Endurance exercise training blunts the deleterious effect of high-fat feeding on whole-body efficiency

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Running head: High-fat feeding and trained men
Abstract

We recently showed that a week-long high-fat diet reduced whole-body exercise efficiency in sedentary men by > 10%. To test if a similar dietary regime would blunt whole-body efficiency in endurance-trained men and, as a consequence, hinder aerobic exercise performance, sixteen endurance-trained men were given a short-term high fat (70% kcal from fat) and a moderate carbohydrate (50% kcal from carbohydrate) diet (MCD), in random order. Efficiency was assessed during a standardised exercise task on a cycle ergometer, with aerobic performance assessed during a one-hour time trial and mitochondrial function later measured using $^{31}$P-magnetic resonance spectroscopy. The subjects then underwent a two-week wash-out period, before the study was repeated with the diets crossed-over. Muscle biopsies, for mitochondrial protein analysis, were taken at the start of the study and on the fifth day of each diet. Plasma fatty acids were 60% higher on the high-fat diet (HFD) compared with MCD ($p < 0.05$). However, there was no change in whole-body efficiency and no change in mitochondrial function. Endurance exercise performance was significantly reduced ($p < 0.01$), most probably due to glycogen depletion. Neither diet led to changes in citrate synthase, ATP-synthase or the mitochondrial uncoupling protein (UCP3). We conclude that prior exercise training blunts the deleterious effect of short-term high-fat feeding on whole-body efficiency.

Keywords: Exercise, magnetic resonance, nutrition, mitochondria
Introduction

The maximum external work rate that can be sustained by a muscle or group of muscles is determined by the rate at which adenosine triphosphate (ATP) can be supplied to the myofibrils, with the overwhelming majority of ATP used during sustained exercise being derived from oxidative phosphorylation at the mitochondria. The maximum sustainable rate of mitochondrial ATP supply is in turn dependent on the rate of delivery of oxygen and reduced intermediates to the respiratory chain and the mitochondrial phosphorylation to oxidation ratio (P/O), which describes the economy of energy transduction.

The major metabolic fuel sources used by the muscle cell to generate reduced intermediates, and thus ATP, are glucose and fatty acids, with substrate preference being determined by different circumstances. For example, glucose oxidation is stoichiometrically more oxygen-efficient than fat oxidation (16), and glycogen breakdown is able to supply ATP at a faster rate than other substrates (32), making it the best fuel when a high sustainable power is required or when oxygen supply is limited. However, energy yield per mole of glucose is low compared to fatty acids, as is the body’s total storage capacity for glycogen and glucose (32), thus in situations where energetic demands are sub-maximal but prolonged, and when oxygen is plentiful, fatty acids are the preferred choice.

Moreover, free fatty acids (FFA) acutely increase proton leak in isolated mitochondria (33), dissipating the proton gradient as heat, and decreasing the phosphorylation to oxidation ratio to a greater extent than that expected from a substrate switch alone. Although the exact mechanism remains poorly understood, several proteins are thought to be important in mediating this loss of efficiency, including uncoupling protein 3 (UCP3) (10) and the adenine
nucleotide translocase (ANT) (29), both of which act to increase futile proton cycling when activated by FFA (2, 3, 10). Furthermore, prolonged exposure of muscle cells to increased FFA activates the fat responsive transcription factor PPARα thereby increasing the expression of fat metabolism genes including UCP3(27). Thus, exposure to elevated plasma FFAs by high-fat feeding might cause a loss of efficiency in skeletal muscle due to the stoichiometry of fatty acid oxidation, and both the acute activation of proton leak in mitochondria and the increased expression of UCP3 (12, 26), which could be reflected in decreased whole-body efficiency and reduced physical performance.

