Quadriceps Muscle Fatigue in Trained and Untrained Boys

Authors M. Callewaert[1], J. Boone[1,2], B. Celie[1], D. De Clercq[1], J. Bourgois[1,2]

Affiliations
[1] Department of Movement and Sport Sciences, Ghent University, Ghent, Belgium
[2] Centre of Sports Medicine, Ghent University Hospital, Ghent, Belgium

Key words
- NIRS
- EMG
- reoxygenation
- isometric
- knee-extension

Abstract

This study aimed to explore muscle oxygen extraction and muscle activation pattern during bilateral intermittent submaximal isometric knee-extensions by combining Near-infrared Spectroscopy (NIRS) and Electromyography (EMG) measurements from the M. Vastus Lateralis. A group of highly specifically trained boys (youth sailors) (n=10) and untrained matched controls (n=10) performed 12 bouts of 90s bilateral submaximal (30–40% MVC) isometric knee-extension interspersed with 6s recovery-periods. Patterns of deoxygenated haemoglobin and myoglobin concentration (Deoxy[Hb+Mb]) were observed during each bout and the entire protocol. Reoxygenation Index (RI) was assessed for each recovery period as the amplitude of Deoxy[Hb+Mb]-decrease relative to amplitude of Deoxy[Hb+Mb]-increase during each bout. Root Mean Square (RMS) and Mean Power Frequency (MPF) were calculated for each bout as an average of the final 60s. Deoxy[Hb+Mb], RI, RMS and MPF were analyzed by repeated-measures ANOVA. Results indicated significantly higher Deoxy[Hb+Mb]-increase and lower RI in specifically trained boys compared to untrained controls. These differences are presumably related to the differences in EMG-measurements which demonstrated lower RMS-increase and MPF-decrease for trained compared to untrained boys. In conclusion, specifically trained boys indicate delayed onset of muscle fatigue in comparison to untrained controls, which might be associated with the different pattern of muscle O_2-extraction or muscle activation pattern (i.e., a more accurate recruitment of slow-twitch fibres).

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Correspondence
Prof. Jan Bourgois
Department of Movement and Sport Sciences
Ghent University
Watersportlaan 2
9000 Ghent
Belgium
Tel.: +32 92 64 6297
Fax: +32 92 64 6484
Jan.Bourgois@UGent.be

Introduction

Localized muscle fatigue during bilateral intermittent submaximal isometric knee-extension exercise is a complex phenomenon, and could be defined as an inability of the muscle (group) to maintain the required or expected torque [29]. Fatigue mechanisms during isometric exercise can be influenced by numerous factors such as: exercise intensity [3,9,10], muscle length [3,21], number of extremities involved [24], number of muscles involved [20], age [13,16], gender [22] and physical fitness level [7,14,23,29,32,33].
Isometric contraction is characterized by restricted local blood flow [10,27,34], since the intramuscular pressure increases even at very low isometric contraction intensities [9]. Therefore, the delivery of oxygen and wash out of muscle metabolites may become limited, which affects the duration of such a contraction [33]. However, literature suggests that when fatiguing isometric contractions are performed at low levels (≤30% maximal voluntary contraction (MVC)), fatigue measured by electromyographic changes, is mainly due to neural changes, whereas at higher contraction levels (>45% MVC) mainly metabolic factors contribute to fatigue [9,16,30].
Fatigue mechanisms have already been investigated by means of Near-infrared Spectroscopy (NIRS) or electromyography (EMG). NIRS-measured deoxygenation during isometric knee-extension is influenced by both capillary blood flow and muscle oxygen uptake [12]. However it is suggested that deoxygenation reflects capillary oxygen extraction rather than oxygen supply [2,5,12,18]. For EMG-measurements during sustained submaximal isometric knee-extension, a significant increase in Root Mean Square (RMS) and decrease in Mean Power Frequency (MPF) [9,27,36] have been demonstrated, reflecting respectively an increase in additional motor unit recruitment and a decrease in the frequency of the EMG-spectrum, indicating muscle fatigue [6].
Recently, there has been an increased interest in

Table 1 Mean (±SD) of age, height, weight, body fat percentage, practice hours/week and sailing experience in specifically trained and untrained boys. (no significant differences were found).

