present study was that the increase in VO_2 max was significantly greater in the CM group than the CHO or PLA groups. The average increase in absolute VO_2 max for the CM group was 12.5% higher

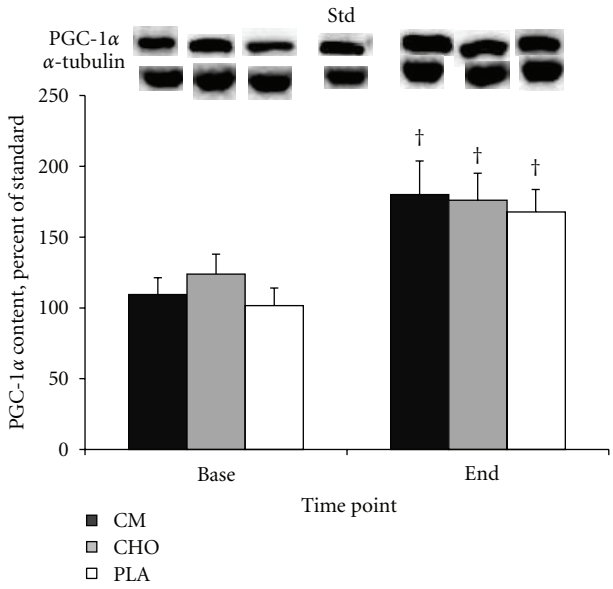

Figure 3: PGC-1α content before and after 4.5 wks of cycling exercise training. No significant treatment differences were found. †, significant time effect ($P < .05$).

than baseline levels, a twofold improvement over the increase found in the CHO and PLA groups. The average absolute VO_2 max (L/min) increase for all subjects and treatment groups combined was 9.2% over the 4.5 wk training period, which is in agreement with other investigations of aerobic training and VO_2 max improvements using a similar time period [28, 29].

It has been established that the primary determinants of VO_2 max are an increased ability of the cardiovascular system to transport oxygen to the working skeletal muscle, and the improved ability of the muscle to utilize the delivered oxygen. The former is a result of increased stroke volume, which improves cardiac output; the latter is determined by the increases in oxidative enzymes and mitochondrial content [1, 2]. We measured the activity of two key oxidative enzymes that are indicative of muscle oxidative capacity, CS and SDH. Both are found in the mitochondria and are key enzymes of the Krebs cycle, and each has been demonstrated to increase in response to endurance training [3–7, 9, 10, 30].We also measured total protein content of the transcription coactivator peroxisome proliferator-activated receptor γ coactivator-1α(PGC-1α) as a marker for increased mitochondrial biogenesis. PGC-1α is a transcriptional coactivator of transcription factor PPARγ, and together, they regulate the expression of genes that encode mitochondrial proteins. An acute bout of exercise or stimulated skeletal muscle contraction induces an increase in both PGC-1α mRNA and protein in skeletal muscle [31–34], and it has been shown that increased PGC-1α activation and total protein amount leads to increased mitochondrial biogenesis [24].

In the present study, we demonstrated that the activity of CS and SDH, and the total protein content of PGC-1α increased significantly in response to 4.5 wks of training.

Table 5: Body composition.

	Baseline	End
Weight (kg)		
CM	71.7 ± 5.5	71.7 ± 5.5
CHO	71.4 ± 3.4	71.4 ± 3.4
PLA	73.2 ± 4.5	72.9 ± 4.4
Lean mass, whole body (kg)		
CM	49.6 ± 4.1	51.0 ± 4.1†
CHO	49.4 ± 3.7	50.0 ± 3.74†
PLA	47.7 ± 3.7	48.5 ± 3.5†
Fat mass, whole body (kg)		
CM	19.1 ± 2.2	17.7 ± 2.15†
CHO	19.0 ± 2.1	18.5 ± 1.9†
PLA	22.5 ± 2.6	21.5 ± 2.6†
Lean mass, trunk (kg)		
CM	23.7 ± 1.8	24.6 ± 1.7§
CHO	22.6 ± 1.7	22.7 ± 1.7
PLA	20.9 ± 1.6	21.3 ± 1.6
Fat mass, trunk (kg)		
CM	11.6 ± 1.6	10.7 ± 1.6†
CHO	10.2 ± 1.3	9.6 ± 1.1†
PLA	10.7 ± 1.3	10.0 ± 1.3†
Lean mass, legs (kg)		
CM	17.8 ± 1.4	18.3 ± 1.4†
CHO	16.7 ± 1.3	17.1 ± 1.3†
PLA	15.6 ± 1.2	16.0 ± 1.2†
Fat mass, legs (kg)		
CM	7.0 ± 0.9	6.6 ± 0.9†
CHO	6.7 ± 0.8	6.8 ± 0.8†
PLA	7.7 ± 0.8	7.5 ± 0.8†
Bone mineral density (g/cm^2)		
CM	1.2 ± 0.1	1.2 ± 0.1
CHO	1.2 ± 0.0	1.2 ± 0.0
PLA	1.2 ± 0.0	1.2 ± 0.0

Values are mean ± SE. Significant differences: †, significant time effect; §, CM versus CHO ($P < .05$).

However, no significant treatment differences in these measures were detected. There was a slight but nonsignificant trend for a greater increase in CS and SDH activity in CM compared to CHO and PLA. It may be that the training period was not long enough to detect any potential differences that could emerge in response to chronic nutritional supplementation. Thus, our results suggest that the greater VO_2 max improvements with CM supplementation are most likely due to cardiovascular adaptations rather than increases in oxidative enzymes or in mitochondrial biogenesis.

As mentioned previously, endurance training leads to an adaptive increase in cardiac output, and this increase is due to augmented stroke volume [1]. While we did not measure these variables in the present study, our results suggest that the significant improvement in VO_2 max in the CM group is likely due to increased stroke volume and cardiac output, which is likely due to increased plasma volume.

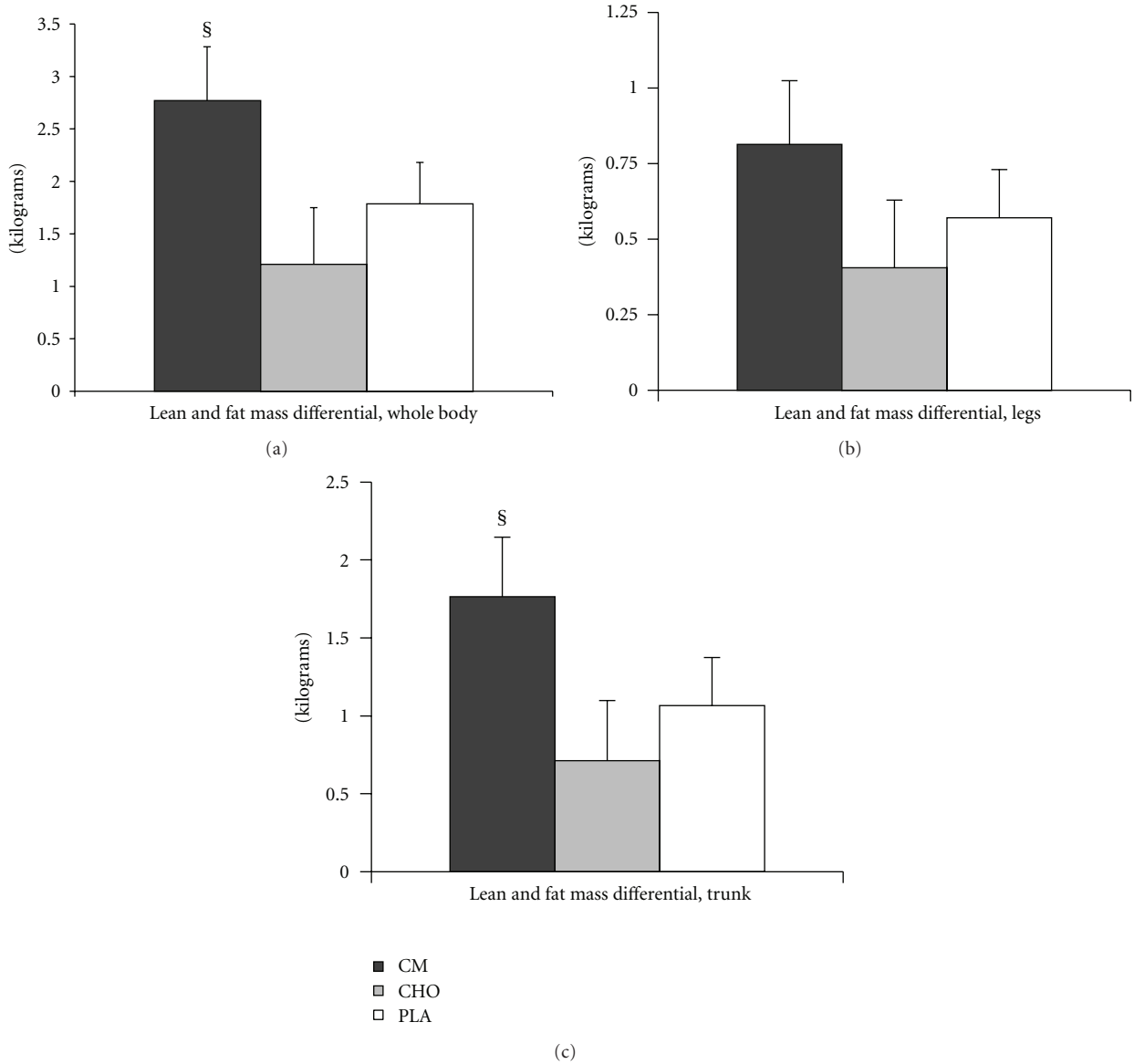

Figure 4: Body composition lean and fat mass differentials. (a) Whole body differential. Lean mass (kg) gained and fat mass (kg) lost was used to calculate a whole-body differential to quantify overall body composition changes in response to 4.5 wks of cycling exercise training. (LM) − (FM) = differential. Example: (0.900 kg lean mass) − (−0.350 kg fat mass) = 1.250 kg. (b) Lean and fat mass differential for the legs. (c) Lean and fat mass differential for the trunk region. Values are mean ± SE. Significant treatment differences: §, CM versus CHO ($P < .05$).