The relationship between mitochondrial and whole-body efficiency can best be understood by considering the intact human as a thermodynamic machine, an approach that was exemplified by Whipp and Wasserman in their seminal 1969 paper (38). In this context, efficiency is equal to the fraction (or percentage) of energy liberated during chemical catabolism of metabolic substrates that is transduced to external work. The remainder is lost as heat. There are several key transduction steps that connect the oxidation of substrates to the performance of external work, any or all of which will influence overall efficiency. These are biomechanical efficiency (or effectiveness), contractile efficiency (in other words, the energy produced by the contractile proteins per mole ATP), mitochondrial efficiency (the ATP/O₂ ratio) and the energy equivalence of oxygen (which is a property of the metabolic substrate(s)). Excluding biomechanical efficiency, this can be written as:

\[
\text{Efficiency (\%) = 100\% \times contractile efficiency (J/mol ATP)}
\times \text{mitochondrial efficiency (ATP/O₂) \times energy equivalence of oxygen (J/mol O₂)}
\]

Thus an increase in mitochondrial ATP/O₂ increases ATP supply to the contractile proteins for a given O₂, so that more contractile work can be performed.
Given its role in determining whole-body efficiency, reduced muscle mitochondrial efficiency has several important physiological and clinical implications. In human subjects whose oxygen transport or delivery is compromised (for example, patients with anaemia or lung disease) the efficiency with which the muscle mitochondria can utilise a scarce resource (oxygen) may be a key determinant of quality of life. Yet another application of changed muscle mitochondrial efficiency is in the realm of athletic performance, where whole-body efficiency is a key determinant of aerobic performance (8). We have recently shown that short-term high-fat feeding impaired physical performance in rats alongside increased skeletal muscle mitochondrial uncoupling and UCP3 content (26) and subsequently showed that a week of high-fat feeding blunted whole-body efficiency in sedentary men by more than 10% (12). There are obvious and important metabolic differences, however, between exercise-trained individuals and those who are sedentary. For example, endurance-trained subjects have higher mitochondrial density and lower expression of UCPs compared with sedentary controls (22), and this correlated with improved cycling efficiency. We therefore examined the effect of short-term high-fat feeding on whole-body efficiency, mitochondrial function and aerobic performance in a cohort of endurance-trained men. We hypothesised that such a diet would raise plasma FFA and, as a result, decrease mitochondrial efficiency and aerobic exercise performance.
Subjects and Methods

Subjects

We recruited sixteen endurance-trained men from the Oxford University rowing crews. This study was approved by the Central Oxfordshire Research Ethics Committee and was conducted in accordance with the principles outlined in the Declaration of Helsinki. Fully informed written consent was obtained from all subjects prior to the intervention. Twelve subjects consented to a muscle biopsy, although two subsequently withdrew their consent.

Experimental design

Subjects attended the Oxford Centre for Clinical Magnetic Resonance Research (OCMR) for an initial assessment. Subjects were screened to exclude impaired glucose tolerance, diabetes, hypercholesterolemia, thyroid dysfunction, abnormal hepatic and renal function, and standard magnetic resonance contraindications. A physical examination was performed to exclude major cardiac, respiratory or abdominal pathology and ensure baseline heart rate, blood pressure and electrocardiograms were normal. At this initial visit, subjects’ peak aerobic capacity (\(\dot{V}\text{O}_2\text{peak}\)) was measured as described previously (12). The percentage of \(\dot{V}\text{O}_2\text{max}\) at which the ventilatory threshold occurred was calculated according to the V-slope method (4).

After one week, subjects were started on the main protocol commencing with three days of a standardised, normal human diet, on day two of which a biopsy was taken from most subjects’ left or right vastus lateralis. Biopsies were taken under local anaesthetic using a modified Bergstrom technique that has been described elsewhere (15), immediately frozen in liquid nitrogen and stored at -80 °C. On the morning of day three, fasted subjects were transported to OCMR and where a venous blood sample was collected, after which subjects
were transferred to the exercise physiology laboratory where they performed a structured warm-up followed by a one-hour time trial on the bicycle ergometers. The subjects were then randomly assigned to either a high-fat (identical in macronutrient composition to the one we used earlier) or moderate-carbohydrate diet (Table 1) for five days and the blood collection, warm-up and exercise testing were repeated as described above for the four remaining days. Diet adherence and composition was assessed post-hoc using nutritional assessment software (DietPlan 6, Forestfield Software Ltd, West Sussex, UK). On day five of the diet intervention, another biopsy was taken. There was then a ‘wash-out’ period of two weeks, after which the protocol was repeated, with the diets crossed over.

