ORIGINAL ARTICLE

Exercise over-stress and maximal muscle oxidative metabolism: a ^{31}P magnetic resonance spectroscopy case report

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23 November 2004**Objective:** ^{31}P magnetic resonance spectroscopy (MRS) was used to document long lasting losses in muscle oxidative capacity after bouts of intense endurance exercise.**Methods:** The subject was a 34 year old highly fit female cyclist ($\text{VO}_{2\text{MAX}} = 53.3 \text{ ml/kg/min}$). Over a five month period, she participated in three separate intense bouts of acute unaccustomed exercise. ^{31}P MRS measurements were performed seven weeks after the first bout and every two weeks for 14 more weeks. In all cases, ^{31}P MRS measurements followed three days after each bout.**Results:** The subject showed a decreased ability to generate ATP from oxidative phosphorylation and an increased reliance on anaerobic ATP production during the 70% and 100% maximal voluntary contractions after the exercise bouts. Increased rates of fatigue and increased indicators of exercise difficulty also accompanied these reductions in muscle oxidative capacity. Increased oxidative and anaerobic ATP production were needed to maintain the work level during a submaximal 45% maximal voluntary contraction exercise.**Conclusions:** Acute increases in intensity accompanied by a change in exercise mode can influence the ability of muscle to generate ATP. The muscles were less economical and required more ATP to generate force during the submaximal exercises. During the maximal exercises, the muscle's mitochondria showed a reduced oxidative capacity. However, these reductions in oxidative capacity at the muscle level were not associated with changes in whole body maximal oxygen uptake. Finally, these reductions in muscular oxidative capacity were accompanied by increased rates of anaerobic ATP production, fatigue, and indicators of exercise difficulty.

Unaccustomed exercise is known to result in significant damage to skeletal muscle in both trained and untrained people. Specifically, exercise in excess of that to which a muscle has become adapted can be termed acute unaccustomed stress (AUS). AUS can be an acute increase in volume, intensity, and/or mode of exercise. Resultant damage to the muscle can be structural¹ and/or metabolic.^{2,3} Although reduced metabolic function after AUS has been shown,^{2,3} the underlying causes remain unclear.

It has been shown that sustained elevated oxidative stress can have adverse effects on mitochondrial function. Hochli *et al*⁴ reported reductions in mitochondrial volume after the 600 km, 30 day, Paris-Dakar foot race. Asp *et al*⁵ reported increased muscle glycogen dependence during concentric exercises two days after fatiguing eccentric exercise, which they attributed to an increased dependence on anaerobic glycolysis. Hikida *et al*⁶ found evidence that marathon running induced muscle necrosis similar to rhabdomyolysis for up to seven days after the precipitating event. In that study, biopsies of the gastrocnemius revealed that mitochondria, erythrocytes, leucocytes, and other phagocytic cells had leaked into the extracellular and extravascular spaces. Taken together, these studies support the hypothesis that sustained oxidative stress will have adverse effects on mitochondrial function after long duration exercise bouts.

^{31}P magnetic resonance spectroscopy (MRS) can be used to evaluate both oxidative and anaerobic metabolism in exercising skeletal muscle. Numerous studies have shown that the net forward ATP production from the creatine kinase reaction, oxidative phosphorylation (OxPhos), and anaerobic glycolysis can be modelled from high time resolution ^{31}P magnetic resonance spectra.^{6–10} Unlike maximal enzyme

activities from biopsy samples, ^{31}P MRS provides a non-invasive in vivo measure of skeletal muscle metabolism while all of the physiological and biochemical control and feedback mechanisms are functioning.

Unfortunately, little is known about the effects of AUS on oxidative and anaerobic ATP production measured in such an in vivo system. Previous ^{31}P MRS studies have reported changes in the inorganic phosphate/creatine phosphate (P_i/PCr) ratios in resting muscle after muscle damage.^{11,12} There have also been reports of no change in P_i/PCr ratios in muscle during submaximal concentric exercise.¹² To our knowledge, ^{31}P MRS has not been used to measure muscle oxidative and anaerobic ATP production after weight bearing AUS bouts.

While studying reliability of ^{31}P MRS oxidative capacity measures, we observed a large fall in muscle oxidative capacity in an endurance trained cyclist after a severe bout of weight bearing AUS and two additional AUS bouts over five months to document her recovery after these AUS bouts. The purpose of this paper was to describe the metabolic changes in this athlete's muscle metabolic function after these AUS bouts.

