Fat adaptation followed by carbohydrate restoration increases AMPK activity in skeletal muscle from trained humans

Wee Kian Yeo,1 Sarah J. Lessard,1 Zhi-Ping Chen,2 Andrew P. Garnham,3 Louise M. Burke,4 Donato A. Rivas,1 Bruce E. Kemp,2 and John A. Hawley1

1Exercise Metabolism Group, School of Medical Sciences, RMIT University, Victoria; 2St. Vincent’s Institute, University of Melbourne, Victoria; 3Exercise, Muscle and Metabolism Unit, School of Exercise and Nutrition Sciences, Deakin University, Victoria; and 4Department of Sports Nutrition, Australian Institute of Sport, Belconnen, Australian Capital Territory, Australia

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Yeo WK, Lessard SJ, Chen ZP, Garnham AP, Burke LM, Rivas DA, Kemp BE, Hawley JA. Fat adaptation followed by carbohydrate restoration increases AMPK activity in skeletal muscle from trained humans. J Appl Physiol 105: 1519–1526, 2008. First published September 18, 2008; doi:10.1152/japplphysiol.90540.2008.—We have previously reported that 5 days of a high-fat diet followed by 1 day of high-carbohydrate intake (Fat-adapt) increases rates of fat oxidation and decreased rates of muscle glycogenolysis during submaximal cycling compared with consumption of an isoenergetic high-carbohydrate diet (HCHO) for 6 days (Burke et al. Am J Physiol Endocrinol Metab 290: E380–E388, 2006). To determine potential mechanisms underlying shifts in substrate selection, eight trained subjects performed Fat-adapt and HCHO. On day 7, subjects performed 1-h cycling at 70% peak O2 uptake. Muscle biopsies were taken immediately before and after exercise. Resting muscle glycogen content was similar between treatments, but muscle triglyceride levels were higher after Fat-adapt (P < 0.05). Resting AMPK-α1 and -α2 activity was higher after Fat-adapt (P = 0.02 and P = 0.05, respectively), while the phosphorylation of AMPK’s downstream target, acetyl-CoA carboxylase (pACC at Ser221), tended to be elevated after Fat-adapt (P = 0.09). Both the respiratory exchange ratio (P < 0.01) and muscle glycogen utilization (P < 0.05) were lower during exercise after Fat-adapt. Exercise increased AMPK-α1 activity after HCHO (P = 0.03) but not Fat-adapt. Exercise was associated with an increase in pACC at Ser221 for both dietary treatments (P < 0.05), with postexercise pACC Ser221 higher after Fat-adapt (P = 0.02). In conclusion, compared with HCHO, Fat-adapt increased resting muscle triglyceride stores and stored AMPK-α1 and -α2 activity. Fat-adapt also resulted in higher rates of whole body fat oxidation, reduced muscle glycogenolysis, and attenuated the exercise-induced rise in AMPK-α1 and AMPK-α2 activity compared with HCHO. Our results demonstrate that AMPK-α1 and AMPK-α2 activity and fuel selection in skeletal muscle in response to exercise can be manipulated by diet and/or the interactive effects of diet and exercise training.

short-term (<1 wk) manipulation of dietary macronutrient intake is associated with marked changes in skeletal muscle gene expression (1, 5, 24), substrate stores (36), metabolic flux, and fuel oxidation (10, 22, 23). Exercise training also results in striking modifications in muscle gene expression (14), energy reserves, and the relative contribution of fuels to the energetic demands of muscle (9). Accordingly, the extent to which acutely altering substrate availability might modify the training impulse has been a key research area among exercise physiologists and sport nutritionists for several decades (for review, see Ref. 19). Indeed, evidence is accumulating that nutrient manipulation can serve as a potent modulator of many of the acute responses to both endurance (15) and resistance exercise (7, 11).

During recent years, our laboratory has undertaken a series of independent but related studies into the effects of a practical “dietary periodization” strategy in well-trained endurance athletes on selected aspects of metabolism and exercise performance (3–6, 32, 33). We have shown that short-term (5-day) exposure to a high-fat diet, while undertaking high-volume, intense training, followed by 1 day of rest and “carbohydrate restoration,” results in greater rates of whole body fat oxidation and decreased rates of muscle glycogenolysis during submaximal (70% peak O2 uptake [VO2peak]) cycling when subjects undertake the same training and consume an isoenergetic high-carbohydrate (HCHO) diet (3, 32). The decreased rate of muscle glycogenolysis during exercise following fat adaptation with carbohydrate (CHO) restoration can, in part, be explained by decreased pyruvate oxidation via pyruvate dehydrogenase flux (32). However, it is not clear what precise metabolic signal(s) accounted for the marked shifts in substrate utilization during exercise. In this regard, it has been suggested that AMP-activated protein kinase (AMPK) may provide a direct link between intracellular signaling events and subsequent substrate selection in exercising muscle (16, 34). In support of this hypothesis, diet-exercise manipulation that results in low muscle glycogen content is associated with increased resting AMPK activity (31, 35) and elevated phosphorylation of AMPK’s downstream target, β-acetyl-CoA carboxylase (ACC-β Ser221), compared with high glycogen stores (35). Furthermore, the degree of AMPK activation during submaximal exercise was also shown to be dependent on the fuel status of the contracting musculature, with AMPK activity elevated to a greater extent in muscle with low compared with high glycogen levels (35).