Measurement of whole-body efficiency

The protocol used to measure gross and delta efficiency was similar to that described previously (11, 12). Testing was performed on a CycleOps ergometer (CycleOps Pro 300PT, Saris Cycling Group, Inc., Madison, Wisconsin, USA). Subjects performed a structured warm-up that consisted of three 5-minute work periods at 50 W, 100 W and 150 W, without break, in ascending order, (9, 22, 24). Cadence was fixed at 90 rev min⁻¹ for all testing. Expired gases were collected breath-by-breath using a Metamax portable gas analyser (Cortex Biophysik, Germany). In all cases, data used were the mean averages taken over the last minute at each workload. Metabolic cost was calculated using the equation in (24):

\[
\text{Metabolic cost} = \frac{\text{work done}}{\text{metabolic cost}}.
\]

Power output was measured directly and continuously at the ergometer’s back wheel using a PowerTap hub (14, 30). Gross efficiency was calculated at each work rate as: gross efficiency (%) = 100% × (work done/metabolic cost). Delta efficiency was calculated as the slope of the linear regression of the relationship between metabolic cost and external work done (24).
Aerobic exercise performance testing

Aerobic exercise performance was assessed during a one-hour time trial on the bicycle ergometer. After the structured warm-up described above, each subject was given a starting power output for the time trial. On day one, this target was set as 60% of each subject’s peak one-minute power from the maximal test. On subsequent days it was 10 W less than the mean power achieved during the time trial on the day before. For the first eight minutes of the test, power was fixed to these targets. After this initial period, subjects were free to vary workrate as necessary. Power output and gas analysis data were recorded at one-second intervals on the ergometer computer and downloaded later for analysis. Capillary blood was collected from an earlobe at 25 and 50 minutes. The sample was immediately used to determine the subjects’ blood lactate concentrations using a small handheld device (Lactate Pro, Arkay Ltd. (31)).

Magnetic resonance spectroscopy exercise/recovery protocol

The magnetic resonance protocol was similar to one described previously (13). Subjects performed a series of plantar flexion exercises while in the bore of a 3 Tesla Siemens Trio clinical magnetic resonance system. Subjects lay supine in the magnet with their foot secured to a custom-built plantar flexion ergometer. A dual-tuned $^{31}$P and $^1$H surface coil was placed under the widest part of the right gastrocnemius in a specially designed wooden housing. The subject was immobilised with the leg straight, and strapped across the shins, knees, hips and shoulders. The exercise protocol consisted of 5 minutes of rest followed by three bouts of exercise, each 5 minutes long, interspersed with 7-minute recovery periods. The workrates, which were established during pilot studies, were 4, 5 and 6 W at a pedal rate of 1 Hz. The subjects were continuously monitored; no subject had any difficulty keeping time, although
one subject was unable to complete the exercise bouts. The first exercise bout was treated as a warm-up and familiarisation effort; reported values are the means of the bouts at 5 and 6 W.

**Magnetic resonance acquisition protocol and calculations**

Spectra were acquired as described previously (13). All spectra were processed using jMRUI version 2.2 (28) and quantified using a non-linear least squares algorithm (AMARES(37)).

Resting ATP and total creatine concentrations were assumed to be 8.2 mM·L⁻¹ and 42 mM·L⁻¹ respectively (20). These commonly-used concentrations are based on extensive published values, and are reliable in healthy humans (20). The chemical shift of the inorganic phosphate (Pi) peak relative to phosphocreatine (PCr) (σ, in parts per million) was used to determine intracellular pH, according to the equation:

\[
pH = 6.75 + \log\left(\frac{\sigma - 3.27}{5.63 - \sigma}\right)
\]

In the absence of large changes in pH, the time taken for muscle PCr to recover halfway to its resting value after a bout of moderate exercise was taken as an inverse index of mitochondrial function (19).

