Improvements in Skeletal Muscle Can Be Detected Using Broadband NIRS in First-Time Marathon Runners

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Abstract Skeletal muscle metabolic function is known to respond positively to endurance exercise interventions, such as marathon training. Studies investigating skeletal muscle have typically used muscle biopsy samples or magnetic resonance spectroscopy (MRS) to interrogate metabolic function. We aimed to non-invasively detect exercise-training-induced improvements in muscle function using broadband near-infrared spectroscopy (NIRS). We used NIRS to determine concentration changes in oxygenated haemoglobin (HbO_2) and the oxidation state of cytochrome-c-oxidase (oxCCO) in gastrocnemius during arterial occlusion in 14 volunteers. We also used a cardio-pulmonary exercise test (CPET) to assess peak total body oxygen uptake (peakVO_2; a measure of fitness). Measurements were made at baseline (BL) which was prior to a period of at least 16 weeks of training for the 2017 London Marathon, and then within 3 weeks after completion of the marathon, follow-up (FU). We observed an increase in locally measured muscle oxygen consumption and rate of oxCCO concentration change, but not in cardio-respiratory fitness measured as whole-body peak oxygen consumption (peakVO_2).

Keywords Near-infrared spectroscopy · Metabolism · Cytochrome-c-oxidase · Endurance exercise · Vascular

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1 Introduction

Skeletal muscle metabolic function is typically interrogated via muscle biopsy or magnetic resonance spectroscopy (MRS). While these techniques have the capacity to phenotype muscle in detail, they are not always appropriate, or available, in research and clinical environments. There is a need to develop skeletal muscle assessment tools which are non-invasive, widely available and sensitive enough to detect changes in function in response to intervention.

Continuous wave NIRS devices, applying 2–4 wavelengths of near-infrared light, have previously been used to measure local muscle oxygen consumption, which is represented by the rate of change in oxygenated haemoglobin/myoglobin (HbO_2) during an arterial occlusion [1]. Broadband NIRS can additionally monitor metabolism via the oxidation state of cytochrome-c-oxidase (oxCCO), from redox-dependent spectral changes in its copper A centre (Cu_A) [2]. Changes in oxCCO in skeletal muscle during arterial occlusions have not been fully examined previously.

Skeletal muscle metabolic function is known to respond positively to endurance exercise interventions. Evidence that has been gathered by measuring increases in the overall concentration of mitochondrial enzymes in biopsy samples, shows that exercise training stimulates mitochondrial growth, oxidative capacity, and capacity for syntheses of glycogen and lipid [3, 4].

In this study we use a miniature broadband NIRS system, called mini-CYRIL [5], to assess the effect of marathon training on muscle haemodynamic and metabolic functions at rest and after exercise.

2 Methods

Participants were healthy, non-athletic adults (>30 years old) enrolled in a study investigating cardiac adaptations to at least 16 weeks of endurance training for their first marathon. All procedures were in accordance with the Declaration of Helsinki; all participants gave written informed consent and the study was approved by the London Queen Square National Research Ethics Service Committee (15/LO/0086).

Before commencing training (baseline; BL), participants underwent measurements of HbO_2, deoxy-haemoglobin (HHb) and oxCCO concentration changes in the lateral gastrocnemius using an in-house built laboratory broadband NIRS device (mini-CYRIL) [5]. Two 3-minute arterial occlusions were applied proximal to the measurement site using a leg cuff inflated to supra-systolic pressure. The first occlusion was carried out at rest; the second, following a maximal exertion exercise test.

Height and weight were measured using a stadiometer and scales (BC-418, Tanita, USA), respectively. Adipose tissue thickness (ATT) was measured by ultrasound at the NIRS measurement site. Cardio-respiratory fitness was measured using a maximal cardio-pulmonary exercise test (CPET) carried out on a supine cycle ergometer (Ergoselect1200, Ergoline, Germany) to determine peak oxygen consumption (peakVO_2) by analysis of expired gases (Quark CPET, Cosmed, Italy).

All measurements were repeated within 3 weeks of participants completing the 2017 London marathon (follow-up; FU).

Data analysis was carried out in MATLAB 2015b (Mathworks, USA). NIRS spectral data were assessed for adequate signal to noise (SNR) and resolved concentration changes were assessed for cross-talk using residual analysis [2]. Data sets with poor signal to noise or evidence of cross-talk were excluded from further analysis. Concentration changes were determined from the mini-CYRIL spectral data using the modified Beer-Lambert law [2] with a differential pathlength factor of 5.51 [6] that was corrected for the wavelength dependency of the pathlength [7]. To check that the occlusion period did not induce changes in the optical pathlength, a dynamic measured pathlength was calculated for a sub-set of data sets using the second differential of water spectral peaks [8].