While the findings of Wojtaszewski et al. (35) imply that glycogen availability per se may modulate AMPK activity, it cannot be ruled out that the diet-exercise regimens used to differentiate glycogen concentration in that study also resulted in significant changes in muscle lipid availability [i.e., increased muscle triacylglycerol (TG)]. Accordingly, the ob-

Address for reprint requests and other correspondence: J. A. Hawley, School of Medical Sciences, RMIT Univ., PO Box 71, Bundoora, Victoria 3083, Australia (e-mail: john.hawley@rmit.edu.au).

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served changes in AMPK activation may have been more a consequence of lipid-induced rather than CHO-induced effects on this enzyme. In this regard, we have recently shown that endurance training in high-fat-fed rats was associated with increased AMPK-\(\alpha_1\) activity, whereas AMPK-\(\alpha_2\) activity was elevated by high-fat feeding alone and was not increased further by exercise training (25). Accordingly, the aim of the present study was to investigate the effect of a diet-exercise protocol known to be associated with elevated rates of fat oxidation, independent of muscle glycogen availability on AMPK signaling at rest and during subsequent submaximal exercise. We hypothesized that fat adaptation followed by CHO restoration would increase resting muscle triglyceride concentrations and that changes in lipid availability per se would modify AMPK signaling at rest and during exercise.

**METHODS**

**Subjects and Preliminary Testing**

Eight endurance-trained male cyclists or triathletes [body mass (BM) 70.2 ± 1.8 kg; age 31.6 ± 2.3 yr; \(\dot{V}O_2\) peak 61.5 ± 1.5 ml·kg\(^{-1}\)·min\(^{-1}\), peak sustained power output (PPO) 339.85 ± 5.8 W; values are mean ± SE] were recruited to participate in this study, which was approved by the Human Research Ethics Committee of RMIT University. Subjects were fully informed about the possible risks of all procedures before providing their written consent. One week before experimental testing, each subject undertook an incremental cycling test to exhaustion on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands). The initial testing protocol has been described in detail previously (20). During the maximal test and the subsequently described experimental trials, subjects breathed through a Hans-Rudolph two-way non-rebreathing valve and mouthpiece attached to a gas analysis system (Parvomedics) interfaced to a computer, which calculated the instantaneous rates of \(O_2\) consumption (\(\dot{V}O_2\)), \(CO_2\) production (\(\dot{V}CO_2\)), minute ventilation, and the respiratory exchange ratio (RER) every 15 s from conventional equations (20). Before each maximal test and all experimental trials, the analyzers were calibrated with commercially available gases of known \(O_2\) and \(CO_2\) content. \(\dot{V}O_2\) peak was defined as the highest \(\dot{V}O_2\) a subject attained during any 60 s of the test, whereas PPO was calculated from the last completed work rate plus the fraction of time spent in the final noncompleted work rate multiplied by 25 W. This value was used to determine the power output corresponding to 70% of each subject’s \(\dot{V}O_2\) peak (63% of PPO) to be used in the subsequently described experimental trials. The maximal test session and all experimental trials were performed under standard laboratory condition (18–22°C, 40–50% relative humidity), and subjects were fan cooled during all these sessions.