In order to estimate contractile economy, mono-exponential functions were fitted to the time course of [PCr] at exercise onset. As there is a time lag before other metabolic processes make a significant contribution at exercise onset, the initial rate of PCr hydrolysis can be used to estimate the total ATP-cost of the work being done (\(J_{ATP}\)) (17):

\[
J_{ATP} = \frac{0.008 \text{ mM s}^{-1}}{\text{external workrate}}
\]

where 0.008 mM s⁻¹ is an estimate of the resting ATP turnover of skeletal muscle (5). Contractile economy (in mM J⁻¹) can then be calculated as \(J_{ATP} / \text{external workrate} \).
Plasma FFA and immunoblotting

Fasting venous blood samples were taken from each subject before commencing the diets and at the end of the diet period. Samples were immediately centrifuged and the plasma supernatant frozen with a final concentration of 30 μg/ml lipoprotein lipase inhibitor (tetrahydrolipstatin, Xenical, Roche) for determination of free fatty acids (FFAs). Plasma concentrations of FFA were measured using an ABX Pentra Clinical Chemistry bench-top analyser (Horiba ABX, Montpellier, France). Abundance of metabolic proteins was measured in muscle biopsy lysates by immunoblotting as described in (25). Muscle UCP3 was detected using Chemicon AB3046 (Millipore UK) at a concentration of 1:1000 in 5% BSA. The specificity of this antibody was confirmed using tissue from two transgenic mouse strains (UCP3KO and UCP3tg, data not shown). All other antibodies were as described in (12).

Statistical analysis

Data were tested for normality using Shapiro-Wilk. Where data were available for all five days of each protocol (for example, aerobic performance), differences were tested for significance using a 5 × 2 repeated-measures analysis of variance (ANOVA). Day-by-day differences were subsequently tested using paired t-tests. When day-1 (pre) and day-6 (post) data only were available (for example, 31P NMR measurements) differences were assessed using a two-way repeated-measures ANOVA. For unpaired data that failed the tests of normality, a Mann-Whitney U test was used. Statistical analyses were conducted using PASW 18.0 (SPSS Inc., Chicago, Illinois, USA). All data are reported as mean ± SEM with alpha = 0.05.
Results

Descriptive measures, diet adherence and fasting plasma FFA

Subjects were aged 22 ± 1 years, weighed 82 ± 2 kg and had a mean peak oxygen uptake of 4.7 ± 0.2 L·min⁻¹, confirming their trained status (Table 1). There was no significant difference between the total caloric content of the two diets. Calories derived from fats in the high-fat diet comprised 74% of total intake (the target was 70%), while calories from carbohydrates comprised ~50% of total intake (the target was 50%) on the moderate-carbohydrate diet (Table 2). As a result of the high-fat diet, plasma FFA were 60% higher compared with the moderate-carbohydrate diet ($p < 0.01$) (Figure 1). There was no significant effect of either diet on body mass (data not shown).

Aerobic exercise performance and whole-body efficiency

The high-fat diet significantly reduced exercising RQ when measured at the three standardized workrates of 50W (0.75 ± 0.01 vs. 0.86 ± 0.01), 100W (0.78 ± 0.01 vs. 0.90 ± 0.02) and 150W (0.81 ± 0.01 vs. 0.92 ± 0.02, all $p < 0.001$) (Figure 2A). As would be expected, this tended to increase VO₂ at all three work rates, becoming significant at 150 W (2.6 ± 0.1 vs. 2.5 ± 0.1 L min⁻¹, $p < 0.05$). We then calculated the oxygen uptake that would be required at each workrate based solely on the new, lower RQ. There was no disparity between the predicted and actual VO₂ (Figure 2C) so that the increased VO₂ we observed could be entirely explained by the altered RQ. Consequently there was no significant effect of diet on whole-body efficiency at any workrate (50W: 11 ± 0.6% (HFD) vs. 11 ± 0.3% (MCD); 100W: 15 ± 0.4% vs. 16 ± 0.2%; 150W: 18 ± 0.4 vs. 18 ± 0.3%; all $p > 0.05$) (Figure 2B). Likewise, delta efficiency was not systematically different between diets (26 ± 1% (HFD) vs. 27 ± 1% (MCD), $p = 0.19$). Performance in the 1-hour time trials was significantly poorer from day 2 on the high-fat diet (Day 5: 215 ± 13W (HFD) vs. 243 ± 11W...
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(MCD), \( p < 0.001 \), with a correspondingly lower blood lactate concentration during exercise diet (Day 5: 1.6 ± 0.1 mM (HFD) vs. 2.9 ± 0.4 mM (MCD), \( p < 0.01 \)), highly significant when tested across the four days of the dietary intervention using a repeated-measures ANOVA (\( p = 0.001 \)), as well as on separate days.

