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AEROBIC FUNCTION AND MUSCLE DEOXYGENATION DYNAMICS DURING RAMP EXERCISE IN CHILDREN

Melitta A. McNarry[1], Colin Farr[2], Andrew Middlebrooke[3], Deborah Welford[4], Brynmor Breese[5], Neil Armstrong[3] and Alan R. Barker[3]

[1] A-STEM, College of Engineering, Swansea University, Swansea, UK

[2] Medical Research Council Epidemiology Unit, Princes of Wales Hospital, Ely, UK.

[3] Children's Health and Exercise Research Centre, Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK

[4] Bishop Burton College, Beverly, UK

[5] Centre for Research in Translational Biomedicine, School of Biomedical and Healthcare Sciences, Plymouth University, UK

Corresponding author:

Dr Alan R. Barker
Children's Health and Exercise Research Centre
Sport and Health Sciences
College of Life and Environmental Sciences
University of Exeter
St Luke's Campus
Exeter
EX1 2LU
Tel: 44 (0)1392 722766
Fax: 44 (0)1392 724726
Email: A.R.Barker@exeter.ac.uk

ABSTRACT

Purpose: To characterise changes in deoxyhemoglobin ([HHb]) response dynamics in boys and girls during ramp incremental exercise to investigate whether the reduced peak oxygen uptake (peak$\dot{V}O_2$) in girls is associated with a poorer matching of muscle O_2 delivery to muscle O_2 utilisation, as evidenced by a more rapid increase in [HHb].

Methods: 52 children (31 boys, 9.9 ± 0.6 years, 1.38 ± 0.07 m, 31.70 ± 5.78 kg) completed ramp incremental exercise on a cycle ergometer during which pulmonary gas exchange and muscle oxygenation parameters were measured.

Results: When muscle [HHb] was expressed against absolute work rate and $\dot{V}O_2$, girls had an earlier change in [HHb] as evidenced by the lower *c/d* parameter (Girls: 54 ± 20 W vs Boys: 67 ± 19 W, P=0.023; Girls: 0.82 ± 0.28 L·min^{-1} vs. Boys: 0.95 ± 0.19 L·min^{-1}, P=0.055) and plateau (Girls: 85 ± 12 W vs. Boys: 99 ± 18 W, P=0.031; Girls: 1.02 ± 0.25 L·min^{-1} vs. Boys: 1.22 ± 0.28 L·min^{-1}, P=0.014). However, when expressed against relative work-rate or $\dot{V}O_2$, there were no sex differences in [HHb] response dynamics (all P>0.20). Significant correlations were observed between absolute and fat-free mass normalised peak $\dot{V}O_2$ and the HHb *c/d* and plateau parameters when expressed against absolute work-rate or $\dot{V}O_2$. Furthermore, when entered into a multiple regression model, the [HHb] plateau against absolute $\dot{V}O_2$ contributed 12% of the variance in peak $\dot{V}O_2$ after adjusting for fat-free mass, gas exchange threshold, and body fatness (model R^2=0.81, P<0.001).

Conclusion: The sex-difference in peak $\dot{V}O_2$ in 9-10 year old children is, in part, related to sex-specific changes in muscle O_2 extraction dynamics during incremental exercise.

Keywords: NIRS; O_2 delivery; O_2 utilization; peak $\dot{V}O_2$; pre-pubertal; sex

INTRODUCTION

A perplexing question in paediatric exercise physiology is the sexual dimorphism in peak oxygen uptake ($\dot{V}O_2$) in pre-pubertal and pubertal children. Specifically, when normalised for body mass, boys display a 10-15% greater peak $\dot{V}O_2$ compared to girls (3). This sex difference has been attributed to changes in O_2 delivery due to an elevated peak stroke volume in the presence of a comparable peak heart rate resulting in a higher peak cardiac output in boys. However, when stroke volume and cardiac output are normalised using fat free mass (FFM), the sex difference for cardiac measures disappears (39). Consequently, scaling for FFM (39) or muscle volume (11, 40) reduces the sex difference in peak $\dot{V}O_2$ to $<5\%$. This has led to the notion that the higher peak $\dot{V}O_2$ in boys is predominantly related to their greater FFM.

This notion has recently been challenged, however, by Winsley et al. (43) who compared boys and girls matched for FFM, and demonstrated a ~15% higher peak $\dot{V}O_2$ in boys, which was not explained by differences in cardiac output, stroke volume or haemoglobin concentration. Rather, a wider arterial mixed venous O_2 content difference, estimated by rearrangement of the Fick equation, was found in the boys, suggesting peripheral factors relating to the ability to deliver and utilise O_2 at the contracting muscle were the cause of the boys' higher peak $\dot{V}O_2$. This finding, however, contradicts studies showing no sex-differences in arterial mixed venous O_2 content difference at maximal exercise in children (29, 39) and warrants further investigation.