Linear fitting was applied to the HbO_2 and oxCCO signals during the occlusion periods to determine their rate of change. These represent local muscle oxygen consumption. While the HbO_2 signal captures changes in haemoglobin/myoglobin -bound oxygen in capillaries, small blood vessels and skeletal myocytes, oxCCO represents changes in mitochondrial oxygen availability. Statistical analysis was performed in STATA15 (StataCorp LLC, USA). Participant characteristics are presented as n (%) if data are categorical and mean ± standard deviation is normally distributed. NIRS data are presented as median (interquartile range; IQR). Differences in mean and median values were determined using a paired Student's t-test or signed-rank test, respectively. The level of significance was set at $p < 0.05$. The rate of change of each NIRS signal was compared before/after exercise and before/after marathon training.

3 Results

Complete data sets at both BL and FU were collected from 14 participants. An example NIRS trace is shown in Fig. 1. Participant characteristics and cardio-respiratory fitness at BL and FU are shown in Table 1. Overall, there were no discernable changes in weight or peakVO_2 after marathon training.

The group median for rate of change in HbO_2 during occlusions at rest was more negative (higher consumption) after marathon training (BL median (IQR): −0.027 (−0.025, −0.035) μM/s versus FU: −0.041 (−0.032, −0.046) μM/s, $p = 0.004$; Fig. 2, top left). This was also the case for rate of change measured directly after the CPET (post-exercise) (BL: −0.037 (−0.028, −0.040) μM/s versus FU: −0.047 (−0.061, −0.036) μM/s, $p = 0.04$; Fig. 2, bottom left).

The group median for rate of change in oxCCO during occlusions at rest was more negative (higher rate of concentration change) after marathon training (B: −0.0012 (−0.0025, 0.0032) μM/s versus FU: −0.0037 (−0.0050, −0.0022) μM/s, $p = 0.02$; Fig. 2, top right). This was also the case for rates of change measured directly after the CPET (post-exercise) (BL: −0.0014 (−0.0057, 0.0049) μM/s versus FU: −0.0060 (−0.014, −0.002) μM/s, $p = 0.04$; Fig. 2, bottom right).

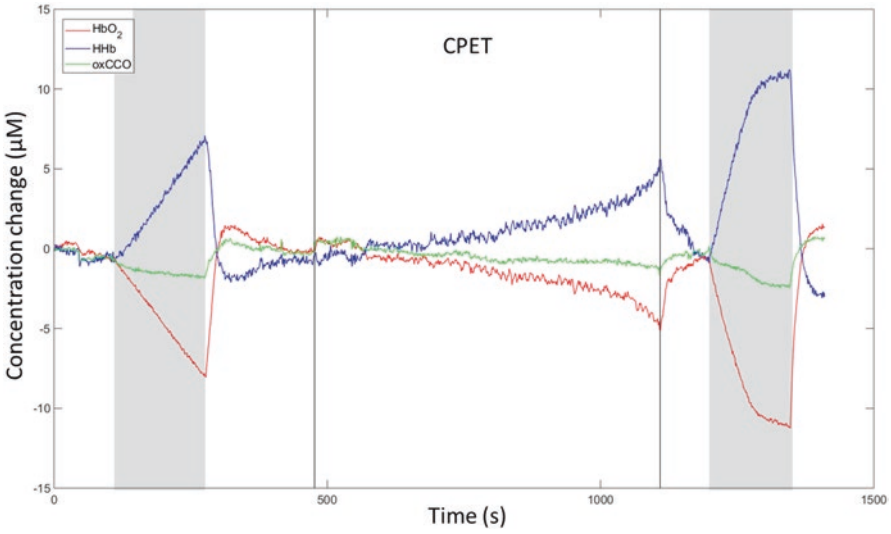

Fig. 1 Example NIRS data at FU during occlusions (grey) pre- and post-CPET (between black lines). Rate of change during occlusions- HbO_2: −0.04 and − 0.07 µM/s, oxCCO: −0.005 and − 0.014 µM/s

Table 1 Group characteristics of participants at baseline and follow-up. Data are mean ± standard deviation

	Baseline	Follow Up	p-value
Male n (%)	6 (43%)	–	–
Age (years)	43.4 ± .6	–	–
Height (cm)	175.4 ± 8.7	–	–
Weight (kg)	76.2 ± 16.7	75.2 ± 16.8	0.18
ATT (mm)	7.3 ± 1.9	6.6 ± 2.1	0.07
Peak VO_2 (ml/min/kg)	31.5 ± 5.7	31.0 ± 4.9	0.41

4 Discussion

We describe the novel application of broadband NIRS to measure changes in oxCCO and HbO_2 during arterial occlusions to estimate oxygen consumption in skeletal muscle. We found that, in middle-aged adults, endurance exercise training was associated with an increase in estimates of local muscle oxygen consumption in the absence of a detectable change in whole-body cardio-respiratory fitness (peakVO_2).

At rest, arterial occlusions induced a more rapid decline in HbO_2 after the period of endurance training (FU resting; Fig. 2, top left). A similar pattern was also seen during arterial occlusions made directly following a period of acute intense exercise (FU post-exercise; Fig. 2, bottom left), a finding that is in line with a previously described effect of marathon training on skeletal muscle oxygen consumption [9].