Plasma volume expansion is a hallmark of aerobic endurance training [35] and is directly associated with increased plasma albumin content. Increased albumin in the plasma causes water to be retained in the vasculature due to increases in the colloid osmotic pressure gradient [36, 37]. Hepatic albumin synthesis has been shown to increase in response to endurance exercise training [38, 39]. Moreover, plasma albumin content was reported increased 23 h after an acute bout of cycling exercise when CHO+PRO supplementation was provided postexercise compared to placebo [40]. These results, along with the findings of the present study, suggest that hepatic albumin synthesis may have been increased to a greater extent in the CM group compared to the CHO or PLA groups and contributed to the significantly greater increase in VO_2 max in the CM group.

Okazaki and colleagues [16] recently demonstrated that CHO+PRO supplementation provided immediately after daily cycling exercise training in older male subjects increased stroke volume and plasma volume compared to a placebo group. Their subjects cycled for 60 min/d, 3 d/wk for 8 wk at 60–75% VO_2 peak and ingested either CHO+PRO or placebo immediately postexercise each session. VO_2peak increased 3.3% in the control group and 6.8% in the CHO+PRO group, with significant stroke volume and plasma volume increases only found in the CHO+PRO group [16]. In the present study, we extend the findings of Okazaki and colleagues [16] by demonstrating that the effect of nutritional supplementation on VO_2 max increases is nutrient specific. In comparing CM against an isocaloric CHO only supplement and a placebo, we have shown that the

increased VO_2 max response is not due to simply providing calories postexercise. In the present study, the VO_2 max increase in the CHO and PLA groups was not significantly different. Thus, these results suggest that the benefit from a CHO+PRO or CM supplement in improving VO_2 max is due to the combined ingestion of carbohydrate and protein. However, we cannot rule out the possibility that a supplement composed of protein alone would not have the same effect.

In addition to well-documented increases in VO_2 max with training, it is known that lactate threshold improves with endurance exercise training of moderate to high intensity [41]. In the current study, LT improved significantly over the 4.5 wks of training although there were no significant treatment differences detected (Table 4). It has been shown that the respiratory capacity of the muscle is the key determinant of LT [42]. Given that muscle oxidative enzyme activity and PGC-1α content increased significantly over time without demonstrating a treatment effect, it would be expected that LT would follow a parallel pattern. Therefore, the results suggest that while LT is increased by exercise training in parallel with muscle oxidative capacity, it likewise may not be affected by nutritional supplementation.

The other key finding of the present study was that body composition improvements, represented by a calculated lean and fat mass differential, were significantly greater in the CM group than the CHO group. Compared to the CHO group, the CM group lost more fat mass and gained more lean mass measured in the whole body, as well as in the trunk region only ($P < .05$). While these differentials were also greater for CM compared with PLA, the differences were not significant.

It is well established that resistance exercise training induces significant gains in lean mass, whereas endurance exercise training is not associated with large increases in lean mass or gains in muscular strength [43]. A previous investigation comparing the effects of aerobic and aerobic + resistance training showed that the aerobic + resistance group increased lean mass in arm, trunk, and total body regions, and the aerobic only group increased lean mass in trunk region only [44]. However, the aforementioned investigation did not use supplementation. The body composition improvement with CM is also in agreement with the findings of Josse and colleagues [22], who recently demonstrated significantly greater muscle mass accretion, fat mass loss, and strength gains with milk supplementation compared to soy and CHO after a 12-wk resistance training program [22]. Therefore, the findings of our study are in line with what is reported in the literature for exercise mode-dependent body composition changes.

As shown in Table 5, all groups demonstrated significant changes over time in whole-body lean mass, trunk fat mass, and legs lean and fat mass, and the CM group demonstrated a significant treatment effect compared to CHO when whole body and regional differentials were calculated (Figures 4(a), 4(b), and 4(c)). The whole body and trunk differentials for PLA were slightly greater than CHO although not significantly different from either CM or CHO. The lack of difference in the PLA treatment from CHO suggests that a component of the CM treatment facilitated the significant body composition change, since simply supplementing with an energy-containing supplement (CHO) did not have a significant effect compared to PLA. In fact, a slight, nonsignificant increase in fat mass in the legs region was detected with CHO, whereas fat mass of the legs decreased in CM and PLA during the training period. To our knowledge, no evidence exists in the literature to suggest that postexercise CHO supplementation would mediate this type of change, given that the subjects' diets were not standardized and controlled during the study. However, this finding further underscores the difference in supplementing with a CHO+PRO-containing supplement versus calories from CHO alone in facilitating body composition changes.

There are two possible explanations for the difference found with the CM treatment compared to CHO: first, the availability of amino acids (AAs) in the milk for anabolism and muscle mass accretion, and second, a fat-loss promoting effect of dairy calcium and protein. It is known that AAs, along with a permissive amount of insulin, are required for muscle protein accretion to occur in response to exercise [45, 46]. The CHO treatment would increase plasma insulin levels and provide glucose as an energy and glycogen-synthesizing substrate, but would provide no AAs for the synthesis of new muscle protein. Thus, AAs availability from the milk proteins whey and casein provided substrate for this adaptive process. In addition, Zemel [47, 48] have shown that the increased consumption of dietary calcium is associated with reduced adiposity and greater weight loss in energy restricted diets. Moreover, the fat and weight loss effects were greater when the source of the dietary calcium was from dairy products rather than a calcium supplement [47, 48]. Additional evidence that the dairy component of the CM treatment likely underlies some of the body composition changes is found in the resistance training study of Hartman and colleagues [21], who demonstrated that fat mass decreased, and lean mass increased, in groups provided either milk, soy, or CHO postexercise but that milk significantly promoted increased hypertrophy compared to soy and CHO [21]. Another well-known benefit of dairy calcium consumption is improved bone mineral density. We did not detect treatment or time differences in BMD (Table 5); however, this is not surprising, given the relatively short duration of the training program and the lack of a resistance training component. Taken together, these data suggest that the dairy component of the CM treatment was instrumental in facilitating the fat mass changes compared to the CHO and PLA groups, while the AAs from milk proteins provided substrate for lean mass accretion in the present study.

There are several limitations to the present study. First, the subjects' normal diets were not controlled nor standardized for the majority of the training period. Although the diets were recorded and replicated for 3 days each week as described above, there could have been within and between-subject variations in the amount of protein, calcium, and total caloric intake on the nonrecorded days during the training period which could have influenced the adaptive response. Second, CM contains many other micronutrients and flavonoids in addition to the major macronutrients and calcium. However, the possible effects of these additional

components on the training adaptations reported here are not known at this time. Third, the taste and appearance of the three treatments were different. However, the subjects were not aware of what the three treatments were, and since they only ingested the treatment for which they were randomized for the entire study period, they did not taste any of the other treatments. Fourth, we did not match the supplement dosing to each individual's body weight, but stratified the amount of supplement for each dose according to body weight ranges. We have previously shown that supplementation with ~1.0 g of CHO and ~0.3 g of PRO per kg body wt postexercise will substantially increase muscle glycogen synthesis and recovery from exercise [14]. In the present study, providing supplement based on a weight range represented a more realistic and practical approach. Finally, while we propose that the greater increase in VO_2 max in the CM group is likely due to albumin synthesis, we did not measure plasma volume or plasma albumin and, therefore, cannot say with certainly that this is the reason for the VO_2 max differences. Further investigation is necessary to expand upon these results and elucidate the mechanisms of the greater adaptive response.

5. Conclusion

Our results demonstrated that CM supplementation postexercise increased the magnitude of VO_2 max improvement in response to a 4.5 wk aerobic exercise-training program. Muscle oxidative capacity and lactate threshold improved significantly in all treatment groups, with no differences found between treatments. This would suggest that the greater improvement in VO_2 max when supplementing with CM as compared with CHO or placebo was cardiovascular rather than cellular in nature. In addition, CM supplementation significantly improved body composition as defined by the combination of an increase in lean mass and a decrease in fat mass compared to CHO. We conclude that CM is an effective postexercise recovery supplement that can induce positive increases in aerobic training adaptations in healthy, untrained humans.

Acknowledgments

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Research Article

Nuts Improve Diet Quality Compared to Other Energy-Dense Snacks While Maintaining Body Weight

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Previous studies have reported that regular nut consumption reduces cardiovascular disease (CVD) risk and does not promote weight gain despite the fact that nuts are energy-dense. However, no studies have investigated the body composition of those regularly consuming nuts compared to similar intakes of other snacks of equal energy density. This parallel study ($n = 118$) examined the effects of providing daily portions (~1100 kJ/d) of hazelnuts, chocolate, or potato crisps compared to a control group receiving no snacks for twelve weeks. Effects on body weight and composition, blood lipids and lipoproteins, resting metabolic rate (RMR), appetite indices, and dietary quality were compared. At week 12, there was no significant difference in any of the outcome measurements between the groups except for dietary quality, which improved significantly in the nut group. Nuts can be incorporated into the diet without adversely affecting body weight and can improve diet quality.

1. Introduction

Nuts are rich in unsaturated fats, fibre, minerals, vitamins, and phytonutrients [1–5]. Regular consumption of nuts has been associated with reductions in blood cholesterol concentrations and the risk of CVD [6–11]. However, nuts are energy-dense, high-fat foods, meaning that they contain high amounts of energy per unit weight. In general, the consumption of energy-dense foods is associated with weight gain and obesity [12, 13]. Therefore, the public health recommendation to consume nuts on a regular basis could potentially result in weight gain and possibly negate their beneficial effects. Also, the general public perceive nuts as "fattening" and thus might not heed the advice to consume nuts regularly as a means of reducing CVD risk [14]. It is, therefore, important for policy makers to determine whether the regular consumption of nuts may promote weight gain and hence increase obesity rates among the general population and, based on findings, change the wording of the health recommendation to consumers as appropriate to clarify any misperceptions.

Epidemiological studies show that nut consumers tend to be leaner than those who do not regularly consume nuts. This research tends to show an inverse or no association between nut consumption and body mass index (BMI) as well as adiposity [15–19]. Also, clinical trials, where the primary outcomes have included cardiovascular risk factors such as blood cholesterol, have failed to show an increase in body weight with regular consumption of different kinds of nuts, albeit over the short term [7, 9, 20–25]. However, these studies were not designed to assess body weight and in many instances the investigators provided dietary advice or adjusted energy intake to prevent weight gain.