**Overview of Study Design**

The experimental protocol is shown in Fig. 1 and has been described in detail previously (3). In brief, each subject undertook two 7-day trials in a randomized, crossover design, separated by a 2-wk washout period. The interventions consisted of prescribed supervised training while undertaking either a 5-day high-fat diet followed by 1 day of HCHO restoration (Fat-adapt) or a 6-day HCHO diet. During Fat-adapt, the subjects were prescribed a high-fat (4.6 g·kg\(^{-1}\)·day\(^{-1}\) fat, 68% of energy), low-CHO (2.5 g·kg\(^{-1}\)·day\(^{-1}\) CHO, 17% of energy) diet supplying 0.25 MJ/kg BM. HCHO was an isoenergetic diet providing 10.3 g·kg\(^{-1}\)·day\(^{-1}\) and 70% of energy from CHO and 1.0 g·kg\(^{-1}\)·day\(^{-1}\) and 18% of energy from fat. Protein content was maintained at 2.3 g·kg\(^{-1}\)·day\(^{-1}\) during both trials, and diets were constructed to maximize, or at least match, absorbable energy. Fiber intake was kept to a daily mean intake of <50 g and matched to within 5–10 g each day between dietary treatments. All meals and snacks were supplied to subjects, with diets being individualized for food preferences as well as BM. Subjects received their food in prepared packages and were required to keep a food checklist to note their compliance to the dietary instructions and their intake of any additional food or drinks. Every 2 days, they met with a dietician to receive new food parcels and check their adherence to the previous days’ diet. The HCHO diet prescribed in this study is similar in composition to that recommended by sports nutritionists for endurance-trained athletes (2). However, the high-fat diet is far removed from the nutritional energy sources typically consumed by our subjects. While an ad libitum diet group may provide insight into the consequences of lipid-induced rather than CHO-induced effects on this enzyme. In this regard, we have recently shown that muscle AMPK activity was increased by exercise training (25). Accordingly, the aim of the current experimental design was to consider changes in AMPK activation may have been more a consequence of lipid-induced rather than CHO-induced effects on this enzyme. In this regard, we have recently shown that endurance training in high-fat-fed rats was associated with increased AMPK-\(\alpha_1\) activity, whereas AMPK-\(\alpha_2\) activity was elevated by high-fat feeding alone and was not increased further by exercise training (25).

![Fig. 1. Overview of the study design and experimental trial. CHO, carbohydrate; \(\dot{V}O_2\) peak, peak oxygen uptake.](http://jap.physiology.org/)

<table>
<thead>
<tr>
<th>Day</th>
<th>Diet</th>
<th>Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>High fat or High CHO</td>
<td>20 min @ 70% (\dot{V}O_2) peak + Interval training</td>
</tr>
<tr>
<td>2</td>
<td>High fat or High CHO</td>
<td>3–4 hour long ride</td>
</tr>
<tr>
<td>3</td>
<td>High fat or High CHO</td>
<td>2–3 hour hill ride</td>
</tr>
<tr>
<td>4</td>
<td>High fat or High CHO</td>
<td>3–4 hour long ride</td>
</tr>
<tr>
<td>5</td>
<td>High fat or High CHO</td>
<td>20 min @ 70% (\dot{V}O_2) peak + Interval training</td>
</tr>
<tr>
<td>6</td>
<td>High CHO</td>
<td>Rest</td>
</tr>
<tr>
<td>7</td>
<td>Experimental trial</td>
<td></td>
</tr>
</tbody>
</table>

Gas Collection (for 5 minutes)

- Muscle biopsy
- 60 minutes steady-state ride at 70% \(\dot{V}O_2\) peak
- Muscle biopsy

15 min 35 min 55 min
ately followed by a high-intensity interval training session consisting of eight repetitions of 5-min work bouts at 85% of VO_2peak with 1 min of recovery at 100 W. The metabolic demands associated with this interval training session have been described in detail previously (33).

The intent of this ride was to lower the muscle glycogen concentrations on the first day and initiate a rapid differentiation between dietary treatments on the basis of their ability to restore depleted muscle glycogen stores. On day 6 of both the Fat-adapt and HCHO trials, subjects were required to rest and ingest a HCHO diet (10.3 g/kg BM).

**Experimental Trial**

On the morning of day 7, subjects returned to the laboratory after a 12- to 14-h overnight fast to undertake a 1-h SS ride at 70% of VO_2peak. On arrival in the laboratory, a single leg was prepared for muscle biopsies, and two incisions were made 2–3 cm apart along the vastus lateralis muscle. A resting muscle sample was then taken using the percutaneous biopsy technique with suction applied. After resting for 10 min, subjects began the 60-min SS ride, and a second biopsy was obtained within 10 s on completion of the ride while subjects remained seated on the ergometer. All muscle biopsies were rapidly frozen in liquid N_2 within seconds of the designated time point for collection and stored at −80°C until subsequent analysis.

**Analytic Procedures**

**Rates of fat and CHO oxidation.** Whole body rates of CHO and fat oxidation (g/min) were calculated from the respiratory data collected during the last 5–6 min of the 20-min SS ride on days 1 and 5 and during the last 5 min of every 20 min of the SS ride at 70% VO_2peak. The calculations were made from VCO_2 and VO_2 measurements, assuming a nonprotein RER value, according to the following equations (27).