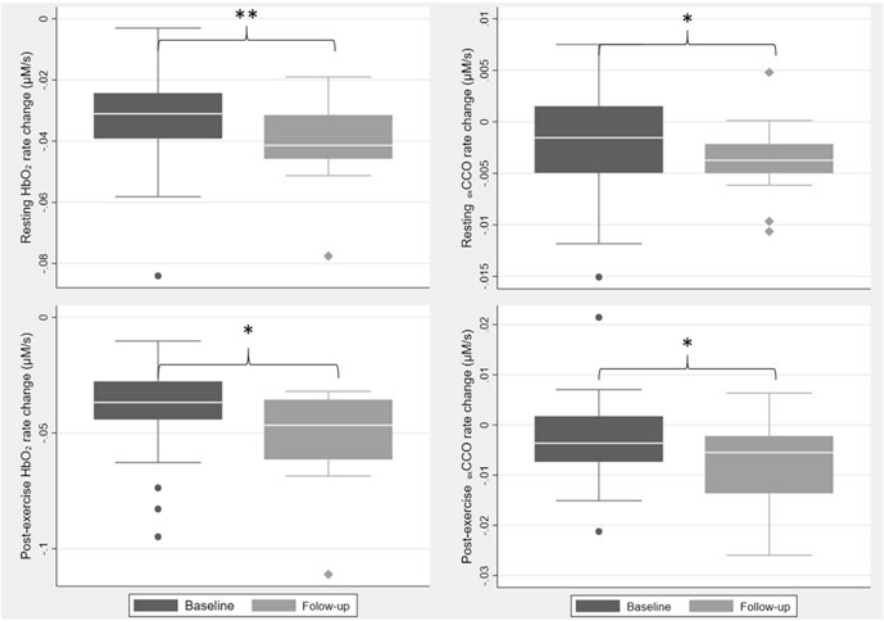

Fig. 2 Boxplots show median (IQR) rate of change of HbO_2 (left side) and oxCCO (right side) during occlusions before (BL, dark grey) and after (FU, light grey) marathon training. Top panels show resting (before CPET) and bottom panels show post-exercise (after CPET). $* p < 0.05$, $** p < 0.01$

We interpret this as an improvement in extraction and/or consumption of oxygen by skeletal muscle. The general view is that oxygen delivery is the limiting factor for oxygen consumption in muscle, but evidence exists that mitochondrial capacity scales with O_2 delivery [10], so the situation is likely more complex.

oxCCO declined more rapidly during occlusions after a period of endurance training both at rest and post-exercise test (FU resting & post-exercise; Fig. 2, right panels). We interpret this as a greater oxidative capacity in the muscle due to training since more rapid reduction in oxCCO indicates a quicker depletion of oxygen, because an absence of oxygen prevents electron transfer leading to a reduced CuA [11]. Further, in brain tissue it has been observed that cytochrome c oxidase (CCO) reduction occurs only under extreme hypoxia and hence, our findings may be an indicator of oxygen depletion in the muscle.

Surprisingly, we observed an increase in oxCCO in several individuals during arterial occlusions at rest (BL: n = 6, FU: n = 3) and post-exercise (BL: n = 7, FU: n = 2). Note that this was observed in fewer individuals at FU, so could be associated with the effects of marathon training. It is possible that these increases are artefacts, or too small to be physiologically relevant. However, these increases are substantial (on the order of 0.1 to 1 μM), and were observed when both a fixed pathlength and an estimated pathlength were used to resolve them. Furthermore, the NIRS intensity data were scrutinized for high SNR, residual analysis was used to check for

cross-talk, and there was no relationship between the ATT and the direction of oxCCO change. A possible explanation for this increase in oxCCO is an increase in nitric oxide (NO) due to ischaemia. NO competes with oxygen to bind to CCO, hence an increase in cellular NO will cause an apparent increase in oxidized CCO [12]. More studies are needed to confirm and understand this result.

There are several limitations to this study. First, while we inflated the cuff to more than 250 mmHg we cannot exclude the possibility of incomplete arterial occlusion in some individuals and, as we considered the change in HbO_2 only, we cannot be sure that volumetric shifts did not occur during the occlusion. Second, participants were not asked to complete a training diary in this study. Therefore, we do not have information about type or intensity of training. Finally, the sample size (n = 14) is small.

To conclude, in healthy middle-aged men and women preparing for a first marathon, training for a period of at least 16 weeks was associated with an increase in the ability of skeletal muscle to utilize oxygen, and an increase in the rate of mitochondrial oxygen depletion. Together these findings suggest an improvement in metabolic capacity with low-intensity endurance training. Metabolic adaptions can be identified non-invasively using broadband NIRS combined with arterial occlusions. However, further work in more subjects is warranted to investigate the changes in oxCCO observed.

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