**Skeletal muscle phosphorus metabolism and pH**

The high-fat diet had no significant effect on phosphorus metabolism, although resting \([\text{PCr}]\) was raised marginally after both diets (for example, pre-HFD: 29.1 ± 0.5 vs post-HFD: 29.6 ± 0.4 mM, \( p < 0.05 \)). Likewise, resting pH decreased fractionally by 0.3% after both diets (\( p < 0.05 \)). Many previous studies have shown \( ^{31} \text{P}-\text{MRS} \) measurements to be stable and reproducible in healthy subjects in the absence of interventions, and so these small changes presumably represent minor metabolic adjustments resulting from the repeated exercise testing. The only significant difference between diets was an increase in exercising pH from 6.98 ± 0.24 to 7.03 ± 0.13 on the high-fat diet (\( p < 0.05 \)). There was no difference in contractile efficiency between the diets (HFD: 67 ± 5 vs. MCD: 70 ± 4 μM ATP / J). There were no effects on muscle PCr recovery halftime, which was 20 sec throughout. An example of typical phosphocreatine recovery kinetics on both diets in a single subject is shown in Figure 3.

**Skeletal muscle protein content**

UCP3, citrate synthase and ATP-synthase protein contents in whole muscle remained unchanged as a result of high-fat feeding.
Discussion

Whole-body efficiency is a key determinant of endurance exercise performance (8) with a substantial part of the variation in the physical performance of runners with similar aerobic capacities explained by their running economy (closely related to their whole-body efficiency) (23). Furthermore, in conditions in which oxygen supply may be limited (for example, at high altitude or in hypoxic disease states), a reduction in the oxygen cost of doing work may be expected to produce improvements in performance (21). In patients suffering from diseases such as cyanotic congenital heart disease, chronic obstructive pulmonary disease or anaemia, therapeutic interventions to improve whole-body efficiency may subsequently improve morbidity and mortality.

We previously found that a week of high-fat feeding reduced whole-body efficiency by ~13% in sedentary men (12) and this has both clinical and human performance implications. Clinical implications aside, however, generalising findings in a cohort of sedentary subjects to a trained population is often unwise, given the well-documented changes in physiology and metabolism that result from training. Therefore we sought to test the hypothesis that a short-term high-fat diet identical in macronutrient composition to the one we used previously (~75% calories from fats with minimal (<2%) carbohydrate) would have the same detrimental effect on whole-body efficiency in endurance-trained as in sedentary men, and that this loss of efficiency would blunt aerobic exercise performance. We therefore measured performance during a one-hour time trial to investigate possible changes in endurance performance (18).

In the present study, a week of high-fat feeding raised plasma FFA by approximately 60% compared with a moderate carbohydrate diet. The diet was therefore even more effective at
raising plasma FFA in this group than in our earlier sedentary cohort (in our earlier sedentary cohort a similar absolute increase from a higher baseline value represented an approximately 40% rise (12)). As might be expected, the increase in plasma FFA was reflected in a significantly reduced RQ when exercising at all three work rates (50, 100 and 150 W) demonstrating a shift in whole-body substrate preference, away from glucose and toward fatty acids. Yet despite a significant increase in plasma FFA and a reduction in RQ at all workrates, we were unable to detect a decline in whole-body efficiency in the endurance-trained subjects consuming a high-fat diet. In order to exclude the possibility that we were committing a Type II error, we calculated the mean differences and 95% confidence intervals for two measures that were directly comparable between this and the earlier study – GE100W and DE (Table 3). First, it was apparent from this analysis that, despite the smaller number of subjects being studied, the confidence intervals under comparable conditions were smaller in the present study, possibly due to the more homogeneous cohort. Second, it was clear that the magnitude of the effect observed earlier was considerably greater (approximately double). Therefore some factor had significantly blunted the effect of high-fat feeding on whole-body efficiency in the present study, compared with our earlier work.