Studies that have looked at the role of nuts in the context of supervised weight loss diets reported that subjects who consumed nut-enriched diets experienced greater weight loss and greater improvement in CVD risk factors compared with a low-fat diet [26, 27], a complex carbohydrate diet [28], or an isocaloric diet without nuts [29]. Thus, incorporating nuts into diets intended for weight loss and weight control has the potential to result in more favourable changes in body weight and CVD risk. Four randomised crossover trials

involving the consumption of nuts have specifically looked at body weight as the primary outcome [30–33]. In general these studies indicate that the daily inclusion of nuts into the diet results in either no weight gain or less weight gain than predicted from the additional energy intake.

There have been several purported reasons why regular nut consumption may not result in the theoretically predicted weight gain [34–37]. Firstly, nuts are high in protein and fibre with low glycaemic index value, which may promote satiety resulting in a reduction in calories from other foods, that is, dietary compensation [30–33, 38, 39]. The crunchy textural property of whole nuts may also promote satiety as the mechanical act of mastication results in the secretion of appetitive hormones such as cholecystokinin and glucagon-like peptide-1 [28, 40–44]. Secondly, previous work has suggested that nut consumption may lead to an increase in energy expenditure. In humans, a high unsaturated-to-saturated-fat ratio in the diet can increase RMR. Thus, the high unsaturated fat content of nuts may increase RMR [30, 41, 45, 46]. Thirdly, some research has suggested that the lipid found in nuts may not be highly bioaccessible [47, 48], meaning that a high proportion of this fat is excreted in the faeces and therefore not available for energy metabolism [32, 40, 49, 50].

All the above-mentioned studies were not designed to answer the question as to whether nut consumption is different to the ingestion of other isoenergetic foods with regard to dietary compensation and energy metabolism. This information is needed to determine whether there is something unique about nuts, setting them apart from other more highly processed energy-dense foods. It is also needed to confirm current public health advice that nuts, though an energy-dense food, are nutrient-dense and thus should be consumed regularly as part of a cardio-protective diet. Therefore, the purpose of this study was to assess the effects of providing daily portions (~1100 kJ/d) of hazelnuts, chocolate, or, potato crisps for twelve weeks on body weight and composition, blood lipids and lipoproteins, RMR, appetite indices, and diet quality compared with the control group.

2. Materials and Methods

2.1. Subjects. One hundred and twenty-four participants were recruited from the general public in Dunedin, New Zealand (NZ). The inclusion criteria were healthy males or females aged between 18 and 65 years. The exclusion criteria were people with BMI $\geq 30\,kg/m^2$, people who have asthma, women who are pregnant or breastfeeding, people with a chronic disease such as cancer, heart disease, or diabetes, and people with food allergies or food aversions. The study protocol was approved by the Human Ethics Committee of University of Otago, NZ. All participants gave written informed consent. The trial was registered at the Australian New Zealand Clinical Trials Registry (http://www.anzctr.org.au/), registration number ACTRN12609000265279.

2.2. Test Products. All the Ennis hazelnuts used in this study were purchased from Uncle Joe's Walnuts (Blenheim, Marlborough, NZ). All nuts were stored at room temperature in darkness prior to opening. Dairy milk chocolate (Cadbury, Dunedin, NZ) and ready salted potato crisps (Bluebird, Auckland, NZ) were chosen as comparison foods because they are both popular snack foods in NZ and have a very similar energy density to nuts, with one being savoury and the other sweet. The energy density for the study hazelnuts was 26 kJ/g, while the energy density for the chocolate and potato crisp was 22 kJ/g.

2.3. Study Design. This study was conducted using a randomised, controlled, parallel design with four arms: ~1100 kJ/d for each of hazelnuts (42 g) chocolate (50 g), and potato crisps (50 g) or no additional food (control group). People who were interested in the study contacted the investigator by phone or email. Participants were informed that the purpose of the study was to assess the effects of consuming three different snacks on energy balance and blood cholesterol concentrations. We purposely did not emphasise body weight so that participants were unaware that this was the focus of the research. They were asked to complete a recruitment questionnaire, which included contact information, demographic and relevant health details that might affect the study outcomes. All participants were asked to consume their regular diets (baseline diet) during a two-week run-in period. Baseline measurements were collected from each participant following this run-in period. These included body weight and composition, blood lipids and lipoproteins, RMR, appetite indices, a three-day diet record (3DDR), and physical activity levels. After collecting all baseline measurements, participants were randomly allocated to one of the four groups for a period of 12 weeks.

Due to the strong possibility of age, sex, and BMI effects, groups were balanced using eight strata covering all possible combinations of age group (18–40, 41–65), sex (male, female), and self-reported BMI group (<25, 25–29.9) categories. Allocation within each strata was conducted by an off-site statistician using blocks of size four. Incomplete blocks remaining at the conclusion of enrolment were randomly allocated first using strata based on sex and BMI and then only BMI, and finally without stratification. The statistician was located in another building and had no involvement in the enrolment process.

All snacks were individually portioned into daily serving sized bags and participants were asked to collect their snacks from the university every three weeks. They received no dietary advice, except that participants in the intervention groups were told that they might consume the snacks however they wished as long as their daily portion was consumed each day. Participants were instructed that the snacks were solely for their own personal consumption and should not be shared with others. They were also asked to return any snacks not eaten on any given day. The control group received no additional food. However, they were given a month supply of the snacks of their choice at the end of the study. Compliance was measured by weighing the returned serving bags and by 3DDR.

2.4. Dietary Assessment. A three-day weighed diet record of all foods and beverages consumed both in and out of the home was collected from participants at baseline and during the intervention using dietary scales (Salter Electronic, Salter House wares Ltd., Kent, UK), accurate to within ±1 gram. The nonconsecutive three days included two weekdays and one weekend day over a one-week period.

The initial 3DDR was issued during the diet record instruction session at the first visit. Detailed instructions on how to collect diet records were verbally presented to each participant by a trained researcher. Written instructions were also included in the 3DDR. Pictures of different portion sizes of common food were given to the participants to help them estimate quantities when they did not prepare the meal themselves. A reminder email and text were sent the day prior to every dietary collection week to improve compliance. Participants were asked to complete a 3DDR at baseline and during the intervention, and all 3DDRs were reviewed by the researcher upon return for completeness and accuracy.

All diet records were analysed to provide an estimate of average energy and nutrient intakes using the computer programme Diet Cruncher for the PC [51]. The programme utilises food composition data from NZ Composition Database [52]. All diet records were entered by a single trained researcher to ensure consistency in data-entry decisions when substitutions had to be made.

2.5. Appetite-Rating Questionnaire. Participants in the intervention groups (hazelnut, chocolate, and potato crisp groups) were also asked to record their appetite ratings on a 100 mm visual analogue scale (VAS) immediately before and after they consumed the study snacks on the same three days that dietary intake was recorded. Mean scores were calculated for the 3-day period, using the mean score from one or two days where there was missing data. The appetite-rating questionnaires included questions on hunger, desire to eat, prospective consumption, fullness, and preoccupation with thoughts of food. Hunger was assessed with the question "How hungry do you feel right now?" preceded with a 100 mm VAS, anchored with "Not at all hungry" on the left side (0 mm) to "Extremely hungry" on the right side of the scale (100 mm). Desire to eat was assessed with the question, "How strong is your desire to eat right now?" and anchored with "Strong desire not to eat" and "Strong desire to eat." Prospective consumption was assessed with the question, "How much food could you eat right now?" and anchored with "Nothing at all" and "The most that I have ever eaten." Fullness was assessed with the question, "How full do you feel right now?" and anchored with "Not at all full" and "Extremely full." Preoccupation with thoughts of food was assessed with the question "Do you have any preoccupation with thoughts of food right now?" and anchored with "No thoughts of food" and "Very preoccupied, difficult to concentrate".

2.6. Physical Activity Assessment. Physical activity may influence the primary outcome measures of interest in this study, namely, body weight and composition and blood lipids and lipoproteins. Therefore, it was important to measure habitual physical activity. Physical activity was measured using NL-1000 accelerometers (New Lifestyles Inc., USA) at baseline and during the intervention. Participants were asked to wear the accelerometer clipped to their waist for a period of seven days. Detailed instructions on how to wear the accelerometer were given to the participants. The accelerometer was sealed so that participants were blinded to the activity reading. After the accelerometer was returned, information including number of steps and duration of activity was retrieved from the 7-day memory and recorded in an Excel spreadsheet.

2.7. Resting Metabolic Rate. Resting metabolic rate was measured by indirect calorimetry after an overnight fast of at least 12 hours. Due to time constraints, RMR was assessed on around half of the study participants ($n = 52$) who were randomly chosen. After a 15-minute rest period, expired gas collection was achieved through a mouthpiece with the nose clipped for a 15-minute period. Participants were asked to consume their normal diet and refrain from exercise in the 24-hour period prior to the test. They were also asked to avoid alcohol, caffeine, or nicotine within 12 hours of the test. Menstruating females were measured during the follicular phase of the menstrual cycle, as metabolic rate could be affected by the thermic effect of progesterone during the luteal phase [53].

2.8. Biochemical Indices. Venous blood samples were taken by a nurse at the Human Nutrition clinic on six occasions; two samples at baseline, twice after six weeks, and twice at the end of the study. Two blood samples on nonconsecutive days were collected during each testing week to account for intra-individual variation in blood lipid measures. Fasting blood tests were collected from participants following a 12-hour overnight fast. Ten millilitres of venous blood was collected into Vacutainers (Belton Dickinson Diagnostics) containing disodium EDTA for the analysis of plasma blood lipids and lipoproteins concentrations. Vacutainers were inverted and stored in a chilly bin containing chilled ice-pads after blood samples were drawn. All blood specimens were separated by centrifugation at 3000 g for 15 minutes at 4°C within two hours of being drawn. Once plasma and red blood cells were separated, aliquots were stored at −80°C until analysis.

Plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride concentrations were measured in all blood samples by enzymatic methods using kits and calibrators supplied by Roche Diagnostics (Mannheim, Germany) on a Cobas Mira Plus Analyser. High-density lipoprotein cholesterol was measured in the supernatant following precipitation of apoprotein B containing lipoproteins with phosphotungstate-magnesium chloride solution [54]. Plasma low-density lipoprotein cholesterol (LDL-C) concentration was calculated using the Friedewald formula [55].