Knowledge of changes in muscle O_2 delivery and utilisation during incremental exercise in children is largely limited to central measures of cardiac output, stroke volume and $\dot{V}O_2$ which may not faithfully reflect peripheral changes in the microcirculation (28). Microcirculatory changes in muscle O_2 delivery and O_2 utilisation can be obtained non-invasively using the near infrared spectroscopy (NIRS) derived signal for muscle [deoxygenated haemoglobin and myoglobin] ([HHb]) (15, 23). Rapid changes in [HHb] reflect an increase in fractional muscle O_2 extraction, which is considered to reflect an inadequate matching of muscle O_2 delivery to O_2 utilisation in the microcirculation. The increase in [HHb] during ramp exercise has been characterised using a sigmoidal (8, 15, 26) or bi-linear (37) model, and used to study the effect of trained status and ageing (8, 18, 26). Interestingly, the rate of change in [HHb] is more rapid in adults (8, 18) and children (26) with a lower $\dot{V}O_2$max, indicating a greater rate of muscle O_2 extraction is required, presumably due to inadequate muscle O_2 delivery. A recent study by Murias et al. (27) examined the [HHb] response dynamics during ramp exercise in men and women and found the latter to be characterised by a more rapid increase in [HHb] and an earlier plateau (i.e. attainment of maximal O_2 extraction) when expressed relative to peak power and $\dot{V}O_2$max. This finding suggests that women have a poorer matching of muscle O_2 delivery to O_2 utilisation during ramp exercise. In girls the rate of increase in [HHb] was recently shown to correlate with peak $\dot{V}O_2$ and the gas exchange threshold (GET) (26). However, it is currently unknown whether similar sex-specific impairments in the matching of muscle O_2 delivery to utilisation during ramp exercise are present in children and whether this can explain, in part, the sexual dimorphism in peak $\dot{V}O_2$.

The primary purpose of the present study was to characterise changes in [HHb] response dynamics in boys and girls during ramp incremental exercise in order to test the hypothesis that the reduced peak $\dot{V}O_2$ in girls is associated with a poorer matching of muscle O_2 delivery to muscle O_2 utilisation, as evidenced by a more rapid increase in [HHb].

METHODS

Participants and anthropometry

In total, 31 boys (mean ± SD age 9.9 ± 0.3 years) and 21 girls (age 10.0 ± 0.4 years) participated in this study. All children and their parent(s)/guardian(s) provided informed assent and consent to partake in the project, which was approved by the institutional ethics committee. The children were healthy, recreationally active, and showed no contraindications to exercise to exhaustion.

An anthropometrical evaluation was performed before the first test for all participants. Stature was measured to 0.01 m using a Holtain stadiometer (Holtain, Crymych, Dyfed, UK) and body mass was determined using Avery beam balance scales to 0.1 kg (Avery, Birmingham, UK). Body fat percentage was determined using an air displacement plethysmograph (BodPod 2000A; Life Measurement Instruments, Concord, California, US) which was initially calibrated according to the manufacturer's instructions and has been validated in children (16). Lung volume was measured and body fat percentage was adjusted according to Lohman's child specific equation (24). Participants were asked to arrive at the laboratory in a rested and fully hydrated state, at least 3 hours postprandial and to refrain from consuming caffeinated drinks in the 6 hours prior to testing.

Experimental procedures

All tests took place on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands), with appropriate adjustments made to the ergometer seat, handlebar and pedal cranks for each participant. Following a 5 minute warm up at 20 W, the participant completed a ramp incremental test in which the work rate increased by 10 $W \cdot min^{-1}$

until volitional exhaustion. Participants were asked to maintain a pedal cadence of 70 rev·min^{-1} throughout the test. A maximal effort was considered to have been given if, in addition to subjective indications such as sweating, hyperpnea and facial flushing, there was a consistent reduction in cadence despite strong verbal encouragement. Although a supra-maximal test was not performed in the current study to validate the determination of $\dot{V}O_2$max, in our laboratory this occurs in ~ 95% of participants despite the absence of a plateau in the $\dot{V}O_2$-work-rate profile at near exhaustion (6). Nonetheless, the term peak $\dot{V}O_2$ will be used throughout to ensure erroneous conclusions with regard to a maximal effort are not made. Peak work rate was defined as the work rate attained at the point of test termination.

Experimental measures

Throughout each test, breath-by-breath gas exchange and ventilation (Metalyser 3B Cortex, Biophysik, Leipzig, Germany) and heart rate (Polar S610, Polar Electro Oy, Kempele, Finland) were measured and displayed online. Prior to each test, the gas analyzers were calibrated using gases of known concentration and the turbine volume transducer was calibrated using a 3 L syringe (Hans Rudolph, Kansas City, MO).

The oxygenation status of the right *vastus lateralis* muscle was monitored using a commercially available NIRS system (NIRO-300; Hamamatsu Photonics K.K, Japan). This system consists of an emission probe which emits four wavelengths of light (776, 826, 845 and 905 nm) and a photon detector. The intensity of incident and transmitted light was recorded continuously at 2 Hz and used to estimate the concentration changes relative to baseline levels for oxygenated, deoxygenated and total haemoglobin. The [HHb] signal was used as an indicator of fractional O_2

extraction within the field of interrogation (10, 15, 17). As the contribution of myoglobin to the NIRS signal is currently unresolved (36) changes in [HHb] are considered to reflect the combined concentration of deoxygenated haemoglobin and myoglobin. The skin was initially cleaned and the probes placed in a rubber holder which was adhered to the skin at the midpoint of the muscle. To ensure the holder and its probes remained stationary during exercise and to minimise the interference of extraneous light with the near-infrared signal a bandage was wrapped around the leg. The NIRS signal was zeroed with the participant at rest in a seated position with the muscle stationary and relaxed.