- CHO oxidation = 4.585 VCO_2 − 3.226 VO_2
- Fat oxidation = 1.695 VCO_2 − 1.701 VO_2

Total fat and CHO oxidation during the 60-min SS ride were estimated by calculating the area under the oxidation (g/min) vs. time curves for each subject. Rates of CHO oxidation (μmol·kg^−1·min^−1) were determined by converting the rate of CHO oxidation (g/min) to its molar equivalent, assuming 6 mol of O_2 are consumed and 6 mol of CO_2 produced for each mole (180 g) oxidized. Rates of fatty acid (FA) oxidation (μmol·kg^−1·min^−1) were determined by converting the rate of triglycerol oxidation (g/min) to its molar equivalent, assuming the average molecular weight of human triglyceride to be 855.3 g/mol and multiplying the molar rate of triglyceride oxidation by 3, because each molecule contains three molecules of FAs.

**Muscle glycogen and TG concentration.** Approximately 40–50 mg of muscle were freeze-dried and powdered, with all visible blood and connective tissue removed. The freeze-dried muscle sample was then divided into two aliquots. The first aliquot (~3 mg) was extracted with 250 μl of 2 M hydrochloric acid, incubated at 100°C for 2 h, and then neutralized with 750 μl of 0.67 M sodium hydroxide. Glycogen concentration was determined via enzymatic analyses, as described previously (7).

The second aliquot (~7 mg) was used for the determination of muscle TG content, as previously described (13). In brief, lipid was extracted with Folch extraction (12), the TG was then saponified in an ethanol/potassium hydroxide solution at 60°C, and glycerol content was determined fluorometrically and expressed as micromoles per gram dry weight.

**AMPK Signaling**

Approximately 60 mg of wet muscle were homogenized in buffer A (50 mM Tris·HCl, pH 7.5, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 50 mM NaF, 5 mM sodium pyrophosphate, 10% glycero, 1% Triton X-100, 10 μg/ml trypsin inhibitor, 2 μg/ml aprotinin, 1 mM benzamidine, and 1 mM phenylmethylsulfonyl fluoride), and the homogenate was then centrifuged at 20,000 g for 25 min. The supernatant was aliquoted, and the total protein concentration was determined by bicinchoninic acid method (Pierce Chemical, Rockford, IL). The aliquots were then stored at −80°C until further analysis.

**Immunoprecipitation**

Approximately 2.5 mg of protein from the supernatants were incubated with AMPK-α1 or AMPK-α2 antibody bound protein A-Sepharose beads (6 MB; Amersham Biosciences, Uppsala, Sweden) for 2 h at 4°C. The polyclonal antipeptide antibodies to AMPK-α1 and AMPK-α2 were raised to nonconserved regions of the AMPK-α1 (rats 231–251) and AMPK-α2 isoforms (rats 351–366). The immunocomplexes were washed with PBS and suspended in 50 mM Tris·HCl (pH 7.5) buffer for the AMPK activity assay. The AMPK activities in the immune complexes were measured in the presence of 200 μM AMP. Activities were calculated as picomoles of phosphate transferred to the SAMS peptide per minute per milligram of protein subjected to immunoprecipitation (pmol·min^−1·mg^−1). The post-AMPK immunoprecipitation supernatants were then incubated in streptavidin-Sepharose high-performance beads (Amersham Biosciences) for 1 h at 4°C for affinity purification of ACC.

**Western Blotting**

The affinity-purified ACC fraction was electrophoresed on 7.5% SDS-PAGE and detected by immunoblotting with anti-phosphoSer232/ACC polyclonal antibody. The blots were then stripped (50 mM Tris·HCl, 2% SDS, 115 mM β-mercaptoethanol) at 55°C for 20 min, blocked, and incubated with horseradish peroxidase conjugated streptavidin (Amersham Pharmacia Biotech UK, Little Chalfont, UK) to determine the total ACC content. Aliquots of the immunoprecipitated AMPK-α1 and AMPK-α2 proteins were electrophoresed on 10% SDS-PAGE and detected by immunoblotting with antibodies specific for the AMPK-α1 and AMPK-α2 isoforms. The post-AMPK immunoprecipitation supernatants containing 60 μg of total protein were electrophoresed on SDS-PAGE [acrylamide concentration either 15% [glucose transporter (GLUT)-4] or 10% [Fat/CD36, peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α], respectively] and detected by immunoblotting with antibodies specific for GLUT-4 (Biogenesis, UK 4670–1704), Fat/CD36 (AbCam, Cambridge, UK), or PGC-1 (Millipore, AB3242). The immunoreactive proteins were detected with enhanced chemiluminescence (Amer sham Biosciences) and quantified by densitometry. α-Tubulin was used as a loading control to ensure that the protein content was the same in all wells.