GROUP	Age (year)	Height (cm)	Weight (kg)	Body fat % (%)	Practice (hours/week)	Experience (years)
trained boys (n=10)	14.0±1.4	157.6±11.4	44.4±7.9	13.2±3.7	9.6±1.6	5.5±1.8
untrained boys (n=10)	13.8±1.3	162.2±6.6	47.4±8.2	11.8±4.5	–	–

combining NIRS and EMG to investigate muscle fatigue mechanisms during submaximal isometric knee-extension exercise in adults [8,11,36]. These research groups report a correlation between NIRS- and EMG-measurements suggesting a relationship between oxygen extraction and fatigue [11,36]. However, only a few studies have been conducted in a paediatric population [27] or in adult populations with specifically trained and untrained subjects [14,29].

By combining NIRS- and EMG-measurements, the purpose of this study was to get an insight into the performance limiting factors of bilateral intermittent submaximal isometric knee-extension exercise at the muscle level by examining the time-course of deoxygenation, reoxygenation and myoelectrical manifestations (i.e., RMS and MPF) of the M. Vastus Lateralis in young, highly specifically trained (sailors) and untrained boys. We hypothesized that (1) specifically trained boys will develop less muscle fatigue and higher deoxygenation and reoxygenation compared to untrained boys and that (2) the appearance of fatigue will be associated with changes in deoxygenation and reoxygenation.

Methods

Subjects

20 male subjects participated in this research: 10 specifically trained boys (international sailing level), highly trained in intermittent submaximal isometric knee-extension exercise, and 10 untrained matched controls, performing no specific organised physical activities. Specifically trained boys and controls were matched for age (±1 year), height (±5 cm) and weight (±5 kg). All subjects and their parents signed informed consent. The study was carried out in accordance with [15] and approved by the Human Research Ethics Committee of Ghent University Hospital. The subjects were instructed to perform no intensive exercise 48 h before this test. The subjects' anthropometrics, practice hours/week and sailing experience are displayed in **Table 1**.

Study design

Each subject performed 12 bouts of 90 s submaximal (30–40% MVC) bilateral knee-extension exercise (120° knee and hip angle) interspersed with 6 s recovery periods. Protocol settings were based on unpublished preliminary race analysis of the temporal pattern, contraction intensity and joint angles during competitive youth sailing (i.e., regattas).

Procedure

The subjects' height (anthropometer GPM, DKSH Switzerland) and weight (Electronic SECA, 815 Elegantia) were determined. Body fat percentage was measured by means of a skinfold caliper (Harpenden) and calculated by the method of Parizková [28].

A 5-min standard warm-up on a cycle ergometer (Monark) and a short familiarization period of knee-extension exercise on an isokinetic dynamometer (Biodex, system 2, USA) were performed 15 min prior to the actual protocol. The isokinetic dynamometer was set at 120° knee and hip angle and subjects performed three 5 s maximal voluntary bilateral isometric knee-extension contractions, interspersed with 90 s of rest. The highest torque developed in the 3 attempts was regarded as the maximal voluntary contraction torque (MVC). Subjects were not fastened with straps to the isokinetic chair, but they were instructed to keep contact with the back support of the isokinetic chair. After 3 min rest, 30 and 40% MVC were set as markers on the dynamometer screen and the subjects performed 12 bouts of 90 s bilateral isometric knee-extension exercise between 30 and 40% MVC (through visual feedback). The bouts were separated by 6 s of rest. The subjects were instructed to use both legs during the knee-extension exercise. They were verbally encouraged by the researchers and all persisted throughout the entire protocol. The protocol is visually displayed in **Fig. 1**.

