High-fat diet elevates resting intramuscular triglyceride concentration and whole body lipolysis during exercise

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Zderic, Theodore W., Christopher J. Davidson, Simon Schenk, Lauri O. Byerley, and Edward F. Coyle. High-fat diet elevates resting intramuscular triglyceride concentration and whole body lipolysis during exercise. Am J Physiol Endocrinol Metab 286: E217–E225, 2004. First published October 14, 2003; 10.1152/ajpendo.00159.2003—This study determined the role of intramuscular triglyceride (IMTG) and adipose lipolysis in the elevated fat oxidation during exercise caused by a high-fat diet. In four separate trials, six endurance-trained cyclists exercised at 50% peak O2 consumption for 1 h after a two-day control diet (22% fat, CON) or an isocaloric high-fat diet (60% fat, HF) with or without the ingestion of acipimox, an adipose lipolysis inhibitor, before exercise. During exercise, HF elevated fat oxidation by 72% and whole body lipolysis [i.e., the appearance rate of glycerol in plasma (Ra glycerol)] by 79% compared with CON (P < 0.05), and this was associated with a 36% increase (P < 0.05) in preexercise IMTG concentration. Although acipimox lowered plasma free fatty acid (FFA) availability, HF still increased fat oxidation and Ra glycerol to the same magnitude above control as the increase caused by HF without acipimox (i.e., both increased fat oxidation 13–14 μmol·kg−1·min−1). In conclusion, the marked increase in fat oxidation after a high-fat diet is associated with elevated IMTG concentration and whole body lipolysis and does not require increased adipose tissue lipolysis and plasma FFA concentration during exercise. This suggests that altered substrate storage in skeletal muscle is responsible for increased fat oxidation during exercise after 2 days of an HF diet.

CONSUMPTION OF A HIGH-FAT DIET necessitates an increased rate of fat oxidation to maintain body composition (10). The rate of adaptation to a high-fat diet depends on the level of physical activity (39, 40) and aerobic fitness (40). In those who exercise regularly, this adaptation is rapid (e.g., <36 h) and is manifest at rest (39) and during exercise (39, 42). Elevated fat oxidation during exercise after a high-fat diet may have additional benefits for physical endurance, because a high-fat diet might spare muscle glycogen use (5). However, elevated fat oxidation created by a high-fat diet has not always been found to significantly improve endurance performance (5, 14). Despite the importance of elevating fat oxidation in the face of elevated fat intake for health and possibly performance, the mechanisms responsible for greater fat oxidation and the source of the augmented fat oxidation after a high-fat diet have not been well described (6).

The primary sources of oxidized fat during exercise in the overnight-fasted state are plasma free fatty acids (FFA) derived from adipose tissue lipolysis and intramuscular triglyceride (IMTG) (48). The consumption of high-fat low-carbohydrate diets is typically reported to result in elevated plasma FFA concentrations (24, 26, 42) and, consequently, elevated plasma FFA uptake by exercising muscle (16, 24). Elevation of plasma FFA by Intralipid and heparin infusion increases fat oxidation during exercise (19, 22), which suggests that elevated plasma FFA after a high-fat diet might be responsible for the greater fat oxidation. Indeed, there are reports concluding that elevated plasma FFA concentration and subsequent increases in FFA uptake are responsible in part (20) or for all of the increase in fat oxidation after a high-fat diet (24). In contrast, Schrauwen et al. (39) reported that, although total fat oxidation was increased by 1-wk high-fat diet, plasma FFA oxidation was not increased.

Increased dietary fat is associated with elevated IMTG concentration (8, 28, 42), and IMTG concentration is positively related to IMTG breakdown (41, 44) and fat oxidation during exercise (15). Therefore, an increase in IMTG concentration and subsequent utilization is another potential mechanism for the increase in fat oxidation following a high-fat diet. Elevated IMTG breakdown and/or adipose tissue lipolysis would be expected to increase whole body lipolysis. However, we are unaware of any direct measurements of lipolysis [i.e., appearance rate of glycerol in plasma (Ra glycerol)] during exercise after a high-fat diet, although there is an indication of increased lipolysis based on the commonly reported elevated plasma glycerol concentrations during exercise. A high-fat diet for 8 wk in sedentary and exercise-trained rats increased whole body lipolysis at rest but was not measured during exercise (36). When we consider that rates of fat oxidation can be markedly affected by acute increases in lipolysis caused by Intralipid and heparin infusion (19, 22), increases in both plasma FFA and/or IMTG concentrations may be the driving mechanisms for the elevated fat oxidation after a high-fat diet.