**Statistical Analysis**

Data from the two experimental trials were compared by using a two-factor (diet and time) ANOVA with repeated measures. Separate analyses were undertaken to compare data from 20-min rides on days 1, 5, and 7, and data were collected at different time points during the experimental ride. Holm-Sidak post hoc tests were undertaken when ANOVA revealed a significant interaction. Muscle glycogen and muscle TG concentration between trials were compared by using Student’s t-tests. Relationships between muscle fuel stores (muscle glycogen and muscle TG) and AMPK activities were studied using Pearson product-moment correlation coefficient. All values are expressed as means and SE, with the critical level of significance established at P < 0.05.
RESULTS

Substrate Oxidation During Exercise

Figure 2A shows the rates of whole body CHO oxidation during 20 min of cycling at 70% \( \text{V} \text{O}_2 \text{max} \) (20-min SS) on days 1, 5, and 7. Rates of CHO oxidation during the 20-min SS were similar during both trials on day 1. There was a significant effect of diet on rates of CHO oxidation, such that HCHO was higher than Fat-adapt at all time points after baseline \((P < 0.05)\). During HCHO trial, 1 day of rest on day 6 increased the rates of whole body CHO oxidation during 20-min SS significantly compared with baseline \((P < 0.05)\). During Fat-adapt, 5 days of high-fat diet and exercise training decreased the rates of whole body CHO oxidation, such that it was significantly lower than all of the other time points.

Figure 2B shows the rates of whole body fat oxidation during 20 min of cycling at 70% \( \text{V} \text{O}_2 \text{max} \) (20-min SS) on days 1, 5, and 7, along with the average RER data during the 1-h SS ride (60-min SS) on day 7 (Fig. 2C). Rates of fat oxidation during the 20-min SS were similar during both trials \((P < 0.05)\) at baseline \((day 1)\). The effect of diet on rates of fat oxidation was significant at all time points after baseline, with Fat-adapt being higher than HCHO \((P < 0.05)\). One day of HCHO diet and rest decreased rates of fat oxidation, such that, on day 7, values were not different from day 1 \((30.20 \pm 3.42 \text{ vs. } 25.79 \pm 3.72 \text{ } \mu \text{mol-kg}^{-1} \text{-min}^{-1}, \text{respectively}; P < 0.05)\). During HCHO, rates of fat oxidation were similar on days 1 and 5 \((23.03 \pm 3.61 \text{ vs. } 18.85 \pm 2.61 \text{ } \mu \text{mol-kg}^{-1} \text{-min}^{-1}; P < 0.05)\) but decreased on day 7 compared with day 1 \((23.03 \pm 3.61 \text{ vs. } 14.44 \pm 1.19 \text{ } \mu \text{mol-kg}^{-1} \text{-min}^{-1}; P < 0.05)\). The average RER over the 1-h SS ride on day 7 was also lower during Fat-adapt \((0.87 \pm 0.004 \text{ vs. } 0.93 \pm 0.004; P < 0.01; \text{Fig. 2B})\), such that fat oxidation accounted for 40.3 ± 3.6% of total fuel oxidation in this trial compared with 21.9 ± 1.5% in HCHO \((P < 0.05)\).

Glycogen and Muscle TG

Table 1 displays muscle glycogen concentrations before and after the two experimental trials. As intended, there were no differences in preexercise muscle glycogen levels between dietary treatments \((547 \pm 40 \text{ and } 694 \pm 55 \text{ mmol/kg dry wt for Fat-adapt and HCHO, respectively})\). Glycogen utilization during 60-min SS was significantly lower after Fat-adapt \((179 \pm 35 \text{ vs. } 264 \pm 45 \text{ mmol/kg dry wt, } P = 0.04)\).