Measurements

Near-Infrared Spectroscopy (NIRS)

The NIRS-model Oxiplex TS^{TM} (ISS, Champaign, Illinois, USA) recorded continuously at a sampling frequency of 1 Hz, using a NIRS-probe consisting of 8 light-emitting diodes operating at wavelengths of 750 and 830 nm and a light detector, with a distance of 2.0–3.5 cm between the light source and the detector [2,5]. The probe was positioned over the belly of the M. Vastus Lateralis (VL), along the vertical axis of the right thigh and attached to the skin secured by Velcro straps and tape. Skin pen marks indicated margins of the probe and belt to check for any displacements of the probe during protocol. The probe was connected to a PC for data acquisition, analogue-to-digital conversion and subsequent analysis, based on the method of Belardinelli et al. [1]. The baseline deoxygenated haemoglobin and myoglobin concentration (Deoxy[Hb+Mb]) was calculated as the average of 2 min rest seated in the dynamometer prior to protocol. This value was set as 100%. From the start of the protocol, Deoxy[Hb+Mb] was averaged in 10 s intervals (as a moving average) and expressed as a function of baseline Deoxy[Hb+Mb] [1]. Reoxygenation Index (RI) was determined immediately after each bout by setting out the amplitude of decrease in Deoxy[Hb+Mb] at the end of 6 s rest (i.e., A2 in **Fig. 2**) to the amplitude of increase in Deoxy[Hb+Mb] during 90 s knee-extension exercise (i.e., A1 in **Fig. 2**). Reoxygenation Index (%) is equal to A2, divided by A1 and multiplied by 100 (RI=A2/A1*100) (**Fig. 2**).

Electromyography (EMG)

EMG (Noraxon) of the M. Vastus Lateralis (VL) was continuously recorded at sampling frequency of 1000 Hz using bipolar 34-mm-diameter Ag-AgCl electrodes (Blue Sensor, Danlee Medical Products, Inc., Syracuse, NY), placed almost on the same location of the VL as the probe of the NIRS device (i.e., in the longitudinal axis of the NIRS-probe). Each electrode site was prepared by shaving, slight abrading and cleaning the site with an alcohol-ether-acetone solution (according to SENIAM recommendations). The EMG signal was checked for movement arte-

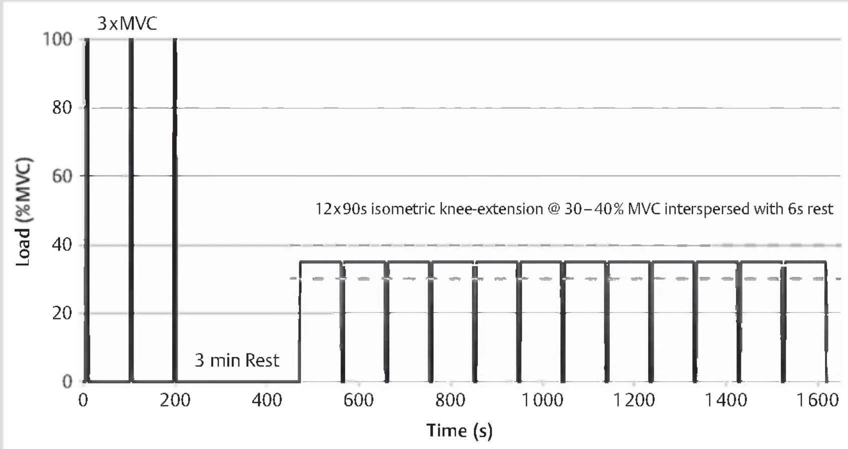

Fig. 1 Visual display of the test protocol: 3 attempts to Maximal Voluntary Contraction (MVC), 3 min rest and 12 times 90 s knee-extension exercise interspersed with 6 s rest.