METHODS

The involvement of human subjects was approved by the University of Alabama at Birmingham Institutional Review Board. This volunteer was screened and briefed about the

Abbreviations: AnMet, anaerobic metabolism; AUS, acute unaccustomed stress; MRS, magnetic resonance spectroscopy; MVC, maximum voluntary contraction; OxPhos, oxidative phosphorylation; PCr, creatine phosphate

Table 1 Body mass and VO_2MAX treadmill data

Week	Event	Body mass (kg)	VO_2MAX (ml/kg/min)	HR_{max} (bpm)	RER_{max}	Treadmill time (min)
0		52.0	53.3	178	1.16	10.1
1	AUS1					
7						
8	AUS2					
9		52.2	54.95	178	1.17	9.5
10						
12						
14						
16		53.4	53.4	177	1.21	9.4
17	AUS3					
18		52.2	53.2	178	1.16	9.6
20						
22						

VO_2MAX , Maximal oxygen uptake; HR_{max} , maximal heart rate at maximal exercise; bpm, beats/min; RER_{max} , maximal respiratory exchange ratio at maximal exercise; AUS1, 35.5 km Grand Canyon rim to rim run; AUS2, 10 km road race; AUS3, 18 mile marathon training weekend.

experimental protocol, and informed consent was obtained before testing.

The subject was a 34 year old endurance trained female cyclist (maximum oxygen uptake (VO_2MAX) = 53.3 ml/kg/min). She was in top cycling shape and had been competing recreationally in endurance cycling events. She was 163 cm tall with a body mass, body mass index, and body fat percentage of 52 kg, 19.6 kg/m², and 13.8% respectively. She was not taking any drugs known to affect metabolism. The subject followed a high carbohydrate, moderate fat vegetarian diet throughout the course of this study. Her body weight remained constant (± 1 kg). She followed her usual training regimen throughout the study (approximately 5.5 hours a week of stationary or road cycling and regular cross training with a weight bearing activity of running two or three days a week averaging 10 miles a week).

The subject was followed over a five month period during which muscle (gastrocnemius/soleus) mitochondrial function and VO_2MAX were evaluated periodically. Three separate AUS bouts occurred during this five month period. The first (AUS1) was a Grand Canyon "rim to rim" run/hike completed in 5.5 hours (approximately a 35.5 km total running distance over rough terrain with an estimated 1525 m descent and 1830 m ascent). This exercise bout corresponded to about 10 times the normal training volume of the subject.

The second AUS bout (AUS2) was associated with a 10 km race seven weeks after AUS1 (about twice the normal training volume). This road race started with a downhill grade, incorporated several large ascents and descents, and ended with an uphill climb (an estimated total vertical change in elevation during the race of over 700 feet). The subject completed the race in 51 minutes and 59 seconds. She reported feeling "awful" afterwards.

The third AUS bout (AUS3) was an 18 mile weekend of running 14 weeks after the initial AUS1 event (two or three times the normal training volume). This 18 mile training weekend coincided with the subject's first time marathon training period. The weekend started with a 13.1 mile half marathon road race and ended with a 5 mile recovery run the next day. Both of these runs occurred on a very hilly asphalt course with multiple ascents and descents. Before this weekend, the subject's longest run had been a 10 mile run the weekend before.

Measurements of whole body VO_2MAX and body composition

Body composition and VO_2MAX were measured before the start of the study. These measurements were repeated

periodically during the study in the morning after an overnight fast. VO_2MAX was determined by indirect calorimetry on a treadmill using a modified Bruce protocol to exhaustion as described in detail elsewhere.^{8,9} The highest oxygen uptake, respiratory exchange ratio, and heart rate achieved were recorded. Plateauing occurred in all tests, showing that VO_2MAX was reached in all tests. Body composition was determined by hydrostatic weighing. Residual lung volume was measured simultaneously by the closed circuit O_2 dilution method.¹³ A correction factor of 0.1 litre was used for gastrointestinal gas. Percentage body fat was calculated from body density using the Siri formula.¹⁴