Figure 3 displays muscle TG concentrations before and after 60-min SS cycling at 70% \( \text{V} \text{O}_2 \text{max} \). Muscle TG concentrations

<table>
<thead>
<tr>
<th>Trial</th>
<th>Day 7 Preexercise</th>
<th>Day 7 Postexercise</th>
<th>Utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat-adapt</td>
<td>547 ± 40</td>
<td>369 ± 22*</td>
<td>179 ± 35</td>
</tr>
<tr>
<td>HCHO</td>
<td>694 ± 55</td>
<td>460 ± 54*</td>
<td>264 ± 45†</td>
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</table>

Values are means ± SE in mmol/kg dry wt for 8 subjects. Fat-adapt, 5 days of a high-fat diet followed by 1 day of high-carbohydrate intake; HCHO, high-carbohydrate diet. *Significantly different from preexercise, \(P < 0.05\). †Significantly greater than FAT-adapt, \(P < 0.05\).
were higher on day 7 after Fat-adapt compared with the HCHO trial (69 ± 10.2 vs. 39 ± 3.4 μmol/g dry wt; P = 0.006). Postexercise muscle TG levels were similar between Fat-adapt and HCHO trial (Fig. 3). There was a small but nonsignificant increase in muscle TG concentration with HCHO after exercise.

**AMPK-α₁ and -α₂ Activity**

Figure 4 displays resting (preexercise) and postexercise AMPK signaling [AMPK-α₁ and -α₂ activity and the phosphorylation of ACC (pACC) at Ser²²¹] after the two dietary treatments and resting total protein content of AMPK-α₁, AMPK-α₂, and ACC. Resting AMPK-α₁ and -α₂ activities (Fig. 4, A and B) were higher after Fat-adapt compared with HCHO (P = 0.02 and P = 0.05, respectively). There were no differences in the protein content of either AMPK-α₁ or -α₂ in response to either treatment (Fig. 4C). Exercise increased AMPK-α₁ activity (Fig. 4A) after HCHO (P = 0.02), but did not have any further effects on either AMPK-α₁ or -α₂ activity after Fat-adapt. AMPK-α₁ and -α₂ activity postexercise were not different between dietary treatments.

There was a significant relationship (r = 0.53; P < 0.05) between resting muscle TG concentration and AMPK-α₂ activity. When this relationship was expressed as the difference in resting muscle TG for each subject between the two dietary treatments vs. the difference in AMPK-α₂ activity between treatment, the correlation improved to r = 0.82 (P = 0.04). Resting muscle glycogen concentration was significantly associated with AMPK-α₁ (r = -0.53; P < 0.05), but not AMPK-α₂ activity. There was a trend for a relationship between muscle TG utilization and changes in AMPK-α₂ activity (r = 0.52; P = 0.06), but no relationship between muscle TG utilization and AMPK-α₁ activity.

**pACC Ser²²¹**

The phosphorylation of the AMPK downstream target ACC at Ser²²¹ (relative to total protein content) on day 7 tended to be higher at rest after Fat-adapt compared with HCHO (P = 0.09). Exercise increased the pACC Ser²²¹ during both HCHO (P = 0.042) and Fat-adapt (P = 0.008), with the postexercise pACC Ser²²¹ being higher during the Fat-adapt compared with the HCHO trial (P = 0.02; Fig. 4D).

**Protein Expression**

Total protein abundance for FAT/CD36, GLUT-4, and PGC-1 was not different between trials (data not shown).

**DISCUSSION**

We have used a human model of short-term (5 days) adaptation to a high-fat diet followed by 1 day of CHO restoration (i.e., a HCHO diet) in well-trained athletes undertaking high-volume, intense training to study the effects of diet-exercise interactions on muscle signaling and metabolism. The novel findings from the present study were that, despite the brevity of the intervention period, 1) the Fat-adapt protocol resulted in elevated resting muscle triglyceride concentrations that were independent of glycogen availability; 2) changes in muscle triglyceride levels were associated with increased basal AMPK activity without concomitant changes in protein levels; and 3) Fat-adapt attenuated the exercise-induced rise in AMPK-α₁ and AMPK-α₂ activity compared with an isonenergetic HCHO diet.

In accordance with our laboratory’s previous investigations (3, 4, 6, 32), we again report that rates of fat oxidation during 60 min of submaximal cycling (70% of VO₂ peak) were elevated approximately twofold after fat adaptation followed by CHO restoration compared with when subjects performed identical training and consumed an isonenergetic HCHO diet throughout the 6-day intervention period (Fig. 2). Furthermore, muscle glycogen utilization was significantly lower after Fat-adapt (Table 1). In our laboratory’s earlier studies (3, 4, 6, 32), we were unable to detect any differences in the utilization of FA between dietary treatments, we were unable to detect any differences in the utilization of FA between dietary treatments, but did show that the FA translocase (Fat/CD36) was expressed greater in muscle from trained athletes after just 5 days of consuming a high-fat diet are in close agreement with those reported by Helge et al. (21) for previously untrained subjects who completed 7 wk of endurance training while consuming a high-fat diet (21). They are also similar to the values of Zderic et al. (36) for endurance-trained cyclists after 2 days of a high-fat diet and Zehnder et al. (37) in muscle from trained athletes after just 1.5 days of lipid supplementation (37). Taken collectively, these findings suggest that muscle TG stores are prone to rapid fluctuations in response to changes in nutrient composition and physical activity levels.