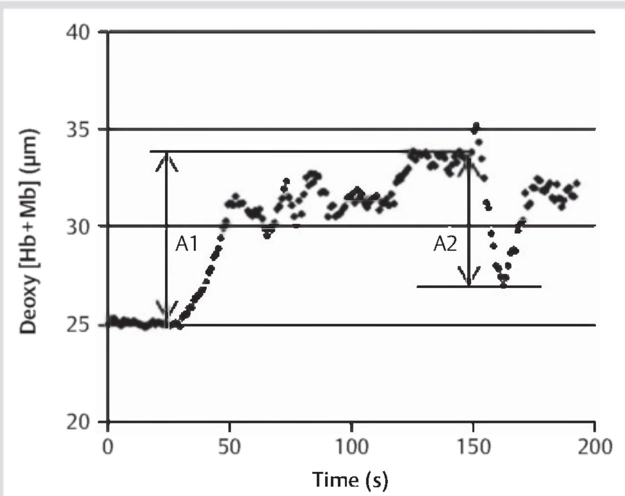

Fig. 2 Visual display of Reoxygenation Index (RI) calculation, as a function of A2/A1.

facts and the wires, connected to the electrodes were taped, to the thigh of the subject. Myoelectric signals were relayed from the bipolar electrodes to a Telemyo device (Noraxon, Inc., Scottsdale, AZ) [2]. The raw EMG signal was rectified, band-pass-filtered (Butterworth filter) and integrated using commercially available software (MyoResearch 2.10, Noraxon, Inc.) [2]. The mean values of RMS and MPF were calculated for each bout as the mean of the final 60 s. The RMS- and MPF-values during the first bout were set to 100% and the values during the following bouts were expressed relative to the first exercise bout.

Statistical analyses

Statistical computations were performed using SPSS® software (version 18; SPSS Lead Technologies Inc., Chicago, IL, USA). All data are presented as mean ± SD and independent sample t-tests were conducted to display significant differences between both groups for age, height, weight, body fat percentage, practice and experience. Bout 1 was analysed separately. Repeated measures ANOVA was used to determine whether changes of recorded Deoxy[Hb+Mb] in time, were significant throughout bout 1. When a significant effect for Deoxy[Hb+Mb] was detected within the 10 s-Deoxy[Hb+Mb]-values of the first bout these were compared 1 on 1 to a post-hoc test (Student's t-test for paired observations, followed by the Bonferroni-type adjustment for multiple comparisons) [35]. To indicate the bouts at which a significant difference between specifically trained and untrained boys could be seen in Deoxy[Hb+Mb], RI, RMS or MPF, an independent sample t-test was used. Surprisingly, every variable showed a significant difference between specifically trained and untrained boys from bout 7 to the end of the protocol. Therefore, repeated measures ANOVA was used to determine significant main effects and interaction effects for all variables throughout the second half of the protocol (from bout 6 to 12). Further, the independent sample t-test was conducted to detect significant differences between both groups for the variables: Deoxy[Hb+Mb], RI, RMS and MPF at bout 12.

Results

Specifically trained boys (151.4 ± 43.9 Nm) showed no significantly higher Maximal Voluntary Contraction Torque (MVC), compared to untrained controls (153.3 ± 55.3 Nm). During 12 bouts of 90 s bilateral submaximal isometric knee-extension interspersed with 6 s rest, deoxygenated haemoglobin and myoglobin concentration (Deoxy[Hb+Mb]) demonstrated a sequence of 12 identical cycles. Each one consists of 3 phases. As a sample, the 10 s-values of bout 1 were statistically analyzed in **Fig. 3**. Significant within-effect for Deoxy[Hb+Mb] ($p = 0.001$ & Wilks' Lambda = 9.017) was reported. Post-hoc comparison indicated first a significant ($p < 0.01$) increase in Deoxy[Hb+Mb] and after approximately 30 s of muscle contraction a levelling off. The following 60 s, Deoxy[Hb+Mb] remained in steady state with a significant ($p < 0.01$) decrease during the 6 s rest.