We previously suggested that the effect we observed in sedentary men might have been due to the acute action of fatty acids on uncoupling proteins (including UCP3). Given that there is a relationship between physical fitness and muscle UCP3 content (6, 34, 35), we directly compared the content of UCP3 and CS in the biopsies taken from our endurance-trained subjects with those from our earlier study (Table 4). Although UCP3 and CS were not systematically different, the ratio of UCP3/CS (which reflects the quantity of uncoupling protein per mitochondrion) was significantly lower in our endurance-trained subjects compared to our sedentary subjects. We suggest, therefore, that the blunted effect of high-fat
feeding on whole-body efficiency in endurance-trained subjects may be due to their low muscle UCP3 content. There was no consistent effect of diet on UCP3 content in whole skeletal muscle in our endurance-trained cohort, a finding that is consistent with earlier work showing that UCP3 is not increased in response to high-fat feeding in endurance-trained subjects (7). That CS did not differ significantly between the trained and untrained cohorts was somewhat surprising. However, the discrepancy was most likely due to the acute variability of CS, particularly in response to training (36).

As in our earlier study, high-fat feeding had no significant effect on mitochondrial function, measured \textit{in vivo} using 31P-MRS. As before, for technical reasons we measured mitochondrial function in the gastrocnemius, not the quadriceps group (which is one of the principal locomotor muscles during cycling exercise, and was our choice for biopsies). Thus there may have been an effect of the high-fat diet on mitochondrial function in the quadriceps that was not apparent in the gastrocnemius. However, mitochondrial function in these two muscle groups is not systematically different (1), and both muscles would have been exposed to the same increases in free fatty acids, so it is unlikely that mitochondrial function would have been blunted in one and not the other.

Although high-fat feeding did not impair whole-body efficiency, the diet significantly blunted exercise performance. The loss of performance was most likely due to the depletion of intramuscular glycogen stores, as the high-fat diet would have supplied insufficient carbohydrate for the muscle cells to restock glycogen stores after exercise, and contractile efficiency was unaffected. The low levels of blood lactate during exercise, as well as the reduction in exercise-induced acidosis on the high-fat diet support this interpretation.
Limitations

Given the difficulty of recruiting a homogeneous cohort of endurance-trained subjects who were age-matched with our earlier study, we decided instead to maximise statistical power for the present study by recruiting as homogeneous a group as possible from the Oxford college rowing crews (a decision that is retrospectively justified by the smaller confidence intervals in Table 3). Yet this meant that the group studied here was significantly younger than the cohort in our earlier study (22 ± 1 vs 36 ± 1 years). Several lines of evidence led us to conclude that it was exercise training, rather than age, that explained the blunted effect of high-fat feeding we observed. First, the marked differences in physiology between the two groups (for example, in VO2max) were unquestionably the result of endurance-training rather than age. Second, there was no correlation between age and UCP3/CS across the groups. Third, the cohort in our earlier study, although significantly older, was not particularly old. Finally, the correlation between UCP3/CS and DE across both studies was not significantly affected by the inclusion of age as a control variable.

Perspectives and significance

We previously showed that short-term high-fat feeding blunted whole-body efficiency in sedentary men. We sought here to test our hypothesis that a similar dietary regime would blunt whole-body efficiency and, as a consequence, endurance exercise performance in a cohort of endurance-trained subjects. However, we found that a week-long high-fat diet had no detectable effect on whole-body efficiency in this group. Therefore exercise training appeared to partly offset the deleterious effect of high-fat feeding on whole-body efficiency. We compared uncoupling protein content in muscle from our trained vs. our sedentary cohort and found that the trained subjects had significantly less UCP3 per mitochondrion. Therefore we hypothesise that the blunted effect of high-fat feeding was due to a lower uncoupling
protein content. Our findings suggest that a high-fat diet is contraindicated for sufferers of hypoxic or ischemic diseases, but that exercise may partly offset this effect.