Data Analysis

The gas exchange data were interpolated to 1 s intervals and peak $\dot{V}O_2$ was taken as the highest 10 s stationary average during the test. The GET was determined by the V-slope method (2) as the point at which carbon dioxide ($\dot{V}CO_2$) production began to increase disproportionately to $\dot{V}O_2$ as identified using purpose designed software developed using LabVIEW (National Instruments, Newbury, UK). The location of the GET was confirmed using the ventilatory equivalents for $\dot{V}O_2$ and $\dot{V}CO_2$.

Prior to analysis, the ramp [HHb] response dynamics were averaged in 5 s bins and expressed from 0% (mean from the 5 min of baseline pedalling at 20 W) to 100% (the highest 5 s [HHb] achieved during the test). The [HHb] response dynamics were expressed in relation to work rate (W) and $\dot{V}O_2$ in both absolute and relative terms. In line with previous research (27, 28), the $\dot{V}O_2$ response profile was back-shifted by 20 s in an attempt to account for the phase I-II, muscle to lung transit time. To determine the most appropriate approach to characterise the profile of the

%Δ[HHb] response (as a function of % peak work rate or $\dot{V}O_2$), two models were compared (GraphPad Prism 5). First, the entire %Δ[HHb] response was modelled from the onset of the ramp exercise until exercise cessation using a sigmoid function (8, 12, 26):

$$Y = a/(1+exp^{-(-c+dx)})$$

where *a* represents the baseline corrected amplitude and *c* is a constant dependent upon *d* (the slope of the sigmoid) whereby *c*/*d* reveals the *x* value that yields 50% of the total amplitude. The point at which a plateau occurred in the [HHb] response was determined as the point at which the [HHb] response reached the lower boundary of the 95% confidence interval for the *a* parameter.

Secondly, the increase in %Δ[HHb] observed throughout the middle portion of the exercise protocol (beginning at the point where the %Δ[HHb] signal began a systematic increase above baseline as determined visually) and the plateau which followed were characterised by a piecewise function that included two linear segments (the 'double-linear model')(38). The models were compared by computing the change in corrected Akaike Information Criterion scores (ΔAIC_c). Contrary to previous findings in adults (27, 37), the sigmoid model provided a superior fit in over 95% of cases according to the AIC_c scores. Thus, the parameters derived from the sigmoid model were used for all subsequent analyses.

Analysis of covariance (ANCOVA) on log transformed data was used to determine the allometric relationship between body size (body mass, FFM) and $\dot{V}O_2$max. Common allometric exponents were confirmed for all groups and power function ratios (Y/X^b) were computed and their size-independence was checked and confirmed by performing size-residual correlations against body mass and FFM.

Statistical analyses

Prior to analysis, distribution normality was examined and verified using the Shapiro-Wilk test. Independent samples t-tests were utilised to assess the influence of sex on the ramp test $\dot{V}O_2$ and [HHb] responses. Equality of variances was checked using Levene's test. If significant, the equal variances not assumed P-value was reported. All data are presented as means ± SD. Statistical significance was accepted when $P<0.05$ and effect size (ES) statistics were used to detail the magnitude of the observed effect using the mean difference and the pooled SD. An ES <0.2 was trivial, >0.2 was small, >0.5 was medium and >0.8 was large.

Pearson correlation coefficients were used to assess the strength of relationships between the [HHb] dynamics and peak $\dot{V}O_2$. These correlations informed the multiple regression analyses to determine the independent contribution of [HHb] kinetic parameters in explaining sex differences in absolute peak $\dot{V}O_2$ after accounting for other potentially important predictors (e.g. sex, age, body fat %). Initially, both sex and FFM were entered into the model given their strong relationship with absolute peak $\dot{V}O_2$ ($L.min^{-1}$) in this age group (11). Subsequently, potential predictor variables were considered in a stepwise manner to determine their independent contribution to predicting absolute peak $\dot{V}O_2$. Inclusion into the model was accepted with a significant increase in explained variance at the 0.05 level. The adequacy of the regression model was examined and verified using checks for multicollinearity (variance inflation factor, tolerance) and distribution normality of the residuals.

RESULTS

Anthropometric characteristics were similar between boys and girls (see Table 1).

Parameters of aerobic function

The physiological responses during the ramp test to exhaustion are presented in table 2. Boys achieved a higher peak $\dot{V}O_2$ irrespective of whether expressed in absolute terms (18.0%) or relative to allometrically scaled body mass (16.2%) or FFM (11.7%). This was despite no sex differences in maximum heart rate. The boys achieved a higher peak work-rate at exhaustion. No sex difference was identified for the GET when expressed in absolute terms or relative to peak $\dot{V}O_2$.

Ramp [HHb] response dynamics

A representative profile of the modelled [HHb] response dynamics during ramp exercise for a boy and girl participant is illustrated in figure 1 when expressed as a function of absolute and relative work-rate and $\dot{V}O_2$. The parameter estimates for the sigmoidal model are presented in table 3. When expressed against absolute work-rate boys had a higher *c/d* (P=0.023, ES=0.67) and attained a plateau at a higher work-rate (P=0.031, ES=0.66). However, when expressed relative to peak work-rate, no sex differences were present for all [HHb] response parameters (all P>0.26, all ES<0.35). Plotting [HHb] against absolute $\dot{V}O_2$ showed a strong trend for boys to have a higher *c/d* (P=0.055, ES=0.58) and to achieve a plateau in the response profile at a higher metabolic rate (P=0.014, ES=0.76). When [HHb] was plotted relative to $\dot{V}O_2$ however, there were no sex differences for response parameters (all P>0.20, all ES<0.41).