In a previous study from our group (5), we showed that 5 days of a high-fat diet coupled with a rigorous training program resulted in a small (17%) but significant increase in the protein abundance of FA translocase (Fat/CD36). These data (5) indicated that an increase in dietary lipid availability was, in part, responsible for the rapid upregulation of Fat/CD36 protein expression, suggesting a role for this FA transporter in facilitating greater skeletal muscle FA uptake. In that investigation, we only assessed the effects of 5 days of a high-fat diet without CHO restoration on Fat/CD36. In the present study, we measured Fat/CD36 protein content after fat adaptation followed by 1 day of rest and a CHO diet. Under these dietary conditions, we were unable to detect any differences in the abundance of this FA transporter between the fat adaptation
protocol and the HCHO diet intervention. To the best of our knowledge, no previous studies have measured GLUT-4 or PGC-1 protein expression in response to the short-term fat adaptation and CHO restoration protocol utilized in the present study. However, Steinberg et al. (31) reported higher skeletal muscle GLUT-4 mRNA expression immediately after an acute bout of endurance exercise commenced with low (vs. normal) glycogen content. With regard to PGC-1, Sparks et al. (30) reported that PGC-1α mRNA was reduced by 20% in muscle from healthy young males following 3 days of a high-fat diet, whereas Mortensen et al. (26) found no differences in PGC-1α mRNA expression after a chronic training intervention in previously untrained subjects in which 50% of the training sessions were performed under conditions of low starting muscle glycogen concentration.

A second important finding from the present study was that the high-fat diet was accompanied by increased AMPK-α1 and -α2 activity (Fig. 4, A and B). Wojtaszewski et al. (35) have also demonstrated that resting AMPK activity is sensitive to fuel status (35). These workers reported that AMPK activities for both α1- and α2-isoforms were higher in the face of low (~160 mmol/kg dry wt) compared with high-resting (~900 mmol/kg dry wt) muscle glycogen content. While their results (35) suggest a relationship between muscle glycogen content and AMPK activity, it should be noted that glycogen does not inhibit AMPK activity in vitro (28). Indeed, it is unlikely that altered muscle glycogen availability is the only factor regulating muscle AMPK activity. Further support for this hypothesis is that the diet-exercise intervention used to differentiate resting muscle glycogen stores in the study of Wojtaszewski et al. (35) is also likely to have resulted in elevated muscle TG levels, particularly as arterial plasma free FA concentrations were significantly higher in the low- vs. high-glycogen condition. Although we did not measure plasma FA levels in the

Fig. 4. AMP-activated protein kinase (AMPK) signaling before and after exercise on day 7 during HCHO and Fat-adapt trials. A: AMPK-α1 activity. B: AMPK-α2 activity. C: relative levels of resting (preexercise) total protein content of AMPK-α1, AMPK-α2, and acetyl-CoA carboxylase (ACC) as quantified by Western blot analysis and densitometry. D: phosphorylation of ACC at serine 221 relative to total ACC protein content (pACC Ser221). Significant differences between groups (P < 0.05) are indicated by the P values listed on the figure. Values are mean ± SE. For details of diet-exercise protocols, see METHODS. AU, arbitrary units.
present investigation, our laboratory has previously shown no differences in resting concentrations between the two diet interventions (3, 4, 6).

Our laboratory has recently reported an increase in AMPK-α2 but not -α1 activity in rodent skeletal muscle in response to chronic high-fat feeding (25). Of note in that study was the observation that AMPK-α1 activity was only increased in high-fat-fed rodents when they concurrently undertook an intense endurance training program with a high-fat diet (25). At the time, we suggested that there may be distinct roles for the AMPK-α subunit isoforms in skeletal muscle, with AMPK-α1 activity being linked to exercise training-induced adaptations and muscle glycogen storage, and AMPK-α2 activity being responsive to increased lipid availability (25). In the present study, we provide further data in support of this premise. We observed a significant relationship between resting AMPK-α1 activity (but not -α2) and muscle glycogen content ($r = -0.51$, $P = 0.05$). In contrast, AMPK-α2 (but not -α1 activity) was positively associated with resting muscle triglyceride content ($r = 0.53$, $P = 0.05$). Perhaps the most robust evidence to corroborate the isoform-specific modification of resting AMPK activity and muscle lipid status comes from the paired data from each subject’s resting biopsy samples: compared with a HCHO diet, fat adaptation significantly increased muscle triglyceride concentration (Fig. 3), with such changes being strongly correlated to difference in basal AMPK-α2 activity ($r = 0.82$, $P = 0.04$). Raney and Turcotte (29) have also reported a positive association between FA uptake and oxidation and AMPK-α2 activity in the perfused rat hindlimb model. However, it should be noted that correlational data cannot determine causality, and it is possible that factors other than muscle lipid status may play a role in modifying AMPK-α2 activity. In this regard, while resting muscle glycogen concentrations were not statistically significant between the two dietary groups, values in the HCHO trial were, on average, 27% higher after the high-fat diet. It is well accepted that preexercise muscle glycogen availability influences subsequent rates of muscle glycogenolysis (17, 18). Indeed, it is likely that a 25–30% difference in resting glycogen content is of physiological significance (17).