Calibration and quality control is maintained by participation in the Royal Australasian College of Pathologists Quality Assurance Programme. The mean intra-assay and inter-assay coefficients of variation for plasma TC, HDL-C and triglyceride were 1.12%, 4.74%, 0.73% and 2.76%, 6.91%, 2.08%, respectively.

2.9. Anthropometric Measurements. Standing height was measured at baseline to the nearest millimeter using a stadiometer. Participants were asked to stand with shoes off, and their backs and heels against the back of the stadiometer. They were instructed to take a deep breath and the adjustable lever was then lowered until it was resting on the top of their head.

Body weight was measured in the morning in the fasting state during all clinic visits. Participants were weighed in light clothing without footwear, on a bioelectrical impedance analyser placed on a hard flat surface that measured to the nearest 0.01 kg. The same machine was used throughout the study. Body composition including fat mass, body fat percent, and waist fat percent was measured at baseline and at the end of the study by using dual energy X-ray absorptiometry (DXA).

2.10. Statistical Analysis. In order to have 80% power to detect a difference of 0.46 kg or more in weight gain over the 12 weeks (equivalent to an annual weight change of 2.0 kg) between any two groups and assuming a standard deviation (SD) in weight change of around 0.6 kg for the 12-week period (estimated from an overfeeding study by Diaz et al. [56]) using a two-sided test with the level of significance set to 5%, 27 participants would be required in each group at the end of the study. This detectable effect size was equivalent to roughly 20% of the expected weight gain for the non-control groups based purely on the additional calories consumed with full compliance and assuming no compensatory changes in energy intake. This sample size would also be sufficient to detect effect sizes of 0.8 SD or larger in the appetite ratings in the same way. Allowing for up to 10% attrition and unusable data, 30 participants should be enrolled in each group, 120 participants in total for the four groups.

Baseline characteristics of the participants were presented as arithmetic or geometric means and arithmetic or geometric standard deviations as appropriate. Categorical data were presented as frequencies and percentages. The effects of the four interventions on all outcomes including body weight and composition, blood lipids, RMR, appetite indices, dietary intakes, and physical activity level were examined using either regression models controlling for baseline values where measurements were available only at baseline and follow-up (body composition, RMR, appetite indices, dietary intakes and physical activity level) or linear mixed models with a random subject effect where interim measurements were also available (body weight and blood lipids). Changes within groups were shown along with their associated standard errors. Where the overall test for difference in changes between groups was statistically significant, pairwise comparisons between groups were performed. Log-transformations were used for both final and baseline (and interim where appropriate) values of dependent variables where this improved residual normality and/or homoscedasticity.

The primary analysis was intention-to-treat (ITT) analysis, which included data from all participants who underwent randomisation. A secondary per-protocol (PP) analysis was also performed, using only participants who had at least 70% compliance to the snacks, which would be equivalent to a 2 kg weight gain over the twelve weeks without energy compensation. Stata 11.1 (StataCorp, College Station, Tex, USA) was used for all statistical analyses. All tests were performed at the two-sided 0.05 level.

3. Results

Of the 124 participants who were enrolled and randomised into the study, four participants were retrospectively excluded on their first visit as their BMI was >30 kg/m^2 despite their self-reported BMI being <30 kg/m^2, one participant had to undergo surgery, and another one was pregnant and thus both were retrospectively excluded following their second visits. Hence, 118 participants were included in the ITT analysis: 32 participants from the hazelnut group, 31 from the chocolate group, 29 from the control group, and 26 from the potato crisp group. Of these, one participant from the chocolate group and one from the potato crisp group withdrew from the study due to their dissatisfaction with group assignment, three participants were lost to follow-up and consequently dropped out from the study, two participants withdrew from the study due to personal issues unrelated to the study, and one participant from the hazelnut group and two participants from the chocolate group with no previously noted sensitivity to these foods experienced adverse events after consuming the study snacks and were discontinued from the study (Figure 1). A similar percentage of participants from each intervention group consumed <70% of study snacks (9% in the hazelnut group, 10% in the chocolate group, and 8% in the potato crisp group). Using this criterion, 100 participants were included in the PP analysis.

As shown in Table 1, the groups were well balanced with respect to their baseline characteristics. Among the 118 participants who were randomised into the study, 53% were females ($n = 63$). Participants ranged in age from 18 to 65 years with a mean (SD) age of 37.4 (14.0) years. The mean (SD) height at baseline was 171.0 (9.4) cm, mean (SD) weight was 69.5 (11.4) kg, and mean (SD) measured BMI was 23.8 (3.0) kg/m^2.

The energy and nutrient intakes at baseline and changes from baseline to week 12 for each group are presented in Table 2. There were statistically significant differences in the percentage of energy from total fat, saturated fatty acids (SAFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), carbohydrate and also in the intake of vitamin E. The results from pairwise comparison showed that the percentage of total energy derived from SAFA (all $P \leq 0.045$) and carbohydrate (all $P \leq 0.006$) in the hazelnut group was significantly lower than all the other groups. On the other hand, vitamin E intake (all $P \leq 0.007$), the percentage of energy derived from total fat (all $P \leq 0.011$), MUFA (all $P \leq 0.001$), and PUFA (all $P \leq 0.011$) increased at 12 weeks in the hazelnut group compared to all other groups, with the exception of one pairwise comparison (hazelnut versus control; $P = 0.057$) for PUFA. Very similar results were obtained from the PP analysis.

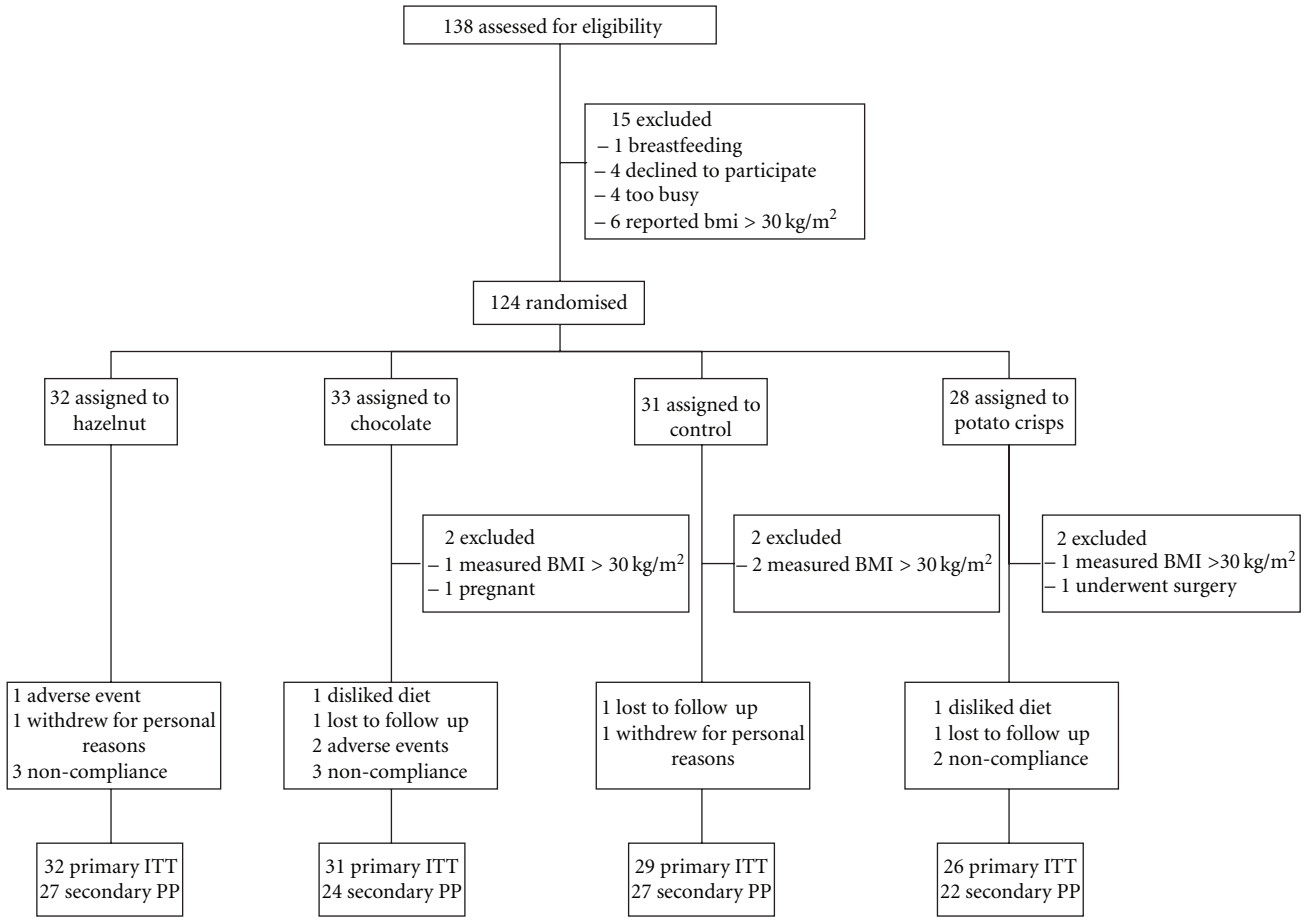

Figure 1: Flowchart of study participants.

Although there was an apparent decrease in physical activity level in the control group, there were no statistically significant differences in the changes of the anthropometric measurements, RMR, and physical activity level from baseline to 12 weeks between the groups (all $P \geq 0.106$, Table 3).

There was, however, a significant interaction between group and baseline BMI for waist circumference using PP analysis ($P = 0.032$). Those with higher BMI reduced their waist circumference in the nut group ($P = 0.005$) and (to a lesser extent) in the potato crisp group ($P = 0.032$) compared to the control group where the association was in the opposite direction.

Compared with baseline, the changes in plasma lipids and lipoproteins at the end of the study did not statistically significantly differ between the groups (all $P \geq 0.136$, Table 4).

However, in a PP analysis including only people who had >70% compliance to the study snacks, there was evidence of a difference in changes in plasma total cholesterol from baseline to week 12 between the groups ($P = 0.035$). Plasma total cholesterol in the hazelnut group was lower compared to the chocolate group ($P = 0.006$), with a tendency for the hazelnut group to be lower than the control ($P = 0.057$). There was an additional tendency for total cholesterol in the chocolate group to be higher than the potato crisp group ($P = 0.099$).