Correlations between aerobic function and [HHb] response dynamics

A significant correlation was evident between absolute peak $\dot{V}O_2$ and the [HHb] *c/d* (r=0.62, P<0.001; r=0.79, P<0.001) and plateau (r=0.70, P<0.001; r=0.77, P<0.001) when expressed as a function of absolute work rate and $\dot{V}O_2$, respectively (see figure 2 for example correlations). When the [HHb] response parameters were derived using relative work rate, similar, although weaker, relationships were manifest between absolute $\dot{V}O_2$max and the *c/d* parameter (r=0.37, P=0.009) and plateau (r=0.30, P=0.035). No correlations were evident between peak $\dot{V}O_2$ and the [HHb] parameters derived using relative $\dot{V}O_2$.

Muscle [HHb] response dynamics were also correlated with peak $\dot{V}O_2$ normalised using allometric models for body mass or FFM, although only the latter results are presented due to the similar outcomes across body size measures. Relationships were observed between FFM normalised $\dot{V}O_2$max and the [HHb] *c/d* (r=0.34, P=0.017 and r=0.52, P<0.001), and plateau (r=0.45, P=0.001 and r=0.53, P<0.001) when expressed using absolute work rate and $\dot{V}O_2$, respectively. However, these relationships disappeared when [HHb] was expressed using relative work rate and $\dot{V}O_2$.

The FFM scaled peak $\dot{V}O_2$ was significantly related to the absolute GET (r=0.52, P<0.001) across the sample. When the GET was correlated against the [HHb] dynamics, a relationship was found for [HHb] *c/d* (r=0.52, P<0.001) and the [HHb] plateau (r=0.47, P<0.001) as a function of absolute $\dot{V}O_2$.

Regression analysis of peak $\dot{V}O_2$ determinants

The output from the multiple linear regression prediction of absolute peak $\dot{V}O_2$ is provided in table 4. Model 1 initially started with sex and FFM entered into the model (R^2=0.41, P<0.001).

Subsequently stepwise regression revealed significant improvements in explained variance due to the addition of absolute GET (ΔR^2=0.23, P<0.001), the [HHb] plateau expressed against absolute $\dot{V}O_2$ (ΔR^2=0.12, P<0.001) and body fat % (ΔR^2=0.03, P=0.034). The final model predicted ~ 81% of the change in absolute peak $\dot{V}O_2$ (R^2=0.81, P<0.001).

DISCUSSION

The primary purpose of the present study was to examine whether sex-specific differences in the temporal response of local muscle fractional O_2 extraction, as indicated by the NIRS-derived Δ[HHb] response, are present in children and account for the sexual dimorphism in peak $\dot{V}O_2$. In agreement with our hypothesis, when muscle [HHb] was expressed against absolute work rate and $\dot{V}O_2$, girls had a greater rate of change in [HHb] as evidenced by the lower *c/d* parameter and plateau. However, when expressed against relative work-rate or $\dot{V}O_2$, the sex difference in [HHb] response dynamics was no longer significant. Significant correlations were observed between absolute and FFM normalised peak $\dot{V}O_2$ and the HHb *c/d* and plateau parameters when expressed against absolute work-rate or $\dot{V}O_2$. Furthermore, when entered into a multiple regression model, the [HHb] plateau against absolute $\dot{V}O_2$ contributed to ~ 12% of the variance in peak $\dot{V}O_2$ after adjusting for FFM, GET, and body fatness. These data, therefore, support the hypothesis that the sex-difference in peak $\dot{V}O_2$ in 9-10 year old children is, in part, related to sex-specific changes in muscle O_2 extraction dynamics during incremental exercise.

In accord with previous studies (1, 11, 13, 39), the magnitude of the sexual dimorphism in peak $\dot{V}O_2$ of the children in the current study varied in relation to the different methods of expressing peak $\dot{V}O_2$. Specifically, boys demonstrated a ~ 18% higher peak $\dot{V}O_2$ compared to girls when expressed in absolute terms, which was reduced following allometric modelling using body mass (~16% difference) and FFM (~12% difference). This residual difference following normalization to FFM is consistent with other studies (11, 34). For example, in a cross-sectional study consisting of 248 children aged 8-11 years, Dencker and colleagues (11) found, through multiple regression, girls to have a lower peak $\dot{V}O_2$ after accounting for differences in body composition, heart size and habitual physical activity. Furthermore, previous data from our laboratory have shown that after matching children for FFM, boys' maintain a ~14% higher peak $\dot{V}O_2$ despite no sex-related differences in blood haemoglobin concentration, cardiac output and heart dimensions (43). The authors attributed the higher peak $\dot{V}O_2$ in boys to a greater muscle O_2 extraction, as evidenced by a ~ 17% wider arterial mixed venous O_2 content difference. This calculation, however, was based on whole-body measures of maximal $\dot{V}O_2$ and cardiac output via re-arrangement of the Fick equation, which is unlikely to reflect the dynamics of muscle O_2 delivery and O_2 utilisation within the microcirculation of the contracting mycocytes over the range of metabolic rates leading to peak $\dot{V}O_2$ (28).

