High-fat diet overrules the effects of training on fiber-specific intramyocellular lipid utilization during exercise

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Van Proeyen K, Szlufcik K, Nielen H, Deldicque L, Van Dyck R, Ramaekers M, Hespel P. High-fat diet overrules the effects of training on fiber-specific intramyocellular lipid utilization during exercise. J Appl Physiol 111: 108–116, 2011. First published May 5, 2011; doi:10.1152/japplphysiol.01459.2010.—In this study, we compared the effects of endurance training in the fasted state (F) vs. the fed state [ample carbohydrate intake (CHO)] on exercise-induced intramyocellular lipid (IMCL) and glycogen utilization during a 6-wk period of a hypercaloric (~+30% kcal/day) fat-rich diet (HFD; 50% of kcal). Healthy male volunteers (18–25 yrs) received a HFD in conjunction with endurance training (four times, 60–90 min/wk) either in F (n = 10) or with CHO before and during exercise sessions (n = 7). The control group (n = 7) received a HFD without training and increased body weight by ~3 kg (P < 0.001). Before and after a HFD, the subjects performed a 2-h constant-load bicycle exercise test in F at ~70% maximal oxygen uptake rate. A HFD, both in the absence (F) or presence (CHO) of training, elevated basal IMCL content by ~50% in type I and by ~75% in type IIa fibers (P < 0.05). Independent of training in F or CHO, a HFD, as such, stimulated exercise-induced net IMCL breakdown by approximately twofold in type I and by approximately fourfold in type IIa fibers. Furthermore, exercise-induced net muscle glycogen breakdown was not significantly affected by a HFD. It is concluded that a HFD stimulates net IMCL degradation by increasing basal IMCL content during exercise in type I and especially IIa fibers. Furthermore, a hypercaloric HFD provides adequate amounts of carbohydrates to maintain high muscle glycogen content during training and does not impair exercise-induced muscle glycogen breakdown.

skeletal muscle; fasted exercise; fat-rich feeding; energy substrate metabolism; muscle lipids

IT IS WELL ESTABLISHED THAT dietary factors play an important role in modulating the acute and chronic metabolic responses to endurance exercise (30). In this regard, increased pre-exercise dietary fat supply stimulates energy provision via fat oxygenation during exercise (8, 33). By analogy, fatty acid infusion elevates the input of free fatty acids (FFAs) in the energy substrate mix fueling muscle contractions (22, 48a, 49). Furthermore, chronic exposure to a high-fat diet (HFD) also enhances the use of FFAs in endurance exercise (8, 9, 25, 29, 32–34, 58). This is, at least partly, due to upregulation of rate-limiting steps in fat metabolic pathways. Thus FFA oxidation can be stimulated with appropriate chronic exercise and/or nutrition (e.g., HFD), which upregulates pivotal steps in FFA catabolism such as fatty acid translocase (FAT)/CD36 (4, 9, β-hydroxyacyl CoA dehydrogenase (β-HAD) (9, 31), and hormone-sensitive lipase (HSL) (58). However, it is still unclear whether these adaptations are specifically due to the increased FFA supply per se or result from decreased glucose availability. Omission of glucose ingestion during exercise also stimulates FFA oxidation rate and upregulates genes involved in fat metabolism such as carnitine palmitoyltransferase I and FAT/CD36 (13, 14).

Fasting is a specific nutritional condition for stimulating FFA supply, relative to carbohydrate supply, to muscles during exercise. The elevated plasma adrenaline-to-insulin ratio, due to fasting, increases circulating FFA concentration via stimulation of peripheral lipolysis (3, 19, 23). At the same time, reduced liver glycogen content impairs glucoseregulation (15, 16), which suppresses the input of blood-borne glucose in energy turnover. Thus during exercise in the fasted state (F), the rate of fat oxidation for a given submaximal exercise intensity is significantly increased (6, 19). This is at least partly due to enhanced degradation of intramyocellular lipids (IMCL) in type I muscle fibers (19). We also demonstrated that consistent training in F can increase membrane-bound fatty acid-binding protein and FAT/CD36 protein content in muscles more than an identical training program with ample carbohydrate intake (CHO) during the training sessions (16a). Furthermore, the fasting endurance-training program also stimulated net IMCL degradation in type IIA muscle fibers during exercise (64). It is reasonable to conclude that exercising with low-carbohydrate availability is an effective strategy to promote fat oxidation rate by enhanced IMCL breakdown in endurance exercise (37, 64, 70). This conclusion is supported by studies showing increased β-HAD activity as well as protein content or elevated activity of citrate synthase and succinate dehydrogenase following training in a carbohydrate-depleted state (26, 37, 48, 64, 70).