Fig. 4 shows the progress of Deoxy[Hb+Mb], Reoxygenation Index (RI), Root Mean Square (RMS) and Mean Power Frequency (MPF) in time, respectively. The independent sample t-test of 12 bouts Deoxy[Hb+Mb], RI, RMS and MPF draws the attention to the second half of the protocol, where significant differences between both groups were detected. Therefore, repeated measures ANOVA was conducted only for the second part of the protocol (bout 6–12). Whereas this statistical analysis showed no interaction effect for Deoxy[Hb+Mb] by group, it did show a significant effect between the groups ($p < 0.05$ and $F = 5.461$) which indicates a higher increase in Deoxy[Hb+Mb] for specifically trained boys, compared to the untrained controls. Furthermore RI shows a significant effect between the groups ($p < 0.05$ and $F = 8.205$). Specifically trained subjects display a significantly lower RI than the untrained controls. Further, only a trend was found to between-group effect for RMS ($p = 0.087$ and $F = 3.272$). However, ▸ **Fig. 4c** displays clearly that untrained boys show a bigger increase in RMS, compared to specifically trained boys. Finally, a group-by-MPF interaction effect ($p < 0.05$ and $F = 3.878$) was found for MPF, which indicates a different progress in MPF for specifically trained boys, compared to untrained controls. Namely, MPF decreased for untrained controls whereas it stayed in a more or less steady state for specifically trained boys.

▸ **Table 2** indicates (at bout 12) a significantly higher increase in Deoxy[Hb+Mb] ($p = 0.016$ and $t = -2.766$), a significantly lower decrease in MPF ($p < 0.001$ and $t = -4.356$) and a significantly lower RI ($p = 0.003$ and $t = 3.479$) for specifically trained subjects compared to untrained controls. However, there were no significant differences found in the increase of RMS.

Discussion

The aim of this study was to investigate performance limiting factors at muscle level during bilateral intermittent submaximal isometric knee-extension by examining the time course of deoxygenation, reoxygenation and myoelectrical manifestations of the M. Vastus Lateralis (VL), by means of Near-infrared Spectroscopy (NIRS) and Electromyography (EMG) in a paediatric population. We also investigated whether there is a different response at muscle level between highly specifically trained and untrained boys. The major findings of this study demonstrate that specifically trained boys show a higher increase in deoxygenated haemoglobin and myoglobin concentration (Deoxy[Hb+Mb]) and surprisingly a lower Reoxygenation Index (RI), compared to untrained controls. Also, the slower rate of increase in Root Mean Square (RMS) and decrease in Mean Power Frequency (MPF) supports the hypothesis that specifically trained subjects develop muscle fatigue more slowly compared to untrained controls, due to a specific response at muscle level.

In the present study, it was demonstrated that during each bout of the bilateral submaximal isometric knee-extension exercise, there was a gradual increase in Deoxy[Hb+Mb], followed by a steady state and a sharp decrease in Deoxy[Hb+Mb] at the onset of muscle relaxation. It should be noted that Deoxy[Hb+Mb] has frequently been used to express the degree of microvascular oxygen (O_2) extraction [2,5,12] and Deoxy[Hb+Mb] is less affected by changes in blood volume under the NIRS probe during exercise compared to oxygenated haemoglobin and myoglobin concentration (Oxy[Hb+Mb]) [12].

Statistical analysis of bout 1 confirmed the appearance of these 3 phases during this exercise. At the onset of submaximal isometric muscle contraction, the balance between O_2-supply and O_2-demand is clearly disturbed. To minimize the O_2-deficit, Deoxy[Hb+Mb] increased sharply as an indication of increase in O_2-extraction. Subsequently, the balance is restored as Deoxy [Hb+Mb] levels off to reach a steady state phase. Finally, as a result of the muscle relaxation (6 s) and a decreased intramuscular pressure, we suggest that there is a sudden outflow of deoxygenated haemoglobin and inflow of oxygenated haemoglobin (i.e., reoxygenation of the tissue under the NIRS-probe). Unfortunately, this study has no reliable measurements of muscle perfusion.