Analysis of skeletal muscle metabolism from ^{31}P MRS

Magnetic resonance images and ^{31}P magnetic resonance spectra were collected on a 4.1 T whole body imaging and spectroscopy system as previously described.^{8,9,15,16} The subject was requested to fast and abstain from caffeinated beverages for at least six hours, and from exercise for at least 24 hours before testing. The subject performed unilateral isometric plantar flexion exercises using a laboratory constructed exercise bench attached to the patient table of the spectrometer. Isometric plantar flexion force was measured with this device as previously described.^{8-10,15}

Figure 1 Total work output (force-time integral) during the submaximal (45% maximal voluntary contraction (MVC)), near-maximal (70% MVC), and maximal (100% MVC) exercises. AUS1, 35.5 km Grand Canyon rim to rim run; AUS2, 10 km road race; AUS3, 18 mile marathon training weekend.

Figure 2 Anaerobic ATP production normalised to total work output during a 90 second isometric plantar flexor exercise at (A) submaximal 45% maximal voluntary contraction (MVC), (B) near-maximal 70% MVC, and (C) maximal 100% MVC. Anaerobic ATP production is defined as the sum of the anaerobic glycolysis and creatine kinase ATP production rates. AUS1, 35.5 km Grand Canyon rim to rim run; AUS2, 10 km road race; AUS3, 18 mile marathon training weekend.

In-magnet exercises consisted of three 90 second isometric plantar flexion exercises at 45% of theoretical maximum voluntary contraction (MVC), 70% of MVC, and 100% of actual MVC. The 45% MVC exercise represented a submaximal exercise bout, the 70% MVC exercise represented a controlled near-maximal exercise bout, and the 100% MVC exercise represented a maximal exercise bout. Each exercise was separated by a 15 minute recovery period. The theoretical MVC force levels were determined from the maximum cross sectional areas of the gastrocnemius/soleus muscles determined from magnetic resonance images as previously described.⁶⁻¹⁰

As previously described, a 7 cm $^1\text{H}/^{31}\text{P}$ surface coil was used to collect two second, time resolved ^{31}P MRS data during 60 seconds of rest, 90 seconds of exercise, and 7.5 minutes of recovery.⁸⁻⁹⁻¹⁵ The coil was fastened to the underbelly of the calf muscle centred about its maximum

circumference. ^{31}P MRS data were collected using the following parameters: TR = 2000 milliseconds, four dummy pulses, one average, and a 90° adiabatic excitation pulse. All peak areas were corrected for saturation using a saturation factor determined for that subject on that experimental day by collecting an unsaturated spectrum of resting muscle (one average, TR = 25 seconds) and a partially saturated spectrum (one average, TR = two seconds, four dummy pulses). All ^{31}P spectra were analysed using time domain fitting as previously described.⁶⁻¹⁰⁻¹⁵ The net forward ATP production rates from the creatine kinase reaction, oxidative phosphorylation (OxPhos), and anaerobic glycolysis were calculated using the methods of Boska *et al*⁶⁻⁷ and Newcomer *et al*.¹⁰⁻¹⁵

In brief, creatine kinase ATP production rate can be calculated from the breakdown of PCr at any time point during exercise. We calculated the creatine kinase rate during the last 14 seconds of exercise. Our model assumes that the OxPhos rate can be calculated from the initial 14 seconds of the PCr recovery after the end of exercise. The choice of a 14 second time window for the calculation of the initial PCr recovery rate eliminates the effects of blood reflow in the muscle on the PCr recovery rate.¹⁷ The calculation of the anaerobic glycolysis rate is based on the rate of change in pH and PCr in the muscle during the last 14 seconds of exercise. Detailed discussions of these calculations have been published.⁶⁻⁷⁻¹⁰⁻¹⁵ Finally, the amount of anaerobic metabolism (AnMet) is defined as the sum of the creatine kinase and anaerobic glycolysis ATP production rates.

RESULTS

The subject reported experiencing extreme delayed onset muscle soreness immediately after her Grand Canyon run/hike (AUS1) which persisted for over seven days. Delayed onset muscle soreness was not experienced before any of the ^{31}P MRS measurements. However, the subject did report a diminished sense of wellbeing after all AUS events and consistently described feelings of muscle burning and a lack of power during physical effort and exercise training.

