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

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Endurance exercise training blunts the deleterious effect of high-fat feeding on whole body efficiency. Am J Physiol Regul Integr Comp Physiol 301: R320–R326, 2011. First published June 1, 2011; doi:10.1152/ajpregu.00850.2010.—We recently showed that a week-long, high-fat diet reduced whole body exercise efficiency in sedentary men by >10% (Edwards LM, Murray AJ, Holloway CJ, Carter EE, Kemp GJ, Codreanu I, Brooker H, Tyler DJ, Robbins PA, Clarke K. FASEB J 25: 1088–1096, 2011). To test if a similar dietary regime would blunt whole body efficiency in endurance-trained men and, as a consequence, hinder aerobic exercise performance, 16 endurance-trained men were given a short-term, high-fat (70% kcal from fat) and a moderate carbohydrate (50% kcal from carbohydrate) diet, in random order. Efficiency was assessed during a standardized exercise task on a cycle ergometer, with aerobic performance assessed during a 1-h time trial and mitochondrial function later measured using 31P-magnetic resonance spectroscopy. The subjects then underwent a 2-wk 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 5th day of each diet. Plasma fatty acids were 60% higher on the high-fat diet compared to changes in citrate synthase, ATP synthase, or mitochondrial un-coupling protein 3. We conclude that prior exercise training blunts the deleterious effect of short-term, high-fat feeding on whole body efficiency.

exercise; magnetic resonance; nutrition; mitochondria

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, 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 with fatty acids, as is the body’s total storage capacity for glycogen and glucose (32); thus, in situations in which energetic demands are submaximal 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-oxygen 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 (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 peroxisome proliferator-activated receptor-α, 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 (38) in their seminal 1969 paper. 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-to-\( \text{O}_2 \) ratio), and the energy equivalence of oxygen [which is a property of the metabolic substrate(s)]. Excluding biomechanical efficiency, this can be written as:

\[
\text{Efficiency} \, (\%) = 100\% \times \text{contractile efficiency} \times \text{mitochondrial efficiency} \times \text{energy equivalence of oxygen} \]

Thus an increase in mitochondrial ATP/\( \text{O}_2 \) increases ATP supply to the contractile proteins for a given \( \text{O}_2 \), 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 anemia or lung disease), the efficiency with which the muscle mitochondria can utilize 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). Our laboratory has 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 1 wk of high-fat feeding blunted whole body efficiency in sedentary men by >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 hypothesized 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 16 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 before 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 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 was measured, as described previously (12). The percentage of maximum \( \text{O}_2 \) uptake (\( \text{V} \dot{\text{O}}_2 \)) at which the ventilatory threshold occurred was calculated according to the V-slope method (4).

After 1 wk, subjects were started on the main protocol, commencing with 3 days of a standardized, normal human diet, on day 2 of which a biopsy was taken from most subjects’ left or right vastus lateralis. Biopsies were taken under local anesthetic using a modified Bergstrom technique that has been described elsewhere (15), immediately frozen in liquid nitrogen, and stored at \(-80^\circ\text{C} \). On the morning of day 3, fasted subjects were transported to Oxford Centre for Clinical Magnetic Resonance Research, 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 1-h 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 (MCD) (Table 1) for 5 days, and the blood collection, warm-up, and exercise testing were repeated as described above for the 4 remaining diets. Diet adherence and composition were assessed post hoc using nutritional assessment software (DietPlan 6, Forestfield Software, West Sussex, UK). On day 5 of the diet intervention, another biopsy was taken. There was then a “wash-out” period of 2 wk, 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 3000PT, Saris Cycling Group, Madison, WI). Subjects performed a structured warm-up that consisted of three 5-min work periods at 50, 100, and 150 W, without break, in ascending order, (9, 22, 24). Cadence was fixed at 90 rpm for all testing. Expired gases were collected breath by breath using a Metamax portable gas analyzer (Cortex Biophysik). 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 Ref. 24:

\[
\text{Metabolic cost} = (3.869 \times \text{V}_2 + 1.195 \times \text{V}_{\text{CO}_2} \times (4, 186/60)
\]