While it is tempting to attribute a causal role for altered muscle substrate availability (i.e., glycogen and/or triglyceride stores) on subsequent changes in AMPK signaling, it should be acknowledged that differences in AMPK responses in the present study could, in part, be due to our well-trained subjects performing prolonged, strenuous training on a high-fat diet (i.e., the interactive effects of diet and exercise). In this regard, Hansen et al. (15) have previously reported that, compared with daily training with normal glycogen reserves, commencing 50% training sessions with low muscle glycogen (and presumably higher triglyceride) levels resulted in a more pronounced increase in citrate synthase activity. The potential mechanism(s) for this augmented training response after “low glycogen” training is hard to define, but it is possible that commencing exercise with reduced glycogen (i.e., high-fat) availability may promote training adaptations through perturbation in circulating systemic factors (i.e., increased catecholamines), altered muscle substrate availability, or a combination of both. Although we did not measure catecholamine levels during training in the present study, Hansen et al. (15) have reported that the catecholamine response to exercise performed with low muscle glycogen levels is higher than when exercise is undertaken with normal glycogen stores, demonstrating a higher stress response. Further work will be needed to determine the precise roles of local (muscle) vs. systemic factors in modifying AMPK responses to diet/exercise manipulations.

As noted previously, one of the residual effects of 5 days of a high-fat diet in combination with intense training is that rates of whole body fat oxidation during submaximal exercise remain elevated above those observed after a HCHO diet, even when CHO stores are restored (3, 6, 32). Accordingly, one would predict that the pattern of activation of the downstream target of AMPK, ACC, would reflect such perturbations. This is indeed the case. pACC Ser221 tended to be higher at rest after fat adaptation compared with the HCHO diet ($P = 0.09$, Fig. 4D), and while there was an exercise-induced increase in pACC Ser221 for both treatments, the phosphorylation state of ACC Ser221 was significantly higher at the end of exercise after fat adaptation ($P = 0.02$; Fig. 4D). Wojtaszewski et al. (35) have previously reported that resting ACC-β Ser212 phosphorylation was higher in subjects with low compared with high resting muscle glycogen stores. In that study (35), concentrations of creatine phosphate and adenine nucleotides in resting and exercised muscles were not altered by the glycogen manipulation, leading these workers to suggest that, in basal conditions at least, fuel-dependent mechanisms independent of energy status may regulate AMPK signaling. Due to insufficient muscle sample in the present investigation, we could not measure high-energy phosphate contents and muscle metabolites, but, using the identical diet-exercise regimen and subjects of comparable training status, our laboratory has previously reported similar resting values for muscle adenine nucleotides (32). However, in contrast to the findings of Wojtaszewski et al. (35), we found that, after 20 min of cycling at the same intensity as employed in the present study, fat adaptation was associated with a lower accumulation of free ADP and AMP after 20 min of submaximal exercise compared with the HCHO intervention (32).

In conclusion, we have used a human model of “dietary periodization” (i.e., fat adaptation followed by CHO restoration) to study the interactive effects of alterations in fuel availability (i.e., muscle lipid stores) and exercise training on AMPK signaling and metabolism. We found that, compared with an isoenergetic CHO diet for 6 days, 5 days of a high-fat diet followed by 1 day of rest and a HCHO diet resulted in higher resting muscle triglyceride levels. The altered muscle fuel status at rest was associated with increased AMPK-α1 and -α3 activity. During exercise, Fat-adapt increased rates of whole body fat oxidation and “spared” muscle glycogen. Accordingly, the increase in ACC Ser212 phosphorylation was greater after fat adaptation compared with the HCHO diet. The changes in AMPK activation observed in the present study could be due to altered fuel status (increased muscle lipid availability), a greater adaptive response when training with low muscle glycogen availability or, most likely, the interactive effects of both of these factors.

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