The purpose of this study was to determine directly, by use of a stable isotope of glycerol, whether a short-term high-fat diet elevates resting IMTG concentration, enhances its utilization during exercise, and increases rates of whole body lipolysis during exercise. Furthermore, we also determined the effect of a high-fat diet on fat oxidation and whole body lipolysis when adipose lipolysis and plasma FFA were severely lowered during exercise by use of acipimox.

METHODS

Subjects and preliminary testing. Six endurance-trained male cyclists [27.0 ± 1.3 yr of age, 76.3 ± 2.7 (SE) kg body wt] volunteered.
to take part in this study after being informed of potential risks associated with participation and after providing written consent. The study was approved by the University of Texas Internal Review Board. Peak O2 consumption (V\textsubscript{O2peak}) was determined while subjects cycled an ergometer (Excalibur Sport; Lode, Groningen, The Netherlands) with an incremental protocol lasting 7–10 min and averaged 4.42 ± 0.12 l/min.

**General procedures.** Four trials were performed, and each required the subject to exercise in the laboratory on three consecutive days. On the first 2 days of each trial, subjects consumed either a control or a high-fat diet that we will describe in detail. Cycle ergometer exercise was performed for 2 h at 65% V\textsubscript{O2peak} on the 1st day and for 1 h at 65% V\textsubscript{O2peak} on the 2nd day. On the 3rd day of each trial, after a 12-h overnight fast, subjects exercised at 50% V\textsubscript{O2peak} and indirect calorimetry. Subjects performed this exercise 2 h after either consuming 250 mg of acipimox (ACP), a nicotinic acid analog, or not consuming acipimox. Acipimox is a potent inhibitor of FFA release from adipose tissue and thus lowers plasma FFA concentration and uptake (13). The trials are referred to as Control diet (CON), high-fat diet (HF), ACP-Control diet (ACP-CON), and acipimox-high-fat diet (ACP-HF). All trials were separated by at least 1 week (6). ACP-HF trials were performed before CON and HF trials. Three subjects performed the control diet trials before the high-fat diet trials, and the other three subjects performed the high-fat diet trials before the control diet trials.

**Diet.** Subjects were provided all of their food for the 8 (total) days of dietary intervention, and they were asked to carefully and honestly report any food not eaten, as well as food eaten in addition to that provided. The control diet (CON) was composed of 22% fat (1 g kg\textsuperscript{-1}·day\textsuperscript{-1}), 65% carbohydrate (9 g kg\textsuperscript{-1}·day\textsuperscript{-1}), and 13% protein, whereas the high-fat (HF) diet was 60% fat (3 g kg\textsuperscript{-1}·day\textsuperscript{-1}), 24% carbohydrate (3 g kg\textsuperscript{-1}·day\textsuperscript{-1}), and 16% protein. On the evening before the 3rd day of all trials, before initiating the overnight fast, subjects consumed a 0.5 g/kg snack of high glycemic carbohydrate. This final meal was maintained constant for all trials to ensure that the metabolism measured on the 3rd day was a reflection of the cumulative effects of the 2-day diet on metabolism and not the effect of the last meal.

**Experimental trial (day 3).** Subjects arrived at the laboratory in the morning, 10 h after the standardized last meal described above. Upon arrival, Teflon catheters were inserted into an antecubital vein in each arm for infusion and blood sampling, respectively. After a 90-min infusion of glycerol and glucose isotope tracers at rest (see **Isotope infusion**), subjects pedaled a cycle-ergometer (Excalibur Sport) for 1 h at 50% V\textsubscript{O2peak}. Approximately 40 min before the start of exercise and immediately after exercise in HF and CON, a biopsy of the vastus lateralis muscle was obtained for determination of IMTG and muscle glycogen concentrations. For ACP-CON and ACP-HF trials, subjects also ingested 250 mg of ACP 2 h before exercise, and muscle biopsies were not performed.

**Isotope infusion.** Upon catheterization, blood was sampled (8 ml) for determination of background isotopic enrichment. Then, a primed constant-rate infusion of [1,1,2,3,3-\textsuperscript{2}H\textsubscript{5}]glycerol (prime = 3.7 mmol/kg, constant = 0.25 mmol kg\textsuperscript{-1}·min\textsuperscript{-1}; Isotec, Miamisburg, OH) and [6,6-\textsuperscript{2}H\textsubscript{2}]glucose (prime = 35 mmol/kg, constant = 0.40 mmol kg\textsuperscript{-1}·min\textsuperscript{-1}) was initiated and maintained for 90 min before exercise and during the 1 h of exercise by use of calibrated syringe pumps (Harvard Apparatus, South Natick, MA).