From the ITT analysis, there was no evidence of a difference in changes in the recordings of subjective appetite sensations among the intervention groups (all $P \geq 0.384$, Table 5). Overall there was an increase in fullness ratings, decrease in hunger, desire to eat, prospective consumption, and preoccupation with thoughts of food ratings after consuming the study snacks. Similar results were obtained from the PP analysis.

4. Discussion

The regular consumption of nuts is recommended in many national dietary guidelines. One concern with this recommendation is that, because nuts are high in fat and thus energy-dense, frequent consumption may lead to weight gain. However, epidemiological studies report that nut consumers tend to be leaner than those who do not consume nuts and clinical trials show lower than predicted weight gain from the addition of nuts. The potential mechanisms can be summarised into two routes [34–37]. Firstly, decreased energy intake via increased satiety levels and food displacements [28, 30–33, 38–44], and energy

Table 1: Subjects' characteristics for the total, hazelnut group, chocolate group, control group, and potato crisp group at baseline.

Variable	Total $n = 118$ mean (SD)	Hazelnut group $n = 32$ mean (SD)	Chocolate group $n = 31$ mean (SD)	Control group $n = 29$ mean (SD)	Potato crisp group $n = 26$ mean (SD)
Age (years)	37.4 (14.0)	38.9 (14.3)	38.2 (13.9)	36.1 (15.2)	35.9 (12.8)
Height (cm)	171.0 (9.4)	170.8 (8.9)	171.0 (10.4)	171.7 (9.6)	170.3 (9.2)
Weight (kg)	69.5 (11.4)	72.0 (11.1)	69.2 (13.0)	67.3 (9.5)	69.5 (11.6)
BMI (kg/m^2)	23.8 (3.0)	24.6 (2.8)	23.6 (3.3)	22.9 (2.8)	23.9 (3.0)
Body fat (%)	26.9 (10.2)	28.1 (10.3)	26.7 (9.5)	25.8 (9.9)	26.9 (11.4)
Waist circumference (cm)	80.7 (9.4)	82.1 (8.5)	80.2 (9.6)	79.0 (8.8)	81.7 (11.1)
Women, no. (%)	63 (53%)	17 (53%)	16 (52%)	17 (59%)	13 (50%)

Table 2: Energy and nutrient intake at baseline and changes in intakes from baseline to week 12 for each group.

Variable	Baseline ($n = 113$) mean (SD)	Change in hazelnut ($n = 29$) mean (SE)	Change in chocolate ($n = 26$) mean (SE)	Change in control ($n = 25$) mean (SE)	Change in potato crisp ($n = 23$) mean (SE)	Overall P value*
Energy (kJ)^	8669.81 (1.35)	1.06 (1.06)	0.98 (1.05)	0.96 (1.05)	1.07 (1.04)	0.721
Total fat (g)	80.12 (27.95)	22.52 (5.61)	4.85 (7.40)	−2.87 (6.90)	3.74 (3.60)	0.077
% of TE	32.56 (6.69)	6.36 (1.00)	1.27 (1.32)	−0.10 (1.46)	−0.35 (1.28)	< 0.001
SAFA (g)	31.68 (12.27)	1.38 (2.39)	4.29 (3.85)	1.58 (2.85)	1.72 (1.93)	0.620
% of TE	12.83 (3.27)	−0.37 (0.65)	1.63 (0.90)	0.89 (0.66)	0.01 (0.75)	0.005
MUFA (g)^	24.72 (1.90)	1.67 (1.07)	1.01 (1.08)	0.95 (1.08)	1.09 (1.06)	< 0.001
% of TE	11.22 (3.14)	5.86 (0.54)	0.38 (0.61)	−0.34 (0.60)	−0.01 (0.60)	< 0.001
PUFA (g)^	10.98 (1.71)	1.23 (1.08)	0.90 (1.09)	0.89 (1.08)	1.09 (1.07)	0.024
% of TE^	4.68 (1.48)	1.16 (1.06)	0.90 (1.08)	0.92 (1.06)	1.03 (1.07)	0.012
Protein (g)^	82.22 (1.43)	1.04 (1.07)	0.93 (1.06)	1.01 (1.07)	1.04 (1.06)	0.482
% of TE	16.49 (3.44)	−0.41 (0.56)	−0.99 (0.70)	0.59 (0.80)	−0.26 (0.86)	0.537
CHO (g)^	252.16 (1.37)	0.93 (1.06)	0.97 (1.05)	0.96 (1.06)	1.08 (1.04)	0.509
% of TE^	46.54 (1.17)	0.89 (1.03)	0.98 (1.03)	1.00 (1.03)	1.01 (1.02)	0.002
Dietary fibre (g)^	24.69 (1.46)	0.98 (1.08)	0.90 (1.06)	1.02 (1.07)	0.95 (1.05)	0.276
Cholesterol (mg)	253.04 (127.85)	45.38 (26.53)	−4.88 (36.35)	−23.62 (31.81)	16.52 (45.73)	0.794
Sodium (mg)^	2497.37 (1.46)	1.00 (1.11)	1.00 (1.07)	1.12 (1.11)	1.11 (1.08)	0.672
Vitamin E (mg)^	9.13 (1.51)	1.60 (1.07)	0.90 (1.06)	0.94 (1.09)	1.30 (1.07)	< 0.001

TE: total energy; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; CHO: carbohydrate.
*Overall P-values adjust for baseline value, sex, baseline age, and baseline BMI.
^Geometric mean, accompanied by ratio of geometric mean.

malabsorption as a result of a reduction in bioaccessibility of the fat [32, 40, 47–50], or, secondly, increased energy expenditure via increased diet-induced thermogenesis or increased RMR due to the high unsaturated fat content [30, 41, 45, 46]. It is, therefore, plausible that these unique properties of nuts help maintain energy balance. However, there is limited data comparing the body composition of those regularly consuming nuts compared to the intake of other snacks of equal energy density. It was hypothesised that consuming nuts may provide some protection against weight gain compared to other energy-dense snacks based on the aforementioned mechanisms. However, the present study found no evidence that changes in body weight or composition differed between the control group and those offered regular consumption of any of the three energy-dense snacks. In addition, blood lipid and lipoprotein response to the different snack foods did not differ significantly. Nevertheless, the diet quality among the nut consumers was appreciably improved compared to the other groups.

It was somewhat unexpected that changes in body weight and composition were not different amongst the four groups. We hypothesised that the nut group would gain less weight than predicted and that body weight would be lower compared to the other snack groups. Our study shows that the reported satiety levels after consuming the study snacks were found to be similar across all intervention groups.

Table 3: Anthropometric measurements, resting metabolic rate and physical activity level at baseline and changes in these measurements from baseline to Week 12 for each group.

Variable	Baseline mean (SD)	Change in hazelnut mean (SE)	Change in chocolate mean (SE)	Change in control mean (SE)	Change in potato crisp mean (SE)	Overall P value*
Anthropometry	($n = 118$)	($n = 32$)	($n = 29$)	($n = 27$)	($n = 25$)	
Body weight^{Y}	69.55 (11.37)	0.83 (0.23)	0.59 (0.43)	0.46 (0.26)	0.50 (0.31)	0.655
BMI (kg/m^2)	23.76 (2.99)	0.28 (0.08)	0.21 (0.14)	0.14 (0.08)	0.15 (0.11)	0.725
Fat mass (kg)$^{\infty}$	18.77 (7.92)	−0.23 (0.28)	−0.42 (0.35)	−0.29 (0.27)	−0.53 (0.30)	0.889
Body fat (%)$^{\infty}$	26.89 (10.17)	−0.75 (0.32)	−1.23 (0.39)	−0.84 (0.33)	−1.09 (0.35)	0.713
Waist fat (%)$^{\infty}$	29.62 (11.08)	1.51 (0.52)	0.86 (0.64)	0.67 (0.54)	0.76 (0.69)	0.800
Waist circumference (cm)	80.74 (9.45)	2.13 (0.89)	1.30 (0.64)	1.36 (0.62)	−0.53 (0.71)	0.106
RMR	($n = 52$)	($n = 14$)	($n = 12$)	($n = 13$)	($n = 10$)	
RMR (kcal/day)	1489.29 (275.82)	−56.86 (58.40)	−69.92 (89.94)	−79.62 (38.39)	11.70 (69.37)	0.922
RQ^	0.86 (1.08)	1.01 (1.02)	1.03 (1.04)	1.00 (1.02)	1.01 (1.02)	0.876
PA level	($n = 114$)	($n = 29$)	($n = 29$)	($n = 28$)	($n = 22$)	
No. of steps/day	9215 (3691)	152 (515)	−260 (685)	−1205 (576)	190 (481)	0.217
Duration (mins)^	28 (16)	9 (10)	9 (10)	8 (10)	9 (10)	0.668

BMI: body mass index; RMR: resting metabolic rate; RQ: respiratory quotient; PA: physical activity; no.: number.
*Overall P-values adjust for baseline value, sex, baseline age, and baseline BMI.
^Geometric mean, accompanied by ratio of geometric mean.
^{Y}Body weight was measured with the use of bioelectrical impedance analyser.
$^{\infty}$Fat mass, percent body fat and waist fat were measured with the use of dual-energy X-ray absorptiometry.

Table 4: Blood lipid profile and changes in the biochemical indices from baseline to Week 12 for each group.

Variable	Baseline mean (SD)	Change in hazelnut mean (SE)	Change in chocolate mean (SE)	Change in control mean (SE)	Change in potato crisp mean (SE)	Overall P value*
	($n = 118$)	($n = 32$)	($n = 29$)	($n = 27$)	($n = 25$)	
TC (mmol/L)	4.79 (0.95)	−0.06 (0.07)	0.22 (0.11)	0.10 (0.07)	0.05 (0.08)	0.211
LDL-C (mmol/L)	2.94 (0.84)	−0.09 (0.06)	0.13 (0.09)	0.09 (0.07)	−0.06 (0.07)	0.231
HDL-C (mmol/L)^	1.32 (1.30)	1.02 (1.02)	1.04 (1.03)	1.00 (1.02)	1.04 (1.02)	0.385
TC:HDL-C ratio^	3.57 (1.34)	0.97 (1.02)	1.01 (1.03)	1.02 (1.02)	0.97 (1.02)	0.136
TAG (mmol/L)^	0.98 (1.48)	0.99 (1.04)	1.05 (1.04)	1.03 (1.05)	1.04 (1.05)	0.600

TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TAG: triglyceride.
*Overall P-values adjust for baseline value, sex, baseline age, and baseline BMI.
^Geometric mean, accompanied by ratio of geometric mean.