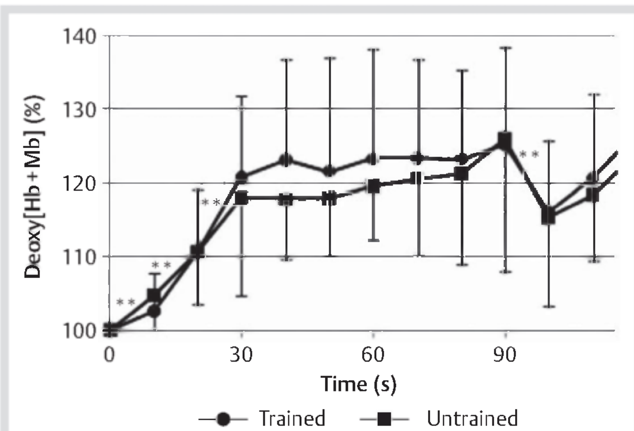

Fig. 3 Deoxy[Hb+Mb]-progress in time throughout bout 1. (Within posthoc-test for Deoxy[Hb+Mb]: $^*p < 0.05$ $^{**}p < 0.01$).

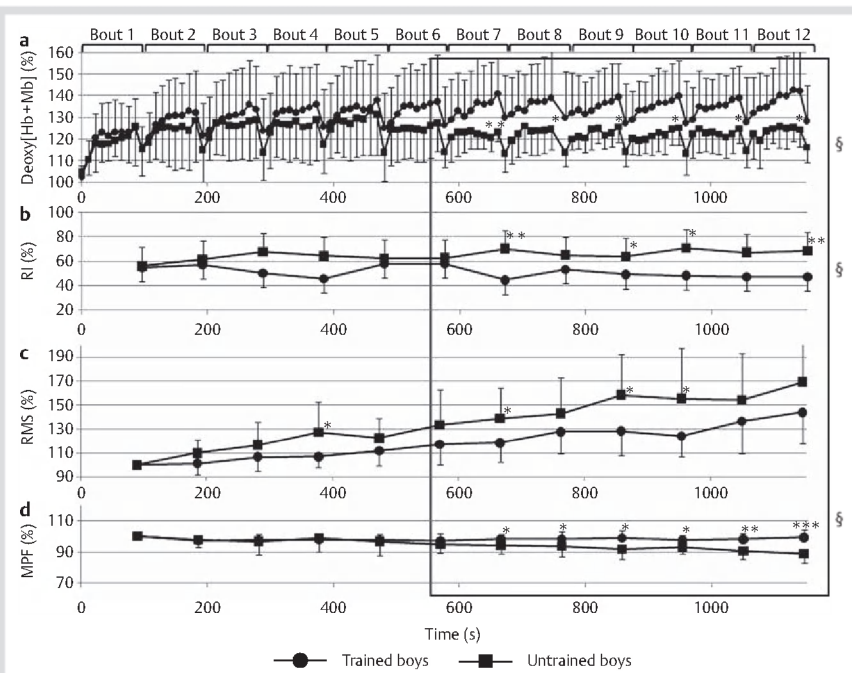

Fig. 4 Deoxy[Hb+Mb] **a**, Reoxygenation Index (RI) **b**, Root Mean Square (RMS) **c** and Mean Power Frequency (MPF) **d** progress in time (Independent sample t-test: $^*p < 0.05$ $^{**}p < 0.01$ $^{***}p < 0.001$) (Repeated Measures ANOVA: § significant between-group effect).

Table 2 Deoxy[Hb + Mb], Reoxygenation Index (RI), percentage Root Mean Square (RMS) and Mean Power Frequency (MPF) at protocol-end (bout 12) in specifically trained and untrained boys (Independent sample t-test).