**Blood sampling and analysis.** For determination of plasma glucose, glycerol, FFAs, insulin concentrations, and rates of plasma glycerol and glucose kinetics, a blood sample (10 ml) was taken immediately before exercise (10 min during exercise and placed in tubes chilled on ice. Eight milliliter was placed into tubes containing 0.4 ml of EDTA (25 mg/ml), and 2 ml were placed in tubes with 0.2 ml of EDTA (25 mg/ml) and aprotinin (0.5 trypsin inhibitor units/ml). Plasma was separated by centrifugation (i.e., 3,000 rpm for 20 min at 4°C) and frozen at —80°C until analysis. Plasma FFA concentrations were determined with a colorimetric assay (33), and plasma glycerol was analyzed with a fluorometric assay (9). Plasma glucose concentration was determined by a colorimetric assay (Trinder; Sigma, St. Louis, MO) and plasma insulin concentration by radioimmunoassay (Linco Research, St. Charles, MO).

**IMTG and glycerol turnover.** Muscle biopsies (48.0 ± 3.0 mg wet wt) were freeze-dried at −50°C for 48–54 h, and aliquots were weighed to the nearest 0.01 mg for muscle glycerol [14.2 ± 0.3 ml of 0.25 mol L\textsuperscript{-1} NaOH was passed down an ion-exchange column, which was then rinsed 5 times with 400 ml of deionized distilled water. The eluant was captured in glass 13×100-mm test tubes and then dried overnight with compressed air. Acetic anhydride (75 μl) and pyridine (75 μl) were added to dried samples, incubated at 100°C for 1 h, and then dried with N\textsubscript{2} gas. Samples were then resuspended in ethyl acetate and injected into a gas chromatograph-mass spectrometer with autosampler (Hewlett Packard 5890 Series II gas chromatograph-5988A mass spectrometer). The masses 145 and 148 of the triacetate derivative of glycerol and the masses 200 and 202 of the pentaacetate derivative of glucose were monitored with selective ion monitoring (35).

**Glycerol and glucose kinetics.** Glycerol and glucose kinetics during exercise were calculated with a modified one-pool non-steady-state model (43)

\[
R_\text{G} = \frac{F}{V_d} \left( \frac{C_1 - C_2}{\eta (t_2 - t_1)} \right) - \left( V_d \left[ (C_2 - C_1)/(t_2 - t_1) \right] \right)
\]

where \( F \) is the isotope infusion rate, \( V_d \) is the effective volume of distribution, \( C \) is the plasma concentration of the tracer, and \( (t_2 - t_1) \) is the change in enrichment (i.e., \( E = \text{tracer/tracer ratio} \)) between two consecutive samples (\( t_2 - t_1 = 10 \) min). \( V_d \) was estimated to be 230 ml/kg for glycerol and 150 ml/kg for glucose (3). \( R_\text{G} \) is the validated measurement of hepatic glucose production during exercise (3). \( R_\text{G} \) is an index of muscle glucose uptake and oxidation during exercise (25). GCR is the glucose clearance rate, an index of the ability of tissues to take up glucose relative to the prevailing plasma glucose concentration. The rate of appearance of glycerol in plasma (\( R_\text{G} \)) was measured as an index of whole body lipolysis, with the assumption that all glycerol released in the process of lipolysis appears in the plasma. \( R_\text{G} \) glycerol could potentially underestimate whole body lipolysis if glycerol is utilized by tissues instead of being released into plasma. Glycerol kinase activity is low in mammalian skeletal muscle, although it appears sufficient to allow some glycerol to be used for muscle triglyceride resynthesis at rest (17). However, during exercise, it is unlikely that glycerol...
Fat and carbohydrate oxidation were calculated from VO₂ and VCO₂ measurements (11). Because at 50% maximal VO₂, it has been reported that 96–100% of Rₐ glucose is oxidized, minimal glycosgen oxidation was calculated as total carbohydrate oxidation minus Rₐ glucose (25).

Statistics. A three-way repeated-measures ANOVA (ACP × diet × time) and mean contrasts according to Bonferroni inequalities were used to analyze dependent variables at specific time points during exercise when there was a significant main effect or interaction. Dependent sample t-tests were used to determine the effect of diet on resting muscle glycosgen and IMTG concentrations. Statistical significance was defined as P < 0.05. The results are presented as means ± SE.