In the present study we used NIRS to non-invasively measure microcirculatory changes in [HHb] in the *vastus lateralis* muscle to provide insight into changes in the rate of fractional muscle O_2 extraction dynamics during ramp exercise. In agreement with previous studies in children (26, 35) and adults (8, 12), the [HHb] response during ramp exercise was well characterized using a sigmoidal model, when compared to a bi-linear model (37). It has been suggested that under conditions in which muscle O_2 delivery is compromised (e.g. disease, detraining) a leftward shift (i.e. more rapid increase) of the muscle [HHb] response is manifest (15). Consistent with this notion are data showing a more rapid increase in muscle [HHb] in untrained children (26) and adults (8), the elderly (18) and adult women compared to men (27). In agreement with the latter study, the girls in the current study were similarly characterised by a greater rate of change in [HHb] during ramp exercise compared to boys. Specifically, at a given work-rate or metabolic rate, the change in [HHb], expressed as a percentage of the total [HHb] amplitude, was greater in girls compared to boys resulting in the earlier attainment of a plateau (i.e. maximal rate of O_2 extraction) in the [HHb] response. As the pattern of muscle [HHb] during ramp exercise reflects the ratio of muscle O_2 delivery to consumption, this finding implies that microvascular blood flow (15) was reduced in girls at sub-maximal work-rates and $\dot{V}O_2$ compared to boys, such that the ‘linear’ portion of the muscle O_2 delivery to utilisation relationship (plateau) was reached earlier in the test while $\dot{V}O_2$ was still increasing.

Interestingly, the current study’s data cohere with a recent study showing female adolescents and adults to have a shorter [HHb] time delay at the onset of high-intensity quadriceps exercise, suggesting impaired muscle O_2 delivery (42). However, such findings are in conflict with data showing women to have an increased femoral blood flow to work-rate relationship during incremental knee-extensor exercise (31), suggesting women would be characterised by a lower rate of muscle O_2 extraction during ramp cycling exercise in the current study. However, it should be noted that while adult studies generally show women to have greater muscle perfusion during exercise at similar exercise intensities compared to their male counterparts, this is dependent on the type (sustained vs. intermittent) of muscle contraction and recruited muscle mass (20). Compared to knee-extensor exercise, cycling exercise involves recruitment from muscles across the lower limbs and is not restricted to the quadriceps (33). Thus, as highlighted by Murias et al. (27), in contrast to knee-extensor exercise the additional muscle mass recruited during cycling exercise will elicit a maximal cardiac output response which needs to be effectively redistributed to the metabolically active fibres. Taken collectively, our data and that of Murias et al. (27) suggest that under conditions of ramp cycling exercise to exhaustion, females are characterised by an impaired muscle O_2 delivery in both prepubertal children and young adults.

While the mechanistic basis for the more rapid rate of change in muscle [HHb] for a given work rate and $\dot{V}O_2$ in girls cannot be explained with our data, a reduction in bulk blood flow, poorer regional matching of blood flow to the metabolically active mycocytes and/or lower muscle oxidative capacity may be implicated. It has been suggested that the mechanical effects of muscle contraction and/or localised vasodilators may play a role in altering the [HHb] dynamics during ramp exercise (8, 15), but these factors are likely to predominate during the early portion of the ramp test. Alternatively, Murias and colleagues (27) suggested that the haemodynamic response in women may be compromised due to sex-specific differences in sympathetic activation limiting the re-distribution of blood flow to the contracting muscles. Unfortunately, complementary data on muscle blood flow at rest or during exercise in children are not available, although studies have shown micro- and macro- vascular function to be sex-independent in healthy children (19, 32). Furthermore, although limited to rest and maximal exercise, our laboratory has previously reported that with boys and girls of similar FFM there is no difference in cardiac dimensions, stroke volume and cardiac output (43). Muscle oxidative capacity is likely to be an important determinant of the muscle [HHb] response, but no data are available on sex-differences in muscle oxidative enzyme activates in pediatric groups. In contrast, the recovery of muscle PCr following exercise can be used as a non-invasive index of the muscles oxidative capacity and is not sex-dependent in prepubertal children (4). Alternatively, it is plausible that sex-differences in the progressive recruitment of higher-order muscle fibres during ramp exercise may account for the more rapid increase in muscle [HHb] in girls. Specifically, it has been shown that type II fibres with a low oxidative capacity are characterised by more rapid muscle O_2 extraction kinetics at the onset of muscle contractions, presumably due to sluggish muscle O_2 delivery dynamics relative to muscle O_2 consumption (7, 25). While, muscle fibre recruitment patterns remain to be elucidated during exercise in children, it is pertinent to note that girls are characterised by slower $\dot{V}O_2$ kinetics during cycling exercise (14) and a greater muscle metabolic perturbation (e.g. PCr breakdown) during high-intensity incremental (5) or squarewave (42) exercise, which may be indicative of a greater reliance on higher-order muscle fibres and reduced muscle O_2 availability. Although not definitive, this suggests that sex-differences in the progressive recruitment of type II muscle fibres during ramp exercise may explain, in part, our observation of more rapid [HHb] kinetics in girls. However, it should be noted, that such sex-differences in muscle phosphate and pH responses are not seen during high-intensity intermittent exercise in children (22) or adolescents (41), suggesting muscle blood flow may not be compromised in females under such experimental conditions and that the findings of the current study reflect the incremental exercise protocol employed.