Table 1 gives physiological measurements. VO_2MAX and body composition remained unchanged during the 22 weeks of study. However, there was a slight reduction in treadmill times after the AUS bouts. Anecdotally, this subject also reported an increased perceived exertion during VO_2MAX testing (personal observations).

Figure 1 illustrates the total work output (force-time integral) during the 45%, 70%, and 100% MVC exercises. This subject was able to maintain total work levels during the 45% MVC exercises, but was unable to maintain the original work levels during the 70% and 100% MVC exercises. Consequently, ATP production rates in the following figures were normalised to total work output to compensate for these reduced exercise levels. This allows us to compare ATP production rates between exercises with different exercise outputs.

Figure 2 illustrates the AnMet rates for the 45%, 70%, and 100% MVC exercises displayed as a percentage of the baseline value for that exercise level. An increase in AnMet was associated with the AUS2 bout at each exercise level. However, only the 70% (fig 2B) and 100% (fig 2C) MVC exercises showed an increased AnMet after the AUS3 bout. Seven weeks after the AUS1 event, this subject needed an increased AnMet rate to perform the 100% MVC exercise (fig 2C). Finally, we see an overall trend towards a reduced reliance on AnMet during the five month study at all three exercise levels.

Figure 3 illustrates the OxPhos rates for the 45%, 70%, and 100% MVC exercises displayed as a percentage of the baseline value for that exercise level. After the AUS2 and AUS3 bouts,

Figure 3 Oxidative phosphorylation ATP production normalised to total work output during a 90 second isometric plantar flexor exercise at (A) submaximal 45% maximal voluntary contraction (MVC), (B) near-maximal 70% MVC, and (C) maximal 100% MVC. AUS1, 35.5 km Grand Canyon rim to rim run; AUS2, 10 km road race; AUS3, 18 mile marathon training weekend.

an increase in OxPhos was needed to perform the 45% MVC exercise (fig 3A). Conversely, a reduced OxPhos rate was observed during the 70% (fig 3B) and 100% (fig 3C) MVC exercises after the AUS2 and AUS3 bouts. An overall trend towards an increased reliance on OxPhos was observed during the five month study in the 45% and 70% MVC exercises.

DISCUSSION

Our data suggest that acute muscle stress may have long term effects on the muscle's ability to generate ATP oxidatively. Several studies looking at trained runners after extreme endurance running have shown decreases in muscle function several days after the event.^{2-5, 18-19} Our subject was a well trained cyclist whose aerobic system was accustomed to exercise over-stress, but her plantar flexors were not accustomed to the weight bearing running exercises of the AUS bouts.

Consequently, the AUS bouts induced a decreased OxPhos rate and an increased AnMet reliance during the 100% MVC exercise up to seven weeks after the initial AUS1 bout. Although our results are anecdotal, we believe this reduction in OxPhos and resulting increase in AnMet is a result of the severe damage induced by the Grand Canyon run/hike. We arrived at this conclusion on the basis of a number of arguments. Firstly, our measure of OxPhos is normally very stable, with a coefficient of variation of only 5.1% for test-retests.⁸ Consequently, maximal OxPhos rates after the AUS bouts decreased by over 50% and far exceed what would be expected by simple random fluctuations in the data. Secondly, the AUS bouts stressed the plantar flexor muscles for more than twice the normal training duration for this subject. In fact, the AUS1 bout was 10 times the normal training volume. Finally, after the subject had recovered from the intense muscle soreness caused by the AUS1 bout, she still reported that she had no muscle "power". Even seven weeks after the AUS1 event, she reported that she could cycle the same distance at a moderate pace but had no power to sprint or "attack" a hill. She also reported burning feelings in her legs during her bike rides and reported a general sense of being "wiped". Taken together, we feel that these anecdotal reports of a continued diminished sense of wellbeing for the seven weeks after the AUS1 event link the reduction in OxPhos to the initial AUS1 event. Further investigation is needed to replicate this kind of extreme exercise bout and follow the time course of the recovery to prove that the OxPhos reductions seen at the seven week time period are linked to AUS1.