Another classical nutritional intervention to stimulate energy turnover via fat oxidation is the administration of a HFD, which increases energy provision via fat oxidation both at rest and during exercise (32, 38, 43, 51). However, previous studies have consistently used isocaloric fat-rich diets when investigating the effects of a HFD on exercise metabolism (8, 9, 25, 29, 32–34, 38, 43, 58). Such strategy implies that increased dietary fat intake is compensated by reduced energy intake in the form of carbohydrates. Inadequate carbohydrate intake between training sessions often results in muscle glycogen depletion (56), which can also alter the metabolic responses to training, independent of increased dietary fat intake (26, 37, 48, 70). Therefore, metabolic adaptations elicited by a hypercaloric HFD, while maintaining adequate carbohydrate supply, may be different from the aforementioned effects of isocaloric HFDs.
Muscle glycogen is the primary energy substrate in high-intensity endurance exercise. In fact, success in endurance competitions often depends on the capacity for high-rate ATP production via glycogen breakdown in the decisive episodes of the competition, such as the pace accelerations when approaching the finish line. However, a number of studies have clearly indicated that the consumption of a HFD impairs the capacity for energy production via glycogen breakdown, even when muscle glycogen stores are ample (32, 33a, 58). Such adaptation is detrimental to endurance exercise performance. We have previously demonstrated that net muscle glycogen breakdown during exercise in F is enhanced compared with exercise with exogenous CHO supply before and during exercise (17). Hence, it is tempting to speculate that fasting exercise may be an effective strategy for stimulating energy provision via fat oxidation, while maintaining the intact potential for glycogenolytic ATP production.

Against the above background, the primary aim of the current study was to investigate the effects of endurance training in F vs. training with CHO intake before and during training sessions on IMCL and glycogen utilization during a period of a hypercaloric HFD. The data demonstrate that a HFD stimulates exercise-induced IMCL breakdown in both type I and IIa fibers. This effect is probably primarily exerted by increased basal IMCL content and overrules the effects of training, independent of whether the training is performed in F or CHO. Finally, a hypercaloric HFD with adequate carbohydrate supply does not impair the capacity for energy provision via glycogen breakdown in endurance exercise.

RESEARCH DESIGN AND METHODS

Subjects

Twenty-eight healthy, young male volunteers (age: 21.2 ± 0.3 yr; body wt: 71.5 ± 1.9 kg) participated in the study, which was approved by the local ethics committee (K.U. Leuven, Belgium) and was in accordance with The Helsinki Declaration. All subjects were involved in regular sports and physical activity for ~3.5 h/wk (range 2–6 h), but none of them was specifically endurance trained in either cycling or running. Subjects were asked not to participate in strenuous exercise and physical activities during the study. Subjects gave their written, informed consent after they were informed of all experimental procedures and risks associated with the experiments. During the study, one subject from CON dropped out due to illness, which was unrelated to the study protocol.

Study Protocol

Preliminary testing and subject randomization. Two weeks before the start of the study and after the intervention period, the subjects performed a maximal incremental exercise test (initial load 100 W + 35 W/3 min) on a bicycle ergometer (Avantronic Cyclus II, Leipzig, Germany) to determine maximal oxygen uptake rate (VO2max) and the corresponding workload. Heart rate (Polar, Kempele, Finland), VO2, and rate of elimination of CO2 (MetaLyzer II, Cortex, Leipzig, Germany) were continuously measured during the test, and the exercise intensity corresponding to the maximal rate of fat oxidation (FATmax) was determined as previously described (1). Furthermore, subjects participated in two familiarization sessions, during which they performed a 2-h exercise bout on the bicycle ergometer following a 10- to 12-h overnight fast. The subjects received 750 ml of water/h during the steady-state exercise. During the first familiarization session, subjects were allowed to adjust the workload (watt) every 10 min to reach a point of exhaustion within 2 h. This workload was further tuned during the second familiarization trial and was eventually used during the experimental sessions (156 ± 5 W). Furthermore, subjects completed a 4-day dietary record to assess their normal dietary habits. Energy intake and diet composition were analyzed using a nutritional software package (Becel 5.00, Unilever Bestfoods, Rotterdam, The Netherlands). Independent of the experimental group, subjects, on average, ingested ~3,000 kcal/day, of which, ~50% in the form of carbohydrates, ~35% fat, and ~15% protein. Based on this preliminary testing, the subjects were matched to obtain triplets with similar values for: VO2max, average workload during the 2-h exercise test, and dietary energy intake (kcal/24 h). At the start of the study, triplet-matched subjects were randomly assigned to the three experimental groups.