GROUP	$Deoxy_{bout12}$ (%)	RI_{bout12} (%)	RMS_{bout12} (%)	MPF_{bout12} (%)
trained boys (n = 10)	142.3 ± 18.5 *	47.4 ± 11.5 **	144.1 ± 26.2	99.3 ± 5.0***
untrained boys (n = 10)	124.4 ± 7.8 *	68.5 ± 15.2 **	169.5 ± 54.6	88.9 ± 5.7***

*$p<0.05$ **$p<0.01$ ***$p<0.001$

At this moment, there is no consensus about whether blood perfusion is occluded during the submaximal (30–40% MVC) isometric knee-extension exercise. Several earlier studies [9], have suggested that during submaximal knee-extension exercise lower than 40% MVC, blood flow is not occluded. On the other hand, De Ruiter et al. [10] registered in VL already an arterial occlusion from 25% MVC torque (at 90° knee and hip angle) and reported that increased fibre-pennation angle, and thus decreased knee angle, leads to a higher intramuscular pressure. Surprisingly, this muscle load which would contribute to muscle occlusion, is quite low. Knowing that intramuscular pressure is higher in deeper parts than in superficial parts of the muscle [31], it can be suggested that when superficial occlusion is measured from NIRS-variables, capillary blood flow into the muscle is certainly occluded. However, in this study, no valid measurements of intramuscular pressure or blood flow were done. Though, the steady state phase in Deoxy[Hb + Mb]-pattern suggests that capillary blood flow in the muscle is probably not occluded. This is in line with the results of the only paediatric study [27] that investigated NIRS and EMG during submaximal knee-extension exercise, since Moalla et al. [27] reported an ischemia at or above 50% MVC isometric knee extension (at 90° knee and hip angle), due to increased intramuscular pressure. However, as demonstrated above, no consensus has been reached on the contribution of restricted blood circulation to the development of fatigue during submaximal isometric knee-extension exercise in adults and only little research [27] in this direction has been done in a paediatric population.

Throughout the entire protocol, specifically trained boys displayed a higher Deoxy[Hb + Mb]-increase compared to untrained controls. In fact, they also showed a continuous increase in Deoxy[Hb + Mb], whereas untrained controls indicated only a slight increase in Deoxy[Hb + Mb] until bout 6 and a steady state in Deoxy[Hb + Mb] from bout 6 to 12. Thus, differences in Deoxy[Hb + Mb] between both groups were present especially from bout 6 to 12. The higher Deoxy[Hb + Mb]-increase in specifically trained subjects suggests that they show a higher increase in capillary O_2-extraction, compared to untrained controls. This is in line with the results of Usaj [33] which showed that after 4 weeks of endurance training an increase in relative deoxygenated haemoglobin concentration and endurance time during sustained submaximal isometric handgrip exercise (at 30% MVC) was visible. The author suggested that a certain number of capillaries may open as a result of isometric endurance training, increasing O_2 consumption capacity as a result of increased O_2 availability. Furthermore, a higher muscle fibre oxidative capacity (i.e., higher muscle capillary density, higher mitochondrial density, higher mitochondrial oxidative enzyme activity, higher glycogen stores, higher creatine phosphate stores and more myoglobin concentration) is suggested due to endurance training adaptation [17,29]. Additionally, the neuromuscular activation (i.e., muscle fibre recruitment) pattern is changed due to training adaptation [4,25]. We suggest that the higher Deoxy[Hb + Mb]-increase in specifically trained compared to untrained boys is probably related to the different muscle fibre recruitment, because changes in RMS and MPF are largely in parallel to those of Deoxy[Hb+Mb] (► **Fig. 4**). In the specifically trained boys, the RMS-increase is not significantly, though remarkably lower and the MPF-decrease is significantly lower than for untrained controls. This expresses less fatigue development and lower additional motor unit recruitment for specifically trained subjects compared to untrained controls [6]. As a result of many hours of quasi-isometric knee-extension exercise during sailing training (on average 5.5 ± 1.8 years of circa 9.6 ± 1.6 h a week), it is suggested that specifically trained boys acquired Slow Twitch (ST) fibres with a higher oxidative capacity [17,29] than those of the untrained controls. This results in a higher strength endurance capacity but not in a higher maximal strength capacity [7]. This is confirmed by the results of this study. Briefly, it is possible that endurance-trained boys will be able to modify their muscle activation pattern [26], presumably by recruiting primarily type I fibres [23], indicated by the continuous increase in Deoxy[Hb + Mb] throughout the protocol, whereas for the same period of time, untrained controls will have to recruit faster their more fatiguable FT fibres, indicated by the steady state in Deoxy[Hb + Mb] during the second half of the protocol.