For the less severe AUS2 bout, the subject showed a decrease in the OxPhos rates and an increased reliance on AnMet during the 70% and 100% MVC exercises. Furthermore, a slight decrease in the OxPhos rate and an associated slight increased reliance on AnMet during the 70% MVC exercise was observed after the AUS3 bout. Once the exercise intensity was increased to the 100% MVC level, these changes become larger after the AUS3 bout. This may reflect muscle adaptation to the acute exercise stresses by the time of the AUS3 bout. Overall, these decreases in OxPhos and increased reliance on AnMet are a result of this trained cyclist's incorporation of running into her training regimen. These changes imply that the muscle has been forced to shift towards more anaerobic pathways during the maximal exercises as a result of the injury sustained during the AUS bouts.

Interestingly, this subject also needed increased OxPhos and AnMet to maintain their force output during the 45% MVC exercise. This suggests that her muscles needed a greater amount of ATP to produce the same relative submaximal work output. This reduction in metabolic efficiency may be a consequence of the damage induced by the AUS.

Using ³¹P MRS we have shown for the first time that maximal and near-maximal muscle OxPhos rates can be reduced after acute fatiguing exercise stress. These OxPhos reductions were associated with increases in AnMet and occurred when a clear increase in exercise stress occurred. The greatest decrease in OxPhos was after the Grand Canyon run/hike (AUS1). This represented the most extreme intensity of the three AUS events and is well in excess of what most people experience during physical activity. This reduction in oxidative metabolism appears to have lasted well over seven weeks and was associated with the subject's self report of a reduced sense of wellbeing. The metabolic changes that followed the AUS2 and AUS3 bouts were not as long lasting as those experienced after the AUS1 event. This may be due to the AUS2 and AUS3 bouts being less stressful than the AUS1 event. We speculate that our measured reductions in oxidative phosphorylation may be due to compromised

What is already known on this topic

Unaccustomed exercise is known to result in significant damage to skeletal muscle in both trained and untrained people. This damage can be structural and/or metabolic and has traditionally been studied through biochemical analysis of invasively acquired biopsy tissue. The underlying mechanisms of the documented metabolic function reductions after unaccustomed exercise remain unclear.

mitochondrial function during or after the exercise. These compromised functions may have been due to oxidative free radical damage, mitochondrial membrane disruption, and/or damage to the capillaries or other vasculature.

Increases in free radicals have been implicated in losses in membrane integrity of the mitochondria.²⁰ Tonkonogi *et al*²¹ have postulated that there may be damage or altered composition/structure of the mitochondrial membrane after prolonged exhaustive exercise. Loss of calcium (Ca^{2+}) homeostasis in skeletal muscle cells has the potential to initiate several detrimental consequences. High intracellular Ca^{2+} concentrations may promote degradation of cellular structures,²² increased Ca^{2+} uptake by mitochondria,^{23 24} and increased activation of Ca^{2+} activated proteases.²⁵

As in the Paris to Dakar race,² no decrease in $\text{VO}_{2\text{MAX}}$ accompanied the decreases in muscle oxidative capacity, suggesting that whole body $\text{VO}_{2\text{MAX}}$ was not limited at the skeletal muscle level. These data suggest that, in the plantar flexors, if not in all skeletal muscle, a significant reserve in mitochondrial capacity exists. This lends support to di Prampero's²⁶ hypothesis that $\text{VO}_{2\text{MAX}}$ is limited by the ability of the cardiovascular system to deliver oxygen to the working muscles rather than by the metabolic capacity of the muscles.²⁷

A pronounced decline in mitochondrial function appears to be a specific metabolic feature of acute unaccustomed exercise stress. This decreased oxidative capacity may affect a person's participation in further strenuous exercise. This phenomenon may serve a physiological purpose by allowing the mitochondria adequate time to recover and adapt without loss of submaximal function. With this information, training programmes can be developed for athletes to enhance recovery and performance. More importantly, this condition may serve as a physiological explanation of why so many people drop out of, or have difficulty adhering to, exercise programmes. More research is needed to determine if the intensity and volume of training programmes for those beginning to exercise can be modified to increase adherence to these programmes.

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What this study adds

This study provides a non-invasive in vivo measure of skeletal muscle metabolism after unaccustomed exercise bouts while all of the physiological and biochemical control and feedback mechanisms are functioning. It shows that acute increases in intensity accompanied by a change in exercise mode can influence the ability of muscle to generate ATP. Reductions in muscular oxidative capacity were found after these unaccustomed exercise bouts and were accompanied by increased rates of anaerobic ATP production, fatigue, and indicators of exercise difficulty.

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