Study design and experimental groups. After the randomization, all subjects were enrolled in a 6-wk dietary intervention program involving hypercaloric fat-rich feeding (see Dietary intervention). Two experimental groups combined the diet with a supervised training program, which consisted either of endurance training in F (n = 10) or similar training with CHO before and during the training sessions (n = 10; see Training intervention). CON (n = 7) received a HFD in the absence of training. For the 6-wk intervention period, the subjects participated in a pretest (before) and posttest (at the end).

Dietary intervention. Before the start of the study, high-fat menus for Monday–Friday were composed by a professional dietician. Four different menus were elaborated, which contained 3,000 kcal, 3,500 kcal, 4,000 kcal, or 4,500 kcal/day, and energy intake was similar on training and rest days. Energy distribution in each dietary program was ~50% fat, ~40% carbohydrates, and ~10% protein. Subjects received supervised lunches, whereas all other meals, snacks, and drinks were provided by the investigators as individual take-home food packages. For Saturdays and Sundays, the subjects were instructed to reproduce the dietary pattern from the weekdays, and they completed a detailed food diary. Based on the dietary analyses prior to the start of the study, triplets were assigned to the menu program (3,000–4,500 kcal), best matching an increase in energy intake of ≥30%. Since body weight increased less than expected in the control group after 2 wk of follow-up (0.46 ± 0.23 kg), all subjects were upgraded to the menu with 500 kcal higher-energy intake from the start of week 3.

Training intervention. F performed all training sessions fasted, whereas CHO received a carbohydrate-rich breakfast (675 kcal, 70% carbohydrates, 15% fat, 15% protein) ~90 min before the start of each training session. In addition, during exercise, CHO ingested a solution containing 1 g maltodextrin/kg body wt in 500 ml water/h, while F received a similar volume of water. To obtain identical daily energy intakes between F and CHO throughout the study, F received the nutrients, which they omitted in the morning, plus the amount of maltodextrin they skipped during exercise in the morning. Subjects participated in two 60-min and two 90-min supervised training sessions/wk between 6:30 and 9:00 AM. Training sessions consisted of a combination of cycling and running exercise. F and CHO subjects always trained simultaneously as matched pairs. Due to the randomization procedure, F and CHO had similar VO2max. Hence, during cycling, F subjects were instructed to adjust the workload to obtain a heart rate corresponding with 70–75% VO2max, while CHO subjects adjusted the workload to correspond with their F companion. A similar procedure was used for the running training. Intensity of running was set at 85% of the maximal heart rate obtained from a Bruce protocol during the first training session in the training center (7). Thus training duration and intensity were identical between F and CHO at all times.

Pretest and posttest. Subjects received a standardized diet for 3 days before testing to avoid fluctuations of IMCL due to uncontrolled dietary fat intake (5). In the pretest, subjects received a well-balanced diet (2,500–3,500 kcal, 60% carbohydrates, 25% fat, 15% protein),
whereas during the posttest, subjects continued to adhere to a HFD as prescribed by the study protocol. At each occasion, subjects reported to the laboratory between 6:00 and 10:00 AM after a 10- to 12-h overnight fast. After a 30-min rest, a blood sample (10 ml) was taken from an antecubital vein. Thereafter, a percutaneous needle biopsy was taken from the right m. vastus lateralis under local anesthetic through a 5-mm incision in the skin (2–3 ml Lidocaine). Subjects then cycled for 2 h at the constant workload obtained from the familiarization sessions (156 ± 5 W). During the exercise bout, the subjects received 750 ml of water/h. The volume consumed during the first trial was recorded and reproduced during the second trial. At the end of the exercise bout, another muscle biopsy and a venous blood sample were taken. Part of the muscle sample was frozen immediately in liquid nitrogen. The remaining part was mounted in embedding medium (Tissue-Tek, Sakura FineTek, Zoeterwoude, The Netherlands) and frozen in isopentane, which was cooled in liquid nitrogen. All muscle samples were stored at −80°C until later analysis. Subjects were instructed to abstain from strenuous exercise for at least 2 days prior to the biopsy. During the posttest, the biopsies in F and CHO were taken ~48 h after the last training session.