Table 5: Changes in appetite indices before and after consuming study snacks during the intervention.

Variable	Before consumption mean (SD)	Change in hazelnut mean (SE)	Change in chocolate mean (SE)	Change in potato crisp mean (SE)	Overall P value*
	($n = 89$)	($n = 32$)	($n = 31$)	($n = 26$)	
Hunger (mm)	56.33 (19.66)	−25.67 (5.16)	−13.37 (5.10)	−19.10 (3.34)	0.384
Desire to eat (mm)	59.39 (20.64)	−25.33 (4.68)	−22.05 (4.72)	−20.66 (3.10)	0.874
Prospective consumption (mm)	50.64 (17.89)	−17.81 (3.76)	−12.31 (3.97)	−12.50 (2.52)	0.921
Fullness (mm)	41.96 (17.68)	17.99 (4.91)	10.59 (4.20)	10.74 (3.33)	0.631
Preoccupation with thoughts of food (mm)	51.80 (19.20)	−22.49 (4.25)	−15.57 (4.58)	−18.61 (3.29)	0.760

* Overall P values adjust for baseline value, sex, baseline age, and baseline BMI.

It appears that participants tended to compensate to a similar extent irrespective of the snack they were provided with, where 61% of the extra energy from the study snacks was displaced by reductions in other foods. This finding is in line with a recent review, which reports that the dietary compensation accounts for 55–75% of the energy from nuts [35]. No change in physical activity level was observed in the intervention groups throughout the study, and the predicted weight gain given the additional calories provided by the snacks was 2.8 kg. However, on average actual weight gain was only 0.64 kg, which equated to 23% of that predicted and did not differ between the snack groups and the control group. This compensatory response has been seen in other studies where nuts have been provided as additional foods and the observed weight gain ranged from 0 to 28% of the predicted weight gain [30–33].

One recent study comparing the effects of the consumption of almonds and cereal bars with the control group on body weight reported similar results to the current study [57]. The addition of either almonds or cereal bars did not result in a significant increase in body weight from baseline indicating a compensatory response for both foods. However, this study had a small sample size ($n = 45$) and the energy provided from the almonds (1430 kJ) was significantly higher than that from the cereal bars (950 kJ). This discrepancy in energy makes comparison difficult but would suggest that compensation was greater in the almond group.

As with our study, research showing some degree of compensation has been conducted in non-obese populations [30, 32, 41, 45]. It is speculated that obese individuals compensate differently than their lean counterparts [41, 58, 59]. Therefore it would be interesting to repeat this study in an obese population. We note that employing a PP analysis (using 70% compliance as an indicator of adherence to the dietary advice to eat the snacks) showed that a higher baseline BMI was associated with a lower waist circumference at follow-up in the nut group compared to the control group ($P = 0.005$) and to a lesser extent in the potato crisp group compared to the control group ($P = 0.034$). Given this, dietary compensation in response to nuts and possibly potato crisps may be more pronounced in overweight individuals compared to those who are of normal weight. However, this result was only marginally statistically significant and should be interpreted with caution unless it can be replicated in other studies.

One purported mechanism whereby nuts may provide a beneficial effect on energy regulation is via an increase in energy expenditure. A previous review suggested this may account for approximately 10% of the energy contributed by nuts [36]. In the present study, RMR was measured in a subsample ($n = 52$) and there was no evidence of a difference in RMR between any of the snack groups. Previous research is somewhat mixed. Three studies showed that there was a significant increase in RMR following 2–19 weeks of peanut consumption in lean [30, 45] and overweight participants [41]. In contrast, daily almond supplementation for ten weeks [32] and six months [31] failed to show any changes in RMR or respiratory quotient. It is unclear whether the increment in RMR is specific to peanut consumption only. Given the inconsistencies among studies, this is an area requiring further research.

Another explanation provided by some researchers for the less than predicted weight gain when consuming nuts involves the reduction in the bioaccessibility of the lipid in nuts. A recent review estimates 10–15% of the energy contributed by nuts is offset by faecal loss [36]. Similar results were obtained in the first and only trial specifically designed to investigate this effect, whereby 5% of dietary fat was excreted in the whole peanut group (70 g/d), which could potentially offset around 9-10% of the energy provided by nuts [50]. However, recent studies reported that there were no statistically significant differences in body weight after consuming three different forms of hazelnuts (30 g/d) [25] and five different forms of peanuts (56 g/d) [22] for four weeks each, suggesting that the bioaccessibility of lipids was similar for all forms of nuts. Thus, the potential difference in bioaccessibility for the amount of nuts (42 g) provided in the present study may be too small to significantly influence body weight.

Changes in blood lipid and lipoprotein concentrations did not differ between the four groups. Most previous literature suggests that regular nut consumption in hypercholesterolemic individuals results in significant reductions in TC and LDL-C [21, 25, 60–64] with some showing increases in HDL-C [25, 62, 63] whilst others do not [21, 60, 61, 64]. It is likely that we did not observe an improvement in blood lipoproteins with regular nut consumption due to the low baseline TC (4.8 mmol/L) and LDL-C (2.9 mmol/L) and relatively high HDL-C (1.3 mmol/L) concentrations of this study population. Recent studies have shown that the magnitude in the reduction in TC and LDL-C following regular nut consumption was dependent on the baseline concentrations [11, 22, 65, 66]. Using PP analysis, plasma TC was significantly lower in the hazelnut group compared to the chocolate group ($P = 0.006$), with a tendency to be lower when compared to the control ($P = 0.057$). In addition, there was a tendency for the chocolate group to have higher TC than the potato crisp group ($P = 0.099$). This would indicate that among those actually following the advice to consume the different snacks, nuts show a more favourable effect on blood lipids. The cholesterol-lowering properties of nuts are largely due to their unsaturated fat content, but also due to other bioactive compounds such as phytosterols [1–5].

One important finding of this study is that the regular consumption of nuts improved diet quality compared to the consumption of other energy-dense snacks. This was particularly evident for dietary fat. The percent of energy derived from SAFA was significantly lower while the energy from MUFA and PUFA was significantly raised in the nut group compared to all the other groups. In addition, the intake of vitamin E during the intervention was significantly raised in the nut group compared to the other groups. These dietary changes support the findings of other studies, which have observed improvements in diet quality with the simple addition of nuts without any further healthy eating advice [2, 19, 38, 67]. Such changes would be expected to reduce the risk of chronic disease, in particular, CVD. A recent study has shown that substituting one unhealthy snack such

as crisps and chocolate bars with one healthy snack such as unsalted nuts or seeds per day has a positive impact on nutrient density and could prevent approximately 6000 cardiovascular deaths every year in the UK [68].

5. Conclusions

Although nuts provided no additional benefits compared to isocaloric quantities of other energy-dense snacks in terms of body weight and composition, blood lipids and lipoproteins in this group of non-obese, normocholesterolaemic individuals, diet quality was substantially enhanced in the nut group. This study supports the findings of other studies, which suggest that nuts can be incorporated into the diet without the risk of adverse weight gain and can improve diet quality.

Authors' Contribution

The authors' responsibilities were as follows S. L. Tey: study coordinator, designing the study, collecting, entering and analyzing the data, disseminating findings, and preparing the paper; R. Brown: study design, supervision of data collecting, data analysis, preparing the paper; A. Gray: assistance with study design, statistical analysis, editing of the paper; A. Chisholm: study design, supervision of data collection, editing of the paper; C. Delahunty: assistance with study design, editing of the paper.

Conflict of Interests

None of the authors had any personal or financial conflict of interests.

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Research Article

The Effect of Dietary Fish Oil in addition to Lifestyle Counselling on Lipid Oxidation and Body Composition in Slightly Overweight Teenage Boys

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Objective. *n*-3 long-chain polyunsaturated fatty acids (LCPUFAs) have shown potential to increase lipid oxidation and prevent obesity. *Subjects*. Seventy-eight boys aged 13–15 y with whole-body fat% of 30 ± 9% were randomly assigned to consume bread with fish oil (FO) (1.5 g *n*-3 LCPUFA/d) or vegetable oil for 16 weeks. All boys were counselled to improve diet and exercise habits. *Results*. Lifestyle counselling resulted in decreased sugar intake but did not change the physical activity level. Whole-body fat% decreased 0.7 ± 2.5% and 0.6 ± 2.2%, resting metabolic rate after the intervention was 7150 ± 1134 kJ/d versus 7150 ± 1042 kJ/d, and the respiratory quotient was 0.89 ± 0.05 versus 0.88 ± 0.05, in the FO and control group, respectively. No group differences were significant. *Conclusion*. FO-supplementation to slightly overweight teenage boys did not result in beneficial effects on RMR, lipid oxidation, or body composition.

1. Introduction

Results from rodent studies have showed that dietary *n*-3 long-chain polyunsaturated fatty acids (LCPUFAs) increase fat oxidation and prevent high fat and sugar-induced obesity [1, 2]. Human studies have indicated similar effects of fish oil. A study by Couet et al. with healthy adults [3] and a study by Kabir et al. with diabetic women [4] both showed reduced body fat mass after fish oil interventions that lasted 3 weeks and 2 month, respectively. Hill et al., however, found no effect of fish oil on fat mass in a group of overweight subjects [5], but they and others [5–7] found that inclusion of fish oil in a hypocaloric diet increased weight loss.

Nakatani et al. found that fish oil supplementation increased the expression of uncoupling protein 2 (UCP-2) in rodent hepatocytes, which could explain the observed protection from weight gain [8]. Tsuboyama-Kasaoka et al. later used a UCP-2 knock out model to show that dietary fish oil prevents weight gain in rodents even in the absence of UCP-2, suggesting more than one pathway in mediating the effects of fish oil on weight control [9]. *n*-3 LCPUFAs have been shown to increase adiponectin concentrations in rodents and obese humans [10], and as adiponectin has been shown to increase fatty acid oxidation [11], this could also be a mechanism for the alleged antiadiposity effects of *n*-3 LCPUFAs.