In order to determine whether the changes in muscle [HHb] dynamics accounted for the sex-differences in peak $\dot{V}O_2$ in the current study, multiple regression analyses were performed. After adjusting for FFM, the model predicted ~ 81% of the variance in absolute peak $\dot{V}O_2$ and revealed significant contributions from the GET, muscle [HHb] plateau and percentage body fat.

In particular, the muscle [HHb] plateau (derived relative to absolute $\dot{V}O_2$) accounted for ~ 12% of the explained variance and rendered the sex term non-significant. This indicates that sex differences in peak $\dot{V}O_2$ can be explained, in part, by muscle O_2 delivery to muscle O_2 utilisation dynamics. The model derived from the present study explains a greater percentage of the variance in peak $\dot{V}O_2$ than previously reported in children by others (11, 30). Interestingly, in the present study, FFM (and sex) accounted for ~ 41% of the variance in absolute peak $\dot{V}O_2$ which is strikingly comparable to previous studies, and presumably accounts for cardiac function and morphology in our participants, although this was not directly measured. The present study extends this observation by demonstrating that an additional ~ 40% of the variance for predicting peak $\dot{V}O_2$ was attributed to the GET and [HHb] plateau, as percentage body fat only improved the model by ~ 3%. To our knowledge, the GET and [HHb] dynamics have not been considered in previous work concerning the determinants of peak $\dot{V}O_2$ in children and is likely to reflect differences in the participants' muscle oxidative capacity and muscle fibre distribution as both the GET (21) and muscle [HHb] responses (as discussed above) are influenced by these factors.

Although hypothesised in initial modelling simulations (15), Boone et al. (8) were the first to demonstrate a relationship between muscle [HHb] dynamics during ramp exercise and peak $\dot{V}O_2$ in adult cyclists and physically active students. Subsequently, McNarry et al. (26) demonstrated a relationship between muscle [HHb] *c/d* and parameters of aerobic function (peak $\dot{V}O_2$ and GET) in girls during cycling exercise. Similar to previous findings in adults and children, in the present study we observed a positive relationship between the [HHb] response dynamics (*c/d*, plateau) and peak $\dot{V}O_2$ (expressed in absolute terms or scaled for FFM) and submaximal (GET) parameters of aerobic function. This supports the putative role of aerobic conditioning on causing a ‘rightward’ shift in the [HHb] response, and is likely to reflect enhanced muscle oxidative capacity and muscle fibre type distribution (8, 26). However, an interesting finding in the current study is that the sex differences in muscle [HHb] dynamics (*c/d* and plateau) disappeared when expressed relative to peak work rate and $\dot{V}O_2$. Both absolute peak $\dot{V}O_2$ and peak work-rate were lower in girls in the current study, meaning that expressing [HHb] at any given $\dot{V}O_2$ or work-rate would represent a greater proportion of their peak response. Similar findings have been reported when comparing younger and older adults (18) and males and females (27), although the differences persisted when expressed relative to peak $\dot{V}O_2$ in the latter study.

It is prudent to note certain limitations with the present study design. Specifically, although chronological age of the participants in the current study is comparable with previous studies (11, 39, 43) and suggests our group were pre-pubertal, this was not determined. Unfortunately, the ethical considerations that surround the utilization of Tanner stages or skeletal age and the inaccuracy associated with age to peak height velocity make the accurate determination of maturity stage challenging. Furthermore, no central measures of bulk O_2 delivery or haemoglobin were collected in the present study, although normalization by FFM has previously been shown to account for differences in these parameters between the sexes (39). Habitual physical activity or participation in structured sports was not measured in the current study. However, after accounting for body size and cardiac dimensions, physical activity (specifically vigorous physical activity) only accounts for ~ 1% of the explained variance in peak $\dot{V}O_2$ in pre-pubertal boys and girls (11). Furthermore, a recent review highlighted that there is no meaningful evidence of a relationship between children’s habitual physical activity and aerobic fitness as expressed by peak $\dot{V}O_2$ (2), suggesting sex-differences in habitual physical activity are unlikely to be a confounding factor in the current study's findings. Finally, the interpretation of the [HHb] kinetics obtained by NIRS requires particular methodological considerations, including i) variations in adiposity beneath the probe between boys and girls; ii) the generalizability of the response dynamics from a localised area to a heterogeneous muscle and iii) the [HHb] response has been shown to be influenced by muscle activation patterns (9). The absence of EMG measures from the present study precludes the possibility that sex differences in muscle activity may explain the altered [HHb] response from being excluded. However, it is important to recognize that there were no differences in FFM between sexes in the current study and changes in [HHb] were normalized to the peak value at exhaustion. Furthermore, the NIRS probe was placed in the same location for all participants, minimizing regional differences.