Biochemical Analyses

Oil Red O staining. Serial sections (4 μm) from biopsy samples were placed on uncoated glass slides for determination of fiber type-specific IMCL content by Oil Red O staining, as we have previously described (18). Fiber type-specific IMCL content was expressed as arbitrary units. For each cross-section, a total of 139 ± 5 fibers was analyzed.

Muscle glycogen. Muscle glycogen content was measured as glucose residues after acid hydrolysis in freeze-dried muscle tissue using a standard enzymatic fluorimetric assay (46).

RNA extraction and reverse transcription. Total RNA from frozen muscle samples was extracted using the Trizol method (Invitrogen, Carlsbad, CA). cDNA was prepared from 1 μg of RNA in a final volume of 20 μl, according to the manufacturer’s protocol (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems, Carlsbad, CA).

Real-time quantitative PCR analysis. Primers and probes for real-time PCR were purchased from Applied Biosystems and supplied as an assay-on-demand gene expression assay mix containing a 20× mix of unlabeled PCR forward and reverse primers, as well as Taqman MGB probe. Assay identification for pyruvate dehydrogenase kinase 4 (PDHK4) was Hs01037712. Real-time quantitative PCR analysis was carried out as previously described (16a). To compensate for variations in input RNA amounts and PCR reaction efficiency, two genes—peptidylprolyl isomerase A (cyclophilin A; Hs99999904) and β-2-microglobulin (Hs00187842)—were chosen using geNorm (65) applet and used as reference genes to normalize the values. Pretest values in CON at rest were assigned the arbitrary value of 1.0, and all other samples were expressed relative to this value.

Muscle lystate production and Western blotting. Muscle lystate production and Western blotting were done as previously described (19). The primary antibodies used were AMP-activated protein kinase α (AMPKα; Cell Signaling Technology, Danvers, MA), phospho-AMPKα Thr172 (Cell Signaling Technology), acetyl-CoA carboxylase β (ACCβ; Cell Signaling Technology), and phospho-ACCβ Ser212 (Cell Signaling Technology). The appropriate secondary antibodies were used (DakoCytomation, Denmark). Band density was calculated by using Kodak 1D Image Analysis software. Results were expressed relative to a standard made from all pretest samples, which was run together with the samples. Pretest values in CON at rest were assigned the arbitrary value of 1.0, and all other samples were expressed relative to this value.

Analysis of blood samples. Plasma nonesterified FFAs were determined using a reagent kit (Wako Chemical GMbH, Neuss, Germany).

Statistical Analyses

Treatment effects were evaluated using a repeated-measures ANOVA. Two-way ANOVA was performed to examine the main effects of treatment and/or time. A planned contrast analysis was used for post hoc comparisons when appropriate. Contrast analysis was also used to evaluate specific preplanned comparisons. The association between variables was analyzed by Pearson product moment correlation analysis. A probability level (P ≤ 0.05) was considered statistically significant. All data are expressed as means ± SE.

RESULTS

Some of the basal values (see Table 1 and Figs. 1–3 and 5) have been previously reported to address the effects of training and a HFD on IMCL and whole-body insulin sensitivity (63).

Body Weight and Exercise Capacity

In the pretest, body weight was similar among the three groups (CON: 70.9 ± 3.4 kg; F: 73.3 ± 3.1 kg; CHO: 70.2 ± 3.6 kg). A HFD increased body weight on average by 3 kg (range +0.4 to +5.7 kg) in CON (73.9 ± 3.2 kg; P < 0.001), +1.2 kg in CHO (71.6 ± 3.4 kg; P < 0.05), and +0.8 kg in F (74.1 ± 2.8 kg; P = 0.30). To evaluate the effect of training on endurance exercise capacity, the subjects performed a maximal incremental exercise test (Table 1). In the pretest, time to exhaustion, VO2max, and FATmax were similar among the three groups, and in CON, values were unchanged by the posttest. The training intervention similarly increased time to exhaustion in F and CHO (+15%; P < 0.05). VO2max was increased by ~7% in CHO (P < 0.01) but not in F (+1.5%; P = 0.53). Training also tended to increase FATmax by ~12% in the two training groups (P ≤ 0.11).

IMCL

In the pretest, baseline IMCL content in both type I and type IIa fibers was similar between the groups, and exercise decreased IMCL content by about ~30% (P < 0.05) in type I fibers but not in type IIa fibers (Fig. 1). Independent of the experimental group, a HFD increased basal IMCL content in the two training groups (Table 1).