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 follows: 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 1-h 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 1, this target was set as 60% of each subject’s peak 1-min 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 8 min of the test, power was fixed to these targets. After this initial period, subjects were free to vary work rate as necessary. Power output and gas analysis data were recorded at 1-s intervals on the ergometer computer and downloaded later for analysis. Capillary blood was collected from

<table>
<thead>
<tr>
<th>Table 1. Subjects’ descriptive data</th>
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<tr>
<td>( n )</td>
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<tr>
<td>-------</td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Weight, kg</td>
</tr>
<tr>
<td>Absolute ( \text{V}_2\text{peak} ), l/min</td>
</tr>
<tr>
<td>Relative ( \text{V}_{\text{CO}_2}\text{peak} ), ml·min(^{-1})·kg(^{-1})</td>
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</tbody>
</table>

Values are means ± SE; \( n \), no. of subjects. \( \text{V}_2\text{peak} \), peak aerobic capacity.
an earlobe at 25 and 50 min. The sample was immediately used to determine the subjects’ blood lactate concentrations using a small handheld device [Lactate Pro, Arkay (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-T 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 31P and 1H surface coil was placed under the widest part of the right gastrocnemius in a specially designed wooden housing. The subject was immobilized with the leg straight and strapped across the shins, knees, hips, and shoulders. The exercise protocol consisted of 5 min of rest, followed by three bouts of exercise, each 5 min long, interspersed with 7-min recovery periods. The work rates, 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 familiarization 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 nonlinear least squares algorithm [AMARES (37)]. Resting ATP and total creatine concentrations were assumed to be 8.2 and 42 mM/l, 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 (P_i) peak relative to phosphocreatine (PCr) (σ, in parts per million) was used to determine intracellular pH, according to the equation:

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

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, monoequational functions were fitted to the time course of PCr concentration 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 could be used to estimate the total ATP-cost of the work being done ($J_{\text{ATP}}$) (17):

$$J_{\text{ATP}} = \lim_{t \rightarrow 0} \frac{d[\text{PCr}]}{dt} + 0.008$$

where 0.008 mM/s is an estimate of the resting ATP turnover of skeletal muscle (5). Contractile economy (in mMJ) can then be calculated as $J_{\text{ATP}}$/external work rate.

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 was frozen with a final concentration of 30 μg/ml lipoprotein lipase inhibitor (tetrahydrodipristat, Xenical, Roche) for determination of FFAs. Plasma concentrations of FFA were measured using an ABX Pentra Clinical Chemistry bench-top analyzer (Horiba ABX, Montpellier, France). Abundance of metabolic proteins was measured in muscle biopsy lysates by immunoblotting, as described in Ref. 25. Muscle UCP3 was detected using Chemicon AB3046 (Milipore) at a concentration of 1:1,000 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 Ref. 12.

Statistical analysis. Data were tested for normality using Shapiro-Wilks. Where data were available for all 5 days of each protocol (for example, aerobic performance), differences were tested for significance using a 5 × 2 repeated-measures 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, Chicago, IL). All data are reported as means ± SE with α = 0.05.

RESULTS

Descriptive measures, diet adherence, and fasting plasma FFA. Subjects were aged 22 ± 1 yr, weighed 82 ± 2 kg, and had a mean peak $\dot{V}_O_2$ of 4.7 ± 0.2 l/min, 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 (HFD) comprised 74% of total intake (the target was 70%), whereas calories from carbohydrates comprised ~50% of total intake (the target was 50%) on the MCD (Table 2). As a result of the HFD, plasma FFA were 60% higher compared with the MCD ($P < 0.01$) (Fig. 1). There was no significant effect of either diet on body mass (data not shown).