CONCLUSION

In conclusion, this is the first study to utilise NIRS derived changes in the muscle [HHb] response dynamics to assess the sexual dimorphism in the peak $\dot{V}O_2$ of boys and girls. In accord with our hypothesis, girls were shown to require a greater fractional O_2 extraction to increase work rate and $\dot{V}O_2$ and thus reached an earlier plateau in O_2 extraction compared to boys during ramp exercise. Parameters of the muscle [HHb] dynamics were related to aerobic function and the plateau in muscle [HHb] was found to account for ~ 12% of the variance in peak $\dot{V}O_2$ after adjusting for FFM, GET and body fatness, and eliminated the sex difference in peak $\dot{V}O_2$. These results may reflect an inferior bulk O_2 delivery and/or regional matching of O_2 delivery in girls.

CONFLICT OF INTEREST

The present study does not engender any conflict of interests and does not constitute an endorsement by ACSM.

REFERENCES

1. Armstrong N, Kirby BJ, McManus AM, Welsman JR. Aerobic fitness of prepubescent children. *Ann Hum Biol*. 1995;22(5):427-41.

2. Armstrong N, Tomkinson G, Ekelund U. Aerobic fitness and its relationship to sport, exercise training and habitual physical activity during youth. *Br J Sports Med*. 2011;45(11):849-58.

3. Armstrong N, Welsman JR. Development of Aerobic Fitness During Childhood and Adolescence. *Pediatr. Exerc. Sci.* 2000;12:128-49.

4. Barker AR, Welsman JR, Fulford J, Welford D, Armstrong N. Muscle phosphocreatine kinetics in children and adults at the onset and offset of moderate-intensity exercise. *J Appl Physiol*. 2008;105(2):446-56.

5. Barker AR, Welsman JR, Fulford J, Welford D, Armstrong N. Quadriceps muscle energetics during incremental exercise in children and adults. *Med Sci Sports Exerc*. 2010;42(7):1303-13.

6. Barker AR, Williams CA, Jones AM, Armstrong N. Establishing maximal oxygen uptake in young people during a ramp cycle test to exhaustion. *Br J Sports Med*. 2011;45:498-503.

7. Behnke BJ, McDonough P, Padilla DJ, Musch TI, Poole DC. Oxygen exchange profile in rat muscles of contrasting fibre types. *J Physiol*. 2003;549(2):597-605.

8. Boone J, Koppo K, Barstow TJ, Bouckaert J. Pattern of deoxy[Hb+Mb] during ramp cycle exercise: influence of aerobic fitness status. *Eur J Appl Physiol*. 2009;105(6):851-9.

9. Chin LMK, Kowalchuk JM, Barstow TJ et al. The relationship between muscle deoxygenation and activation in different muscles of the quadriceps during cycle ramp exercise. *J. Appl. Physiol.* 2011;111(5):1259-65.

10. DeLorey DS, Kowalchuk JM, Paterson DH. Relationship between pulmonary O_2 uptake kinetics and muscle deoxygenation during moderate-intensity exercise. *J. Appl. Physiol.* 2003;95(1):113-20.

11. Dencker M, Thorsson O, Karlsson MK et al. Gender differences and determinants of aerobic fitness in children aged 8-11 years. *Eur J Appl Physiol*. 2007;99(1):19-26.

12. DiMenna FJ, Bailey SJ, Jones AM. Influence of body position on muscle deoxy[Hb+Mb] during ramp cycle exercise. *Respir Physiol Neurobiol*. 2010;173(2):138-45.

13. Eiberg S, Hasselstrom H, Gronfeldt V, Froberg K, Svensson J, Andersen LB. Maximum oxygen uptake and objectively measured physical activity in Danish children 6-7 years of age: the Copenhagen school child intervention study. *Br J Sports Med*. 2005;39(10):725-30.

14. Fawkner SG, Armstrong N. Sex differences in the oxygen uptake kinetic response to heavy-intensity exercise in prepubertal children. *Eur J Appl Physiol*. 2004;93(1-2):210-6.

15. Ferreira LF, Koga S, Barstow TJ. Dynamics of noninvasively estimated microvascular O_2 extraction during ramp exercise. *J. Appl. Physiol.* 2007;103(6):1999-2004.

16. Fields DA, Goran MI, McCrory MA. Body-composition assessment via air-displacement plethysmography in adults and children: a review. *Am J Clin Nutr*. 2002;75(3):453-67.

17. Grassi B, Pogliaghi S, Rampichini S et al. Muscle oxygenation and pulmonary gas exchange kinetics during cycling exercise on-transitions in humans. *J. Appl. Physiol.* 2003;95(1):149-58.

18. Gravelle BM, Murias JM, Spencer MD, Paterson DH, Kowalchuk JM. Adjustments of pulmonary O2 uptake and muscle deoxygenation during ramp incremental exercise and constant-load moderate-intensity exercise in young and older adults. *J Appl Physiol* 2012;113(9):1466-75.

19. Hopkins ND, Stratton G, Tinken TM et al. Relationships between measures of fitness, physical activity, body composition and vascular function in children. *Atherosclerosis*. 2009;204(1):244-9.

20. Hunter SK. Sex differences in human fatigability: mechanisms and insight to physiological responses. *Acta Physiol.* 2014;210(4):768-89.

21. Jones AM, Carter H. The effect of endurance training on parameters of aerobic fitness. *Sports Med*. 2000;29(6):373-86.

22. Kappenstein J, Ferrauti A, Runkel B, Fernandez-Fernandez J, Muller K, Zange J. Changes in phosphocreatine concentration of skeletal muscle during high-intensity intermittent exercise in children and adults. *Eur J Appl Physiol*. 2013;113(11):2769-79.