Table 1. Effect of high-fat diet, alone or in conjunction with training in either the fasted or the carbohydrate-fed state, on exercise capacity

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>F</th>
<th>CHO</th>
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<tr>
<td>VO2max (ml/min/kg)</td>
<td></td>
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<tr>
<td>Pretest</td>
<td>61.3 ± 3.2</td>
<td>60.3 ± 2.2</td>
<td>60.5 ± 1.5</td>
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<tr>
<td>Posttest</td>
<td>60.7 ± 2.9</td>
<td>61.2 ± 2.6</td>
<td>64.7 ± 1.8*</td>
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<tr>
<td>Time to exhaustion (min)</td>
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<tr>
<td>Pretest</td>
<td>22.8 ± 1.5</td>
<td>21.5 ± 1.1</td>
<td>21.6 ± 1.2</td>
</tr>
<tr>
<td>Posttest</td>
<td>23.6 ± 1.3</td>
<td>25.0 ± 1.1*</td>
<td>24.6 ± 1.2*</td>
</tr>
<tr>
<td>FATmax (watt)</td>
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<tr>
<td>Pretest</td>
<td>141 ± 10</td>
<td>120 ± 13</td>
<td>132 ± 10</td>
</tr>
<tr>
<td>Posttest</td>
<td>138 ± 13</td>
<td>136 ± 9.1</td>
<td>147 ± 11†</td>
</tr>
</tbody>
</table>

Data provided are means ± SE [control group (CON): n = 7; fasted state (F): n = 10; carbohydrate-fed state (CHO): n = 10] and represent exercise capacity, as measured by a maximal incremental exercise test. Values before (Pretest) and after (Posttest) a 6-wk hypercaloric fat-rich diet, either in the absence (CON) or presence of training in either F or CHO are shown. Values are adapted from ref. (63). VO2max, maximal oxygen uptake rate; FATmax, rate of fat oxidation.

*P < 0.05 vs. pretest; †P ≤ 0.11 vs. pretest.
type I (~50%) as well as in type IIA (~75%) fibers (P < 0.01). Compared with the pretest, exercise-induced net IMCL breakdown in type I fibers was increased in all groups (P < 0.01) in the posttest. In addition, IMCL content in type IIA fibers also markedly decreased (~40%; P < 0.01) in all groups. Furthermore, in the posttest, we found significant positive correlations between pre-exercise IMCL levels and exercise-induced net IMCL degradation in both type I (r = 0.38; P < 0.05) and type IIA fibers (r = 0.64; P < 0.001).

**Muscle Glycerogen**

In the pretest, basal muscle glycogen content was similar between the groups (~500 mmol/kg dry weight), and exercise decreased glycogen content by ~50–60% (Fig. 2). Furthermore, in CON, values were similar in the posttest. Compared with CON, basal glycogen levels in the posttest were slightly elevated in both F (P < 0.05) and in CHO (P = 0.14). However, exercise-induced net glycogen breakdown tended to be attenuated (P = 0.09). Thus postexercise muscle glycogen contents were higher in F and CHO than in CON (P < 0.001).

**AMPK and ACC**

In the pretest, basal AMPKα, as well as ACCβ protein expression was similar between the groups (Fig. 3). Compared with the pretest, posttest basal AMPKα protein content was slightly increased in F (P < 0.05) but not in CON or CHO, whereas ACCβ protein was unaffected in all groups. Exercise did not change AMPKα and ACCβ protein expression in the pretest or posttest. The phosphorylated fractions of both AMPKα and ACCβ were similar between groups in the pretest before and after the 2-h exercise bout. Independent of the experimental condition, exercise markedly increased AMPKα and ACCβ phosphorylation (P < 0.01). In CON, AMPKα as well as ACCβ, phosphorylation status at all times was similar between the pretest and posttest. However, compared with CON, training in F (P < 0.05) but not in CHO (P = 0.73) upregulated the basal phospho-to-total AMPKα ratio. Furthermore, compared with the pretest, the exercise-induced increase of AMPKα phosphorylation was completely offset in F (P < 0.05) but not in the other groups. The phosphorylation status of ACCβ paralleled the changes in AMPKα phosphorylation.