Aerobic exercise performance and whole body efficiency. The HFD significantly reduced exercising respiratory quotient (RQ) when measured at the three standardized work rates of 50 W (0.75 ± 0.01 vs. 0.86 ± 0.01, 100 W (0.78 ± 0.01 vs. 0.90 ± 0.02), and 150 W (0.81 ± 0.01 vs. 0.92 ± 0.02, all $P < 0.001$) (Fig. 2A). As would be expected, this tended to increase $V_O_2$ at all three work rates, becoming significant at 150 W (2.6 ± 0.1 vs. 2.5 ± 0.1 l/min, $P < 0.05$). We then calculated the $V_O_2$ that would be required at each work rate based solely on the new, lower RQ. There was no disparity between the predicted and actual $V_O_2$ (Fig. 2C), so that the increased $V_O_2$ we observed could be entirely explained by the altered RQ. Consequently, there was no significant effect of diet on whole body efficiency at any work rate [50 W: 11 ± 0.6 (HFD) vs. 11 ± 0.3% (MCD); 100 W: 15 ± 0.4 vs. 16 ± 0.2%; 150 W: 18 ± 0.4 vs. 18 ± 0.3%; all $P > 0.05$] (Fig. 2B). Likewise, delta efficiency was not systematically different between diets [26 ± 1 (HFD) vs. 27 ± 1% (MCD), $P = 0.19$]. Performance in the 1-h time trials was significantly poorer from day 2 on the HFD [day 5: 215 ± 13 (HFD) vs. 243 ± 11 W (MCD), $P < 0.001$], with a corresponding lower blood lactate concentration during exercise diet [day 5: 1.6 ± 0.1 (HFD) vs. 2.9 ± 0.4 mM (MCD), $P < 0.01$], which was highly significant when tested across the

### Table 2. Details of the actual macronutritional composition of the diets

<table>
<thead>
<tr>
<th>Diet Type</th>
<th>Carbohydrate (%total)</th>
<th>Fat (%total)</th>
<th>Protein (%total)</th>
<th>Total kcal</th>
</tr>
</thead>
<tbody>
<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>4,210 ± 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>4,321 ± 261</td>
</tr>
</tbody>
</table>

Values are means ± SE.

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4 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 HFD had no significant effect on phosphorus metabolism, although resting PCr concentration 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 $^{31}$P-magnetic resonance spectroscopy 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 HFD ($P < 0.05$). 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 half time, which was 20 s throughout. An example of typical PCr recovery kinetics on both diets in a single subject is shown in Fig. 3.

Skeletal muscle protein content. UCP3, citrate synthase (CS), 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 anemia, therapeutic interventions to im-

**Fig. 1.** The effect of a high-fat vs. moderate-carbohydrate diet on plasma free fatty acids in endurance-trained men. Values are means ± SE ($n = 16$). *Significant at $P < 0.05$. **Significant at $P < 0.01$.

**Fig. 2.** The effect of a high-fat vs. moderate-carbohydrate (control) diet on exercising respiratory quotient (RQ; A), exercising oxygen uptake ($\dot{V}O_2$; B), and exercise efficiency (D). C: predicted vs. actual $\dot{V}O_2$. Predicted values are based on data from the moderate-carbohydrate diet, adjusted for reductions in RQ with unchanged efficiency. $\dot{V}CO_2$, CO$_2$ consumption; GE50, GE100, GE150: gross efficiency at 50, 100, 150 W, respectively; DE, delta efficiency. Values are means ± SE ($n = 16$). *Significant at $P < 0.05$. **Significant at $P < 0.01$. 

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**Fig. 3.** An example of typical PCr recovery kinetics on both diets in a single subject.
prove whole body efficiency may subsequently improve morbidity and mortality.

Our laboratory previously found that 1 wk of high-fat feeding reduced whole body efficiency by \( \sim 13\% \) in sedentary men (12), and this has both clinical and human performance implications. Clinical implications aside, however, generalizing 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 HFD identical in macronutrient composition to the one we used previously [\( \sim 75\% \) 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 1-h time trial to investigate possible changes in endurance performance (18).