23. Koga S, Kano Y, Barstow TJ et al. Kinetics of muscle deoxygenation and microvascular PO(2) during contractions in rat: comparison of optical spectroscopy and phosphorescence-quenching techniques. *J Appl Physiol*. 2012;112(1):26-32.

24. Lohman TG. Assessment of Body Composition in Children. *Pediatr. Exerc. Sci.* 1986;1:19-30.

25. McDonough P, Behnke BJ, Padilla DJ, Musch TI, Poole DC. Control of microvascular oxygen pressures in rat muscles comprised of different fibre types. *J Physiol*. 2005;563(3):903-13.

26. McNarry MA, Welsman JR, Jones AM. Influence of training and maturity status on the cardiopulmonary responses to ramp incremental cycle and upper body exercise in girls. *J Appl Physiol*. 2011;110(2):375-81.

27. Murias JM, Keir DA, Spencer MD, Paterson DH. Sex-related differences in muscle deoxygenation during ramp incremental exercise. *Respir. Physiol. Neuro.* 2013;189(3):530-6.

28. Murias JM, Spencer MD, Keir DA, Paterson DH. Systemic and vastus lateralis muscle blood flow and O2 extraction during ramp incremental cycle exercise. *Am J Physiol Regul Integr Comp Physiol*. 2013;304(9):R720-5.

29. Obert P, Mandigout S, Nottin S, Vinet A, N'Guyen LD, Lecoq AM. Cardiovascular responses to endurance training in children: effect of gender. *Eur J Clin Invest*. 2003;33(3):199-208.

30. Obert P, Mandigout S, Vinet A, Nottin S, N'Guyen LD, Lecoq AM. Relationships between left ventricular morphology, diastolic function and oxygen carrying capacity and maximal oxygen uptake in children. *Int. J. Sports Med.* 2005;26(2):122-7.

31. Parker BA, Smithmyer SL, Pelberg JA, Mishkin AD, Herr MD, Proctor DN. Sex differences in leg vasodilation during graded knee extensor exercise in young adults. *J Appl Physiol (1985)*. 2007;103(5):1583-91.

32. Radtke T, Khattab K, Eser P, Kriemler S, Saner H, Wilhelm M. Puberty and microvascular function in healthy children and adolescents. *J Pediatr*. 2012;161(5):887-91.

33. Richardson RS, Frank LR, Haseler LJ. Dynamic knee-extensor and cycle exercise: functional MRI of muscular activity. *Int J Sports Med*. 1998;19(3):182-7.

34. Rowland T, Goff D, Martel L, Ferrone L. Influence of cardiac functional capacity on gender differences in maximal oxygen uptake in children. *Chest*. 2000;117(3):629-35.

35. Saynor ZL, Barker AR, Oades PJ, Williams CA. Impaired Aerobic Function in Young Cystic Fibrosis Patients During Ramp Exercise. *Med Sci Sports Exerc*. 2014.

36. Seiyama A, Hazeki O, Tamura M. Noninvasive quantitative analysis of blood oxygenation in rat skeletal muscle. *J Biochem*. 1988;103(3):419-24.

37. Spencer M, Murias J, Paterson D. Characterizing the profile of muscle deoxygenation during ramp incremental exercise in young men. *Eur. J. Appl. Physiol.* 2012;112(9):3349-60.

38. Vieth E. Fitting piecewise linear regression functions to biological responses. *J Appl Physiol*. 1989;67(1):390-6.

39. Vinet A, Mandigout S, Nottin S et al. Influence of body composition, hemoglobin concentration, and cardiac size and function of gender differences in maximal oxygen uptake in prepubertal children. *Chest*. 2003;124(4):1494-9.

40. Welsman JR, Armstrong N, Kirby BJ, Winsley RJ, Parsons G, Sharpe P. Exercise performance and magnetic resonance imaging determined thigh muscle volume in children. *Eur. J. Appl. Physiol.* 1997;76:92-7.

41. Willcocks RJ, Fulford J, Armstrong N, Barker AR, Williams CA. Muscle metabolism during fatiguing isometric quadriceps exercise in adolescents and adults. *Appl Physiol Nutr Metab*. 2014;39(4):439-45.

42. Willcocks RJ, Williams CA, Barker AR, Fulford J, Armstrong N. Age- and sex-related differences in muscle phosphocreatine and oxygenation kinetics during high-intensity exercise in adolescents and adults. *NMR Biomed*. 2010;23(6):569-77.

43. Winsley RJ, Fulford J, Roberts AC, Welsman JR, Armstrong N. Sex difference in peak oxygen uptake in prepubertal children. *J. Sci. Med. Sport*. 2009;12(6):647-51.

FIGURE CAPTION

Figure 1. Deoxygenated haemoglobin plus myoglobin concentration ([HHb]) response as a function of a) absolute work rate (WR), b) relative work rate, c) absolute $\dot{V}O_2$, and d) relative $\dot{V}O_2$ for a representative boy (○) and girl (●).

Figure 2. The relationship between absolute peak $\dot{V}O_2$ and muscle [HHb] c/d (A) and plateau (B) as a function of absolute $\dot{V}O_2$ in boys (○) and girls (●). Results for the Pearson's correlation are presented. See text for further details.