Since specifically trained subjects exhibited a higher increase in capillary O_2-extraction compared to untrained controls, it is very surprising that specifically trained boys displayed a lower RI compared to the controls. However, it is important to note that RI does not reflect muscle reoxygenation, but reoxygenation of [Hb + Mb] under the NIRS-probe, situated in small capillary blood vessels. In line with Kime et al. [18], the slower RI for specifically trained children is presumably due to the higher oxidative capacity and thus the higher capillary O_2-extraction from the VL of specifically trained boys compared to that of untrained controls. As a consequence, the O_2-gradient from capillary blood to myocyte at the onset of blood reperfusion will be higher in specifically trained boys compared to untrained controls. Consequently, the trained subjects will be able to compensate the O_2-imbalance in the VL itself more rapidly, but this will result in a slower reoxygenation of [Hb + Mb] under the NIRS-probe, because of a very fast capillary O_2-extraction of [Hb + Mb]. Additionally, results confirm that the speed and magnitude of capillary O_2-extraction during this type of exercise is a very important factor influencing the RI [18].

To our knowledge, this is the first study to examine modification in both EMG and NIRS activity in trained and untrained children during bilateral intermittent submaximal isometric knee-extension exercise. However, the study shows some limitations, inherent to NIRS- and EMG-measurements. First of all, earlier studies [18] with NIRS performed an arterial occlusion as physiological scale to determine the level of deoxygenation in adult participants. However, this method is very painful, especially for leg occlusion, and rarely used in paediatric populations. Therefore, we set the baseline value at 100% Deoxy[Hb + Mb] and expressed Deoxy[Hb + Mb] relative to the baseline value. Also, since NIRS-techniques rely on light penetration to the tissue which is mainly absorbed by chromophores [27], we assume that no difference can be shown between adults and children. It would be interesting in the future to assess methodologies of the NIRS signal in both children and adults. Secondly, the 2 probes, metabolic and electromyographic (NIRS and EMG), were as close as possible to each other but not exactly on the same muscle proportion. EMG-measurement provides a good representation of total muscle recruitment [6]. However, NIRS shows regional differences in oxygenation [8]. In addition, NIRS-measurement allows the investigation of only a few cubic centimeters of the superficial muscle area, where predominantly FT fibres are situated [19]. Unfortunately, this technical limitation of NIRS in general cannot be solved. In addition, we strongly advise additional investigations with suitable techniques (blood metabolites and electrolytes concentration measurement) combined with NIRS and EMG to get more insight into the mechanisms that limit the bilateral intermittent submaximal isometric knee-extension.

In conclusion, our findings indicate that specifically trained boys show at the muscle level a different response to bilateral intermittent submaximal isometric knee-extension exercise compared to the untrained controls. The differences in Deoxy[Hb+Mb] might reflect a different pattern of muscle O_2-extraction, presumably due to a higher oxidative capacity of the ST fibres in their VL and a more accurate neuromuscular fibre activation pattern, due to training adaptations at muscle level. As a consequence, specifically trained boys showed a delayed onset of muscle fatigue during this specific submaximal bilateral isometric knee-extension exercise.

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