Considering the association between childhood obesity and later lifestyle diseases [12], early prevention of overweight and its sequelae is important. Studies on how children and adolescents respond to dietary interventions are few, and since it is problematic to extrapolate the results from interventions with adult subjects, data from intervention-studies in children are much needed. Therefore, we have investigated if *n*-3 LCPUFAs increase lipid oxidation, and improve body

composition in slightly overweight teenage boys during their pubertal growth spurt.

2. Materials and Methods

2.1. Study Population. The study protocol was approved by The Committees on Biomedical Research Ethics of the Capitol Region of Denmark (H-A-2007-0055) and registered in the Clinical Trials database (http://www.clinicaltrials.gov/no.NCT00929552). The recruitment and inclusion criteria have been previously described [13]. Our inclusion criteria were body mass for height above the 90th percentile and the average BMI of the included boys was comparable to 25 kg/m^2 for adults [14].

2.2. Study Design and Intervention. The participants were randomly assigned to dietary supplementation with fish oil (FO) or a control oil blend for a 16-week intervention. The oils were included in bread, and the participants were asked to consume two pieces of rye bread and one wheat roll pr day. The intervention bread was produced for this study by Kohberg A/S (Bolderslev, Denmark). The fish oil was microencapsulated tuna oil from NuMega (Clover Corporation, Sydney, NSW, Australia), and the control oil was a 6 : 1 : 1 mix of palm shortening, soy oil, and rapeseed oil. We designed the study to provide the FO-group with 1.5 g/d LCPUFAs, which was the dose shown by Couet et al. to increase fat oxidation in adults [3]. However, random sampling of the bread revealed that the fat content was lower, providing the FO-group 4.9 (4.1–5.4) g/d (0.2 g/d eicosapentaenoic acid (EPA) + 0.9 g/d docosahexaenoic acid (DHA)) and 4.2 (3.0–5.0) g/d in the control group. (See full fatty acid composition in the bread in [13].) Both subjects and investigators were blinded to the treatment. Biomedical measurements were taken at baseline and after 16 weeks of intervention.

2.3. Life Style Intervention. The participants in this study were also subjected to a life style intervention, as we tried to educate and influence the boys towards healthier diets and a higher level of physical activity. Subjects in both groups received counselling by a dietician at the first examination visit and were further encouraged and supported in making healthy choices (regarding diet and exercise) every two weeks when they came in to pick up new supplies of bread. The initial counselling was based on a four-day food diary, including three weekdays and one weekend day, and results from a seven-day step recording with a pedometer (Yamax SW-200 Tokyo, Japan). The details about these recordings were published elsewhere [13].

2.4. Anthropometry, Body Composition, and Indirect Calorimetry. The boys were measured in the morning after an overnight fast. Height was measured to nearest cm, and weight to nearest 100 g on an electronic scale (Frederiksberg Vægtfabrik, Frederiksberg, Denmark). A measuring tape was used for waist and hip circumferences. Waist circumference was measured right below the ribs, and hip circumference was measured at the fullest part of the hip. Body composition was assessed by DXA scanning (Prodigy, General Electric Company, Madison, WI, USA). The scanner was calibrated each morning before use. Analysis of all scans was performed using enCore 2008 software (General Electric Company).

Resting metabolic rate (RMR) and respiratory quotient (RQ) was measured by indirect calorimetry in a ventilated hood (Viasys Healthcare, Hoechberg, Germany). Metabolic testing was performed after 15 min supine of rest, and lasted for 25 min, of which the first 5 min were omitted from the later calculations. The calorimeters used room air for ventilation and for comparison with exhaust gas, and the analyzers were therefore calibrated to room air before each use and every 5 min during testing. Analyzers were furthermore calibrated to a standard gas sample (Air Liquide Danmark, Taastrup, Denmark) every day and tested every week with controlled alcohol burns. All readings were subsequently adjusted according to the results from the alcohol burn test.

2.5. Blood Sampling. Blood samples for the analysis of red blood cell (RBC) fatty acid composition, plasma adipokines and growth markers were drawn from the antecubital vein. The blood for RBC fatty acid analysis was collected in lithium-heparin-coated tubes, EDTA-coated tubes were used for analysis of leptin and adiponectin, and uncoated tubes for testosterone, insulin-like growth factor-1 (IGF-1), and insulin-like growth factor binding protein-3 (IGFBP-3). Blood samples were immediately placed on ice before centrifugation at 4°C with 2500 g for 10 min to isolate plasma, which was stored at −80°C until later analysis. RBCs were washed three times in isotonic saline and finally reconstituted 1 : 1 with isotonic saline with 0.005% butylated hydroxytoluene, and stored under nitrogen at −80°C.

2.6. Biochemical Analyses. Adiponectin was analyzed with a Milipore RIA kit (cat no. HADP-61HK, Billerica, MA, USA), and leptin was analyzed with a Human leptin RIA kit (cat no. HL-81K, Linco, St Charles, MO, USA). Interassay CV% was <9% for both adiponectin and leptin, and quality controls in both assays fell within expected limits. Testosterone, IGF-1, and IGFBP-3 concentrations were measured using an Immulite 1000 and kits from Siemens Medical Solutions Diagnostics (Los Angeles, CA, US). All CV% on the Immulite was <9%. The fatty acid composition in RBC was determined as previously described [14].

2.7. Statistical Analysis. Values are reported as mean ± SD if the data follow a normal distribution and as geometric mean (95% CI) if the data are not normally distributed. Potential effects of the intervention were analyzed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA) with general linear models to adjust for the effect of covariates (ANCOVA). Baseline values of the outcome measure in question were included as covariate in all tests. Change in testosterone was furthermore included as covariate in the group comparisons of whole body fat percentage (fat-%), trunk fat-%, fat mass, hip and waist circumferences, and waist/hip ratio. The ANCOVA residuals were plotted to assess the need for transformation of the dependent variable. Within group changes from baseline to followup were assessed by paired t-tests. The

study was based on power calculations to make it possible to show significant differences in the magnitude of 0.4 SD with $\alpha = 0.05$ and $\beta = 0.20$ in a 2-sided test.

3. Results

The intervention was completed by 78 of the 87 teenage boys who originally entered the study. Six subjects dropped out from the FO-group and 3 from the control group. Reasons for withdrawal were: disliking the taste of the bread (5 from the FO-group and 2 from the control group), and insufficient structure in meal habits, which prevented them from eating the daily ration of bread (1 from each group). When asked after completion, 48% of the boys in the control group and 73% of the boys in the FO-group were able to guess which diet they had received. Compliance, estimated by asking the boys how much of the bread they had eaten during the intervention, was about 90% (70–100%) in both groups.

Baseline measurements showed that the boys were successfully randomized with respect to anthropometric measures and habitual diet (Table 1). During the 16-week intervention, the boys grew in height (1.8 ± 1.2 cm) and weight (2.0 ± 2.6 kg), and there was a significant increase in plasma testosterone of 0.6 ± 1.0 μg/L. These developmental changes were similar for both groups. The lifestyle counselling resulted in a significant reduction in sugar intake and an increase in fibre intake for both groups. The boys in the FO-group significantly reduced fat intake compared with baseline, but the change was not different from that in the control group. No further differences were observed in the energy intake, the overall macronutrient composition of the diet, or the level of physical activity (steps per day).

The two groups had similar RBC fatty acid composition at baseline, but the RBC fatty acid composition at the end of the intervention period reflected that of the intervention oils (See [13]). In summary, DHA and EPA had increased by 78 and 100%, respectively, (DHA from 3.9 to 6.7 and EPA from 0.6 to 1.2% of total fatty acids) in the FO-group and by 14 and 20%, respectively, in the control group.

Whole body fat-% was significantly reduced in all boys after the intervention ($P < 0.02$), but no change was seen for the trunk fat-%. There was, however, no effect of fish oil on body composition compared with the control group (Table 2). No group differences were seen for hip and waist circumference, or the waist to hip ratio and RMR and RQ were also unaffected by the treatments.

Leptin tended to decrease for the whole group ($P = 0.06$), and the change was best explained by the change in weight ($r^2 = 0.138$, $P < 0.001$), but the correlation with changes in fat mass was also significant ($r^2 = 0.056$, $P < 0.04$). Changes in adiponectin concentration correlated directly with the change in fat mass ($r^2 = 0.061$, $P < 0.03$). No group differences were found (Table 3), but there was a weak, but significant, inverse correlation between the change in the RBC-content of DHA+EPA and plasma concentrations of adiponectin at the end of the intervention adjusted for baseline values and changes in fat mass (Figure 1).

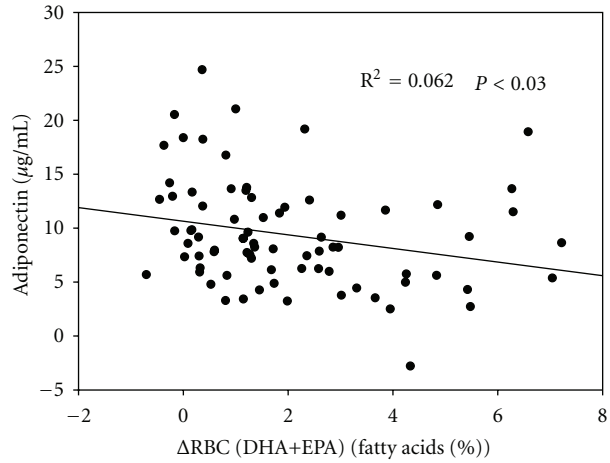

Figure 1: The correlation between the change in DHA+EPA content of RBC and plasma adiponectin at the end of the intervention adjusted for baseline values and changes in fat mass.