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**Fig. 1.** Effect of a high-fat diet (HFD), alone or in conjunction with training in either the fasted (F) or the carbohydrate-fed state (CHO), on intramyocellular lipid (IMCL) content during exercise. Data provided are means ± SE [control group (CON); n = 7; F: n = 10; CHO: n = 10] and represent IMCL content before (pretest) and after (posttest) a 6-wk HFD, either in the absence (CON) or presence of training in either F or in CHO. IMCL content was measured before (Rest) and at the end (Exercise) of a 2-h constant load-cycling bout, and net exercise-induced IMCL breakdown was calculated. Exercise decreased type I IMCL content in the pretest and in the posttest. Type Ia IMCL was reduced by ~40% in the posttest. Fiber type-specific IMCL content was determined by fluorescence microscopy on Oil Red O-stained muscle cross-sections [arbitrary units (A.U.)]. Basal values are adapted from ref. (63). *P < 0.05 vs. pretest.

**Fig. 2.** Effect of a high-fat diet (HFD), alone or in conjunction with training in either F or CHO, on muscle glycogen content during exercise. Data provided are means ± SE (CON: n = 7; F: n = 10; CHO: n = 10) and represent muscle glycogen concentrations [mmol/kg dry weight (dw)] before (pretest) and after (posttest) a 6-wk HFD, either in the absence (CON) or presence of training in either F or in CHO. Muscle glycogen content was measured before (Rest) and at the end (Exercise) of a 2-h constant load-cycling bout, and net exercise-induced glycogen breakdown was calculated. Exercise decreased muscle glycogen content by ~50–60% in the pretest and in the posttest. Basal values are adapted from ref. (63). *P < 0.05 vs. pretest; †P < 0.05 vs. CON; $P < 0.05 vs. F.
including a reduced exercise-induced ACCβ phosphorylation following 6 wk of endurance training, irrespective of the nutritional status during exercise (P < 0.05).

**PDK4 mRNA Expression**

In the pretest, basal PDK4 mRNA contents were similar between the groups, and exercise increased mRNA abundance five- to eightfold (P < 0.01) (Fig. 4). Compared with the pretest, in the posttest, basal PDK4 mRNA content was significantly reduced in F (−64%; P < 0.01) and tended to be lower in CHO (−43%; P = 0.07), whereas no change occurred in CON (P = 0.22). Furthermore, the exercise-induced increase in PDK4 mRNA was markedly blunted in F (P < 0.01) but not in the other two groups.

**Plasma FFA**

In the pretest, the exercise elicited an approximate four- to fivefold increase in plasma FFA concentration in all groups (Fig. 5). Compared with the pretest, posttest baseline plasma FFA level was reduced in F (−42%; P < 0.01) and CHO (−31%; P < 0.05) but not in CON (P = 0.39). Furthermore, in the posttest, the exercise-induced increase in plasma FFA was blunted in all groups, but this effect was more explicit in F than in CON and CHO (P < 0.05).

**DISCUSSION**

In the present study, we investigated the effect of endurance training in conjunction with a HFD on IMCL and glycogen utilization during exercise. We postulated that training in F,
It is well documented that exercise in F, compared with exercise in conjunction with carbohydrate intake, stimulates the contribution of fat oxidation to ATP production in muscles (3, 6, 19, 23). This elevated fat turnover rate is at least partly due to greater utilization of IMCL in type I muscle fibers (19). Against this background, we hypothesized that a fasting exercise training program is superior to consistent training in conjunction with CHO to facilitate exercise-induced IMCL utilization during a HFD. However, independent of the training interventions, a HFD per se markedly increased net IMCL degradation during exercise. In all groups, net exercise-induced IMCL breakdown in both type I and type IIa muscle fibers during exercise without impairing exercise-induced net glycogen degradation.