In the present study, 1 wk of high-fat feeding raised plasma FFA by \( \sim 60\% \) compared with a MCD. 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 \( \sim 40\% \) 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 work rates, we were unable to detect a decline in whole body efficiency in the endurance-trained subjects consuming a HFD. 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: gross efficiency at 100 W and delta efficiency (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 UCPs (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 to CS (which reflects the quantity of UCP per mitochondrion) was significantly lower in our endurance-trained subjects compared with 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 in vivo using \(^{31}\text{P}\)-magnetic resonance spectroscopy. As before, for technical reasons, we measured mitochondrial function in the gastro-

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**Fig. 3. The effect of high-fat vs. moderate-carbohydrate (control) diet on phosphocreatine (PCr) recovery kinetics in a single subject.**

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Table 3. Mean difference and 95% CI for selected measures from this study and that of Edwards et al. (12)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Sedentary Subjects</th>
<th>Trained Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>36 ± 1</td>
<td>22 ± 1†</td>
</tr>
<tr>
<td>( V_{\text{O2max}} ), l/min</td>
<td>3.6 ± 0.2</td>
<td>4.7 ± 0.2†</td>
</tr>
<tr>
<td>( V_{\text{O2max}} ), ml·min(^{-1})·kg(^{-1})</td>
<td>44 ± 2</td>
<td>58 ± 2†</td>
</tr>
<tr>
<td>Muscle CS, AU</td>
<td>0.9 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Muscle UCP3, AU</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Muscle UCP3/CS, AU</td>
<td>1.2 ± 0.2</td>
<td>0.8 ± 0.2††</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), no. of subjects. \( n \) values show total and (sedentary \( n \) + trained \( n \)). \( V_{\text{O2max}} \), maximum \( O_2 \) uptake; UCP3, uncoupling protein 3; AU, arbitrary units; CS, citrate synthase. *Different from sedentary at \( P < 0.05 \); †Different from sedentary at \( P < 0.01 \); ‡Mann-Whitney \( U \)-test; all other tests are unpaired \( t \)-tests.

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Table 4. A comparison of selected measures from this study and that of Edwards et al. (12)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Sedentary Subjects [data from Edwards et al. (12)]</th>
<th>Trained Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ge100W (trained cohort)</td>
<td>-0.4 0.9 16</td>
<td></td>
</tr>
<tr>
<td>Ge100W (sedentary cohort (12)]</td>
<td>-1.1 1.0 20</td>
<td></td>
</tr>
<tr>
<td>De (trained cohort)</td>
<td>-1.6 2.4 16</td>
<td></td>
</tr>
<tr>
<td>De (sedentary cohort (12)]</td>
<td>-3.1 3.0 20</td>
<td></td>
</tr>
</tbody>
</table>

\( n \), No. of subjects. CI, confidence interval; GE100W, gross efficiency at 100 W; DE, delta efficiency.
nemius, 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 HFD 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 FFAs, 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 HFD 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 HFD, 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 maximize 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 yr). 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 observed. First, the marked differences in physiology between age, that explained the blunted effect of high-fat feeding, may partly offset this effect.

Second, there was no correlation between age and UCP3/CS, although significantly older, was not particularly old. Finally, the correlation between UCP3/CS and delta efficiency 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 1-wk-long HFD 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 UCP content in muscle from our trained vs. our sedentary cohort and found that the trained subjects had significantly less UCP3 per mitochondrion. Therefore, we hypothesize that the blunted effect of high-fat feeding was due to a lower UCP content. Our findings suggest that a HFD is contraindicated for sufferers of hypoxic or ischemic diseases, but that exercise may partly offset this effect.

ACKNOWLEDGMENTS

We thank Martin Brand and Jon Arch for gifts of tissue from the UCP3KO and UCP3tg mice, respectively.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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