4. Discussion

In the present study, we did not find an effect of fish oil supplementation on metabolic rate and body composition in slightly overweight boys during puberty growth, nor did our results indicate any effect on fat oxidation as RQ was similar in the groups. These results on adolescent boys are in contradiction to the results from Couet et al., who found reduced RQ (suggesting increased lipid oxidation), and reduced fat mass in adults after only three weeks of fish oil intervention with doses only slightly higher than what we used [3], but in line with a recently published study from DeFina et al. who studied the effect of *n*-3 PUFA intake in combination with diet and excercise. In present study, the oil was a DHA-rich tuna oil, which resulted in a higher average DHA intake than what was attained by Couet and colleagues (0.9 versus 0.7 g/d). Thus, the lack of effect in present study cannot be explained by a too low DHA intake. It is also noteworthy that DeFina et al. used an EPA-rich oil at a higher level of intake than in our study (3 g/d of EPA+DHA) without observing effect on weight, metabolic rate, or RQ beyond those achieved with diet and exercise alone [16]. The weight reducing effect of fish oil has been consistent in rodent studies. However, most of the rodent studies have used high doses of fish oil. Nakatani et al. investigated the effects of different fish oil doses in female C57BL/6J mice and found that less than 40 E% fish oil was insufficient in reducing weight-gain [17]. Setting up a similar experiment to find the minimum dose for an effect on metabolism in humans would be highly interesting and may prove that the dose used in this study was too low for an effect to be detected. However, if the results parallel those in mice, that much fish oil is unrealistic in a human context, and using fish oil in weight management could be futile.

Because childhood obesity has long-lasting consequences, it is important to investigate potential health effects of commonly used foodstuff in children and adolescents. It is difficult to compare our results with studies in adults, as adolescents are not, metabolically, in a steady state, and

Table 1: Anthropometrics, habitual diet, and exercise[1].

	Baseline		16 weeks		
	Control $n = 40$	Fish oil $n = 38$	Control $n = 40$	Fish oil $n = 38$	P^2
Anthropometrics					
Age (yrs)	14.3 ± 0.6	14.3 ± 0.7	14.6 ± 0.6	14.6 ± 0.7	
Weight (kg)	66.6 ± 9.9	69.8 ± 14.3	68.7 ± 10.3*[3]	71.7 ± 14.4*	0.73
Height (cm)	169 ± 9	169 ± 11	171 ± 9*	170 ± 11*	0.45
BMI (kg/m^2)[4]	23.1 (22.5–23.7)	24.1 (23.1–25.2)	23.3 (22.7–23.9)	24.3 (23.3–25.4)*	0.81
Testosterone (μg/L)	3.0 ± 1.7	3.5 ± 1.7	3.6 ± 2.0*	4.0 ± 1.8*	0.48
IGF-1	360 ± 101	333 ± 93	352 ± 84	363 ± 86	0.19
IGFBP-3	5.5 ± 0.9	5.4 ± 0.6	5.4 ± 0.8	5.4 ± 0.6	0.08
Habitual diet					
Energy intake (kJ)	9292 ± 2523	8716 ± 2811	8524 ± 2258	8043 ± 2631	0.89
Carbohydrates (E%)	50.7 ± 5.1	51.3 ± 5.0	52.0 ± 6.7	52.7 ± 5.8	0.90
Protein (E%)	15.3 ± 2.9	15.5 ± 2.7	15.1 ± 2.9	16.2 ± 2.6	0.22
Fat (E%)[5]	33.8 ± 4.7	33.3 ± 5.3	32.9 ± 6.4	31.0 ± 5.7*	0.19
SFA (E%)	14.0 ± 2.4	14.0 ± 2.8	13.7 ± 3.3	12.8 ± 3.0*	0.21
MUFA (E%)	12.2 ± 2.2	11.6 ± 2.2	11.7 ± 2.6	10.9 ± 2.3*	0.56
PUFA (E%)	5.2 ± 1.2	5.2 ± 1.0	5.1 ± 1.3	4.9 ± 1.1	0.43
n-3 PUFA (E%)	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.51
Added sugar (E%)	10.8 ± 5.5	9.0 ± 5.0	8.6 ± 4.9*	6.7 ± 4.7*	0.98
Fibre (g/MJ)	1.9 ± 0.5	2.1 ± 0.6	2.2 ± 0.5*	2.5 ± 0.7*	0.16
Physical activity					
Steps pr day	10 950 ± 3842	10 711 ± 4253	11 901 ± 4234	11 134 ± 5395	0.43

[1] Data are presented as mean ± SD or geometric mean (95% CI).
[2] P values for baseline adjusted group differences at 16 weeks.
[3] * Indicates that the within-group-change from baseline to followup is significant ($P < 0.05$), assessed by paired t-tests.
[4] The usual cut-offs for BMI (>25 kg/m^2 = overweight) is not applicable to children. The corresponding value for 13–15-year-old boys lies between 21.9 and 23.6 kg/m^2 [15].
[5] This table compares habitual fat intake only, that is not including the contribution of energy and fat from supplemental oils.

Table 2: Metabolic rate and body composition[1].

	Baseline		16 weeks		
	Control $n = 40$	Fish oil $n = 38$	Control $n = 40$	Fish oil $n = 38$	P^2
RMR (kJ/d)	6974 ± 913	7032 ± 1067	7150 ± 1134	7150 ± 1042	0.70[3]
RQ	0.88 ± 0.06	0.89 ± 0.07	0.89 ± 0.05	0.88 ± 0.05	0.62[3]
Whole body fat (%)	28.5 ± 8.8	31.1 ± 8.1	27.8 ± 8.8	30.5 ± 7.7	0.67
Trunk fat (%)	31.5 ± 9.5	31.7 ± 8.8	31.4 ± 9.2	31.1 ± 8.8	0.45
Fat mass (kg)	18.9 ± 6.2	21.9 ± 8.1	18.9 ± 6.1	22.1 ± 7.9	0.63
Hip circumference (cm)	94.9 ± 5.6	96.5 ± 8.3	95.9 ± 6.2	97.4 ± 8.7	0.94
Waist circumference (cm)	81.0 ± 6.4	83.1 ± 10.7	81.5 ± 6.0	83.0 ± 9.7	0.83
Waist/Hip	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.88

[1] Data are presented as mean ± SD.
[2] P values describe group differences at 16 weeks adjusted for baseline and Δ testosterone.
[3] The analysis of RMR and RQ was only adjusted for baseline values.

Table 3: Plasma concentrations of adipokines[1].

	Baseline		16 weeks		
	Control $n = 40$	Fish oil $n = 38$	Control $n = 40$	Fish oil $n = 38$	P^2
Leptin (ng/mL)	7.6 (5.8–9.9)	7.4 (5.7–9.7)	7.2 (5.6–9.2)	6.9 (6.8–9.9)	0.99
Adiponectin (μg/mL)	8.7 (7.3–10.4)	8.0 (6.7–9.6)	7.9 (6.4–9.5)	8.2 (6.8–10.0)	0.49

[1] Data are presented as geometric mean (95% CI).
[2] P values describe group differences at 16 weeks adjusted for baseline and Δ fat mass.

changes in body composition in particular are controlled by mechanisms other than those seen in adults. Compared with the study of Couet et al. they had the advantage of a cross over design [3], which we were unable to match considering the unsteady state of our participants. The unsteady state might have increased the variation in the measurements due to differences in the pubertal development, which was a factor we tried adjusting for by including delta testosterone levels as covariate in most analyses. We, furthermore, studied a much larger group for a longer duration of time, which added to the quality of our study. It should be noted that the *n*-3 LCPUFAs in this study were included in bread and not given as pure oil. In one study the metabolic response to *n*-3 LCPUFAs was different when given as a salmon enriched diet compared with capsule supplementation, suggesting a possible matrix effect [18]. However, another study showed similar incorporation of *n*-3 LCPUFAs from capsules and fatty fish [19]. Unfortunately, we did not reach our intended dose of fish oil (1.5 g/d as in Couet et al. [3]), and we saw a slightly higher fat content of the bread in the FO-group, which may have counteracted a potential beneficial effect of the fish oil supplement. The difference in total fat intake caused by this might have been counter balanced somewhat as we also saw a significant reduction in dietary fat intake between baseline and follow up in the FO-group only.

In our design, we tried to influence the participants to change lifestyle, which was included primarily for ethical reasons. The lifestyle intervention had no impact on the physical activity level, but sugar consumption had decreased for both groups, indicating that participation in the study had increased the boys' awareness of candy and soft drink usage. Fiber consumption was also increased during the intervention, but this is likely attributed to the fiber-rich bread provided by us, and not a lasting change in eating habits. The limited success of the lifestyle intervention shows that making a change in the most obvious bad habits is easier than making a commitment to increasing the physical activity level in spite of continuous encouragement to do so. The lifestyle intervention could have increased variation in the outcome measures or might have been a design advantage as previous studies have indicated that the effects of dietary fish oil was augmented when combined with increased exercise or reduced caloric intake [5–7]. There is also the risk that decreased sugar and increased fiber consumption had an impact on the outcome measures overriding that of the fish oil intervention.

Circulating leptin was reduced in a study with rats fed fish oil [20], but a human intervention study by Mori et al. showed no reduction after fish oil supplementation [21]. This agrees with our results as no group differences were found after 16 weeks of fish oil consumption. Leptin is a marker of adiposity [22], and plasma concentration of leptin has been shown to correlate with fat mass [22]. In our data, changes in leptin concentration correlated more strongly with changes in total body weight than with the changes in fat mass. *n*-3 LCPUFAs has been shown to increase adiponectin concentrations in rodents and obese humans [10]. In the present study, fish oil supplementation did not lead to an increase in adiponectin concentration, rather on the contrary, as we found a weak yet significant inverse association between the change in the RBC-content of EPA+DHA and changes in adiponectin concentration. Plasma concentrations of adiponectin have been shown to correlate inversely with fat mass [23], but in the present study changes in adiponectin concentration correlated directly with changes in fat mass. In a previous study in pubertal boys and girls, Schoppen et al. also did not find a significant correlation between plasma adiponectin and fat mass or fat%. However, they did find higher adiponectin levels in normal weight girls compared with overweight or obese girls, but this relationship was not seen for boys [24]. Thus, difference between our results and those from previous studies could be due to the male puberty, which was shown by Andersen et al. to affect adiponectin levels [25]. Girls experience metabolic and hormonal changes during adolescence that are very different from the male developments and might therefore respond differently to a similar treatment. The boys in this study were healthy and only mildly overweight, and it is not known if obese boys with more pronounced metabolic complications would respond differently to a fish oil intervention.

In conclusion, a 16-week fish oil intervention did not influence RMR or body composition in slightly overweight boys during their puberty growth spurt compared with the effect of a vegetable control oil. The antiobesity effect of *n*-3 LCPUFAs in adults has not been consistent, and more work is needed to investigate differences in the response to *n*-3 LCPUFAs in adolescents compared with adults.

Conflict of Interests

The authors declare no conflict of interests.

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