It is well documented that exercise in F, compared with exercise in conjunction with carbohydrate intake, stimulates the contribution of fat oxidation to ATP production in muscles (3, 6, 19, 23). This elevated fat turnover rate is at least partly due to greater utilization of IMCL in type I muscle fibers (19). Against this background, we hypothesized that a fasting exercise training program is superior to consistent training in conjunction with CHO to facilitate exercise-induced IMCL utilization during a HFD. However, independent of the training interventions, a HFD per se markedly increased net IMCL degradation during exercise. In all groups, net exercise-induced IMCL breakdown in type I fibers during a 2-h submaximal exercise bout, corresponding to ~65% of \( V_{\text{O2max}} \), increased from ~25% of initial content in the pretest to ~50% in the posttest. Unexpectedly, however, a HFD also triggered IMCL utilization in type IIa fibers during exercise (~40% of initial content), which did not use IMCL in the pretest (see Fig. 1). Indeed, available literature clearly shows that during prolonged submaximal exercise, significant IMCL utilization only occurs in type I fibers (16a, 19, 61), except in highly trained endurance athletes, who can use IMCL in type II fibers (57a). We recently found that as little as 6 wk of endurance training, while using an isocaloric carbohydrate-rich diet in subjects with no prehistory of specific endurance training, elicited significant IMCL breakdown in type IIa fibers (64). Thus our present and previous (64) findings clearly indicate that even untrained individuals can use IMCL as an energy fuel in type IIa fibers during exercise following short-term adaptation to either endurance training or a HFD (64). Our current observations also corroborate earlier findings by other laboratories showing an elevated contribution of IMCL to energy provision in endurance exercise following a period of a HFD in young volunteers (39, 71, 72). However, in the latter studies, IMCL content was measured in mixed muscle homogenates, and the increased net IMCL degradation was tentatively explained by greater IMCL oxidation in type I fibers. Based on the current results, such interpretation must be reviewed, because we clearly demonstrate that a HFD substantially promoted IMCL utilization also in IIa fibers.

Increased basal IMCL content probably is the primary candidate to explain the higher exercise-induced IMCL utilization following a HFD alone or in conjunction with training. Earlier studies have repeatedly shown that higher pre-exercise IMCL content facilitates exercise-induced IMCL breakdown (39, 54, 57, 57a, 72). In line with these previous reports (41, 55, 71, 72), a short-term HFD indeed caused a significant increase of basal IMCL content in both type I (~50%) and type IIa fibers (~75%; see Fig. 1). In agreement with previous findings by others (16a, 19) and van Loon et al. (61), fiber-specific IMCL degradation was positively correlated with initial IMCL content, particularly at the higher range of IMCL levels seen in the posttest. By analogy, elevated IMCL hydrolysis during exercise in females compared with males probably also is largely driven by higher basal IMCL content (54, 60). Such interpretation is also supported by the observation that 5 days of a HFD were sufficient to enhance HSL activity during moderate-intensity exercise by ~20–30% (58). The precise role of IMCL concentration in regulating HSL activity is at present unknown. However, it is worthwhile mentioning that high initial muscle glycogen content can promote net glycogenolysis during muscle contractions (28, 36) by covalent activation of the glycogen phosphorylase enzyme (36). A similar mech-
anism of substrate regulation might exist for HSL, which in fact is
the only lipase known to be activated by reversible phosphoryla-
tion, similar to glycogen phosphorylase (67). It also has been
previously suggested that increased circulating FFA concentration
late in prolonged exercise may inhibit IMCL degradation by
reducing HSL activity via long-chain fatty acyl CoA accumula-
tion in muscle cells (66). Along this line, it is reasonable to
speculate that the lower plasma FFA concentrations during exer-
cise following a HFD (see Fig. 5) stimulated IMCL breakdown in
the later stage of the 2-h exercise bout.

Another interesting observation in this study is that a HFD
inhibited the potential of endurance training to promote exercise-
induced IMCL hydrolysis. We have recently demonstrated that
an isocaloric carbohydrate-rich diet did not increase basal IMCL
content but markedly stimulated exercise-induced IMCL hydro-
lysis. Moreover, this effect was more explicit when training
sessions were performed in F than in CHO (64). In the present
study, a HFD increased exercise-induced net IMCL utilization,
independent of whether a HFD was administered alone or in
combination with training. There is evidence to indicate that
training per se can increase contraction-induced activation of HSL
via PKC (21, 44). However, if such effect occurred here, then it
seemed to have been overruled by HFD-induced adaptations. We
have previously reported that the lack of net IMCL hydrolysis in
type IIa fibers probably largely reflects poor recruitment of type
IIa motor units in prolonged submaximal exercise (19). For sure,
a HFD, as such, cannot impact on the motor-unit recruitment
pattern during exercise. Still, a HFD substantially increased IMCL
utilization in type IIa fibers. In this regard, it is reasonable to
speculate that a HFD, probably by virtue of higher IMCL content,
stimulated contraction-induced HSL activity in the active fraction
of type IIa motor units while possibly promoting adenraline-
duced lipolysis in inactive type IIa fibers (45, 67). By analogy,
stimulation of glycogen phosphorylase by adrenaline (53) also can
explain net glycogenolysis in inactive muscle fibers during exer-
cise (2). Finally, it is important to note that besides HSL, adipose
triglyceride lipase (ATGL) is also implicated in regulation of
IMCL breakdown (68). However, it is at present unclear whether
nutritional changes can impact on ATGL regulation.