Acknowledgements

Thanks to Professor Martin Brand and Professor Jon Arch for their gifts of tissue from the UCP3KO and UCP3tg mice respectively. This work was funded by the British Heart Foundation.
References


Figure legends

**Figure 1:** The effect of a high-fat vs. moderate-carbohydrate diet on plasma free fatty acids in endurance-trained men ($n = 16$)

**Figure 2:** The effect of a high-fat vs. moderate-carbohydrate (control) diet on **A:** Exercising RQ, **B:** Exercising oxygen uptake and **D:** Exercise efficiency. **Figure 2C:** Predicted actual exercising oxygen uptake. Predicted values are based on data from the moderate carbohydrate diet, adjusted for reductions in RQ with unchanged efficiency. ($n = 16$). GExx = gross efficiency at xx Watts; DE = delta efficiency

**Figure 3:** The effect of high-fat vs. moderate carbohydrate (control) diet on phosphocreatine (PCr) recovery kinetics in a single subject
**Table 1:** Subjects’ descriptive data \((n = 16)\)

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<table>
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<tbody>
<tr>
<td>Age (years)</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82 ± 2</td>
</tr>
<tr>
<td>Absolute (\dot{V}O_2) peak (L/min) ((n = 15))</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>Relative (\dot{V}O_2) peak (mL/min·kg⁻¹) ((n = 15))</td>
<td>58 ± 2</td>
</tr>
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**Table 2:** Details of the actual macronutritional composition of the diets

<table>
<thead>
<tr>
<th></th>
<th>Carbohydrate (% of total)</th>
<th>Fat (% of total)</th>
<th>Protein (% of total)</th>
<th>TOTAL (kcal)</th>
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<tr>
<td>Moderate-carbohydrate diet</td>
<td>47.7 ± 0.3</td>
<td>26.3 ± 0.4</td>
<td>26.6 ± 0.4</td>
<td>4210 ± 214</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>3.4 ± 0.1</td>
<td>74.4 ± 0.5</td>
<td>25.4 ± 3.3</td>
<td>4321 ± 261</td>
</tr>
</tbody>
</table>

**Table 3:** Mean difference and 95% confidence intervals for selected measures from this study and Edwards *et al.* (12)

<table>
<thead>
<tr>
<th></th>
<th>Mean difference</th>
<th>95% CI</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GE100W (trained cohort)</td>
<td>-0.4</td>
<td>0.9</td>
<td>16</td>
</tr>
<tr>
<td>GE100W (sedentary cohort (12))</td>
<td>-1.1</td>
<td>1.0</td>
<td>20</td>
</tr>
<tr>
<td>DE (trained cohort)</td>
<td>-1.6</td>
<td>2.4</td>
<td>16</td>
</tr>
<tr>
<td>DE (sedentary cohort (12))</td>
<td>-3.1</td>
<td>3.0</td>
<td>20</td>
</tr>
</tbody>
</table>

GE100W = gross efficiency at 100W; DE = delta efficiency
Table 4: A comparison of selected measures from this study and Edwards et al. (12)

<table>
<thead>
<tr>
<th></th>
<th>Sedentary subjects (data from Edwards et al. (12))</th>
<th>Trained subjects</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>36 ± 1</td>
<td>22 ± 1**</td>
<td>36 (20+16)</td>
</tr>
<tr>
<td>( \dot{V}O_2 \max ) (L min(^{-1}))</td>
<td>3.6 ± 0.2</td>
<td>4.7 ± 0.2**</td>
<td>35 (20+15)</td>
</tr>
<tr>
<td>( \dot{V}O_2 \max ) (mL min(^{-1}) kg(^{-1}))</td>
<td>44 ± 2</td>
<td>58 ± 2**</td>
<td>35 (20+15)</td>
</tr>
<tr>
<td>Muscle UCP3 (AU)</td>
<td>0.9 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>24 (12+12)</td>
</tr>
<tr>
<td>Muscle CS (AU)</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>24 (12+12)</td>
</tr>
<tr>
<td>Muscle UCP3/CS (AU)</td>
<td>1.2 ± 0.2</td>
<td>0.8 ± 0.2*(^{1})</td>
<td>24 (12+12)</td>
</tr>
</tbody>
</table>

Bracketed \( n \) are those in each group (Sedentary \( n \) + Trained \( n \)). *different from sedentary at \( p < 0.05 \); **different from sedentary at \( p < 0.01 \). \(^{1}\)Mann-Whitney U test, all other tests are unpaired \( t \)-tests.
Fractional PCr vs Time (sec)

- Control diet
- High-fat diet

Time (sec): 0 60 120 180 240 300