Muscle glycogen is the primary substrate fueling muscle con-
tractions during high-intensity endurance exercise. Some earlier
studies have reported impaired capacity for muscle glycogenoly-
sis in endurance exercise following the short-term administration
of a low-carbohydrate HFD (32, 33a, 58). However, these diets
causd basal muscle glycogen contents to significantly drop,
which can help to explain the reduced glycogen utilization during
exercise (28, 36). Conversely, we administered a hypercaloric HFD supplying carbohydrates at an even higher daily dose (g/day)
then the subjects’ habitual diet before the study. Hence, normal
muscle glycogen contents were well maintained throughout the
study, and this expectedly resulted in normal net glycogenolysis
during exercise (see Fig. 2). The 6-wk training intervention,
in conjunction with a HFD, tended to slightly decrease exercise-
induced net muscle glycogen degradation in the training groups,
yet this was likely due to slightly lower relative exercise intensity
post-training. In fact, it is well known that endurance training
reduces net muscle glycogen utilization for a given duration and
relative intensity (% VO_{2 max}) of exercise (11, 40). Taken
gether, our data indicate that high-fat intake per se does not impair
muscle glycogen breakdown during exercise, provided muscle
glycogen levels are well maintained by adequate daily carbohy-
trate intake. The administration of a HFD probably also negated
the effect of training in F to inhibit net glycogen breakdown in
endurance exercise. We previously reported 6 wk of fasting
training in conjunction with a carbohydrate-rich diet to cause
muscle glycogen sparing during exercise (64). Here, in fact, we
used a similar fasting training program, but the dietary context
was switched from carbohydrate-rich to a HFD, which eliminated
the training effect on exercise-induced net glycogen degradation.
It is also worthwhile to note that the similar pattern of glycogen
breakdown between the training groups post-training occurred
against the face of a complete offset of exercise-induced AMPKα
phosphorylation in F but not in CHO (see Fig. 3). This finding
once more indicates a dissociation between initial muscle glyco-
gen content and regulation of AMPKα activity after a period of
exercise training (47, 69). Along the same line, AMPKα activa-
tion was not simply linked to baseline IMCL content, as was
recently proposed by Yeo and coworkers (69). However, the
phosphorylation status of ACCβ, one of the major downstream
targets of AMPKα (27), closely reflected the pattern of AMPKα
activation (see Fig. 3) and as such, may be regarded as a confir-
mation of the AMPK findings. The lower ACCβ phosphorylation
immediately after exercise post-training likely results from a
decreased relative exercise intensity rather than being an indica-
tion of fat oxidation during exercise (10), also supported by our
previous data (16a, 62).

Surprisingly, we found PDK4 mRNA content pre- and postex-
ercise to be decreased in F but not in CHO or in CON (see Fig. 4).
Several studies have demonstrated that isocaloric HFDs can up-
regulate PDK4 expression (4, 12, 51). As mentioned above, such
diets typically reduce muscle glycogen content, while increasing
plasma FFA levels. Both of these adaptations can increase muscle
PDK4 mRNA and protein content at rest as well as during
exercise and recovery (42, 52). However, in the present study, F,
more than CHO, elicited a significant increase in muscle glycogen
content (see Fig. 2), whereas plasma FFA levels dropped both
before and during exercise (see Fig. 5). This may explain the
unexpected reduction in PDK4 mRNA expression. Although we
did not measure PDK4 protein content, based on available litera-
ture data, it is reasonable to assume that the decreased PDK4
mRNA under the conditions of the current study corresponded
with decreased PDK4 protein expression (24, 51).

In conclusion, the current study clearly demonstrates that the
administration of a hypercaloric HFD elevates IMCL content
and increases the contribution of IMCL to energy provision in
endurance exercise. This effect is not altered by exercise
training, independent of whether the training is consistently
performed in F or in CHO. Interestingly, a fat-rich diet elicits
significant IMCL utilization during exercise in type IIa fibers,
which otherwise, do not exhibit exercise-induced IMCL break-
down in young, healthy male volunteers. Furthermore, during
training on a hypercaloric HFD, the capacity for muscle glyco-
gen utilization in endurance exercise is well maintained,
probably because adequate dietary carbohydrate supply pre-
vents a training-induced drop of muscle glycogen content.

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

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