RESULTS

Plasma FFA concentration. ACP-CON and ACP-HF markedly suppressed resting plasma FFA compared with CON and HF, respectively (Fig. 1) (P < 0.05). Acipimox maintained plasma FFA at ~0.10 mM throughout exercise during both ACP-CON and ACP-HF trials. At 50 and 60 min of exercise, HF plasma FFA concentration was slightly but significantly elevated above CON (0.46 ± 0.11 vs. 0.34 ± 0.04 mM, mean 50–60 min, P < 0.05), but there was no significant difference in plasma FFA concentration during the first 40 min of exercise.

Fat oxidation. During exercise, HF elevated fat oxidation by 13.6 ± 1.7 μmol·kg⁻¹·min⁻¹ compared with CON (32.4 ± 2.2 vs. 18.8 ± 2.4 μmol·kg⁻¹·min⁻¹, mean 10–60 min, P < 0.05), which amounted to an increase of 72 ± 5% (Fig. 2). ACP-HF elevated fat oxidation over ACP-CON (24.5 ± 2.4 vs. 11.7 ± 1.9 μmol·kg⁻¹·min⁻¹, P < 0.05) by a similar absolute magnitude (i.e., 12.8 ± 0.7 μmol·kg⁻¹·min⁻¹) compared with the absolute increase in HF vs. CON (i.e., 13.6 ± 1.7 μmol·kg⁻¹·min⁻¹; Fig. 2). In association with the lowering of plasma FFA concentration, acipimox trials also lowered fat oxidation after both control and high-fat diets by 7–8 μmol·kg⁻¹·min⁻¹ (CON vs. ACP-CON and HF vs. HF-ACP, both P < 0.05).

Glycerol kinetics. During the 1st h of exercise, HF significantly elevated RA glycerol above CON (13.63 ± 1.65 vs. 7.62 ± 0.85 μmol·kg⁻¹·min⁻¹, mean 10–60 min, P < 0.05); furthermore, ACP-HF elevated RA glycerol above the ACP-CON trial (6.14 ± 0.48 vs. 3.05 ± 0.61 μmol·kg⁻¹·min⁻¹, mean 10–60 min, P < 0.05). Under both control and high-fat diets, acipimox significantly lowered RA glycerol during exercise (3.05 ± 0.61 vs. 7.62 ± 0.85, and 6.14 ± 0.48 vs. 13.63 ± 1.65 μmol·kg⁻¹·min⁻¹; ACP-CON vs. CON and ACP-HF vs. HF, respectively, both P < 0.05). At rest, RA glycerol was not affected by diet (4.20 ± 0.58 vs. 5.71 ± 1.17, CON vs. HF; 1.91 ± 0.21 vs. 2.68 ± 0.57 μmol·kg⁻¹·min⁻¹, ACP-CON vs. ACP-HF, respectively; neither significant; Fig. 3) but was significantly lowered by acipimox after both diets (P < 0.05). IMTG and glycogen concentrations. IMTG concentration before exercise was significantly elevated by 36% after HF vs. CON (49.9 ± 3.4 vs. 36.8 ± 4.8 mmol/kg dw, P < 0.05; Fig. 4). During 60 min of exercise in CON and HF trials, there was no statistically significant net breakdown of IMTG (4.5 ± 4.3 vs. 8.8 ± 3.3 mmol/kg dw). However, there was a tendency for...
a significant net breakdown during HF ($P = 0.06, n = 5$). Before exercise, resting muscle glycogen concentration was significantly lowered by ~50% with the HF diet (348 ± 36 vs. 716 ± 68 mmol/kg dw), and the net breakdown during exercise was also significantly lower during HF (67 ± 12 vs. 171 ± 40 mmol/kg dw $^{-1}$ 60 min $^{-1}$, HF vs. CON, $P < 0.05$; Fig. 4). Although muscle biopsies were not taken in acipimox trials (ACP-CON and ACP-HF), it was expected that the preexercise muscle substrate concentrations were similar to CON and HF, because the same subjects replicated the diet and exercise protocols.

Carbohydrate and minimal glycogen oxidation. HF decreased carbohydrate oxidation during exercise by 38% below CON (i.e., 146 ± 8 vs. 90 ± 7 mmol·kg$^{-1}$·min$^{-1}$ in CON vs. HF; $P < 0.05$; Table 1). Similarly, ACP-HF decreased carbohydrate oxidation by 30% from 170 ± 10 in ACP-CON to 119 ± 7 mmol·kg$^{-1}$·min$^{-1}$ ($P < 0.05$). Acipimox raised carbohydrate oxidation after both control and high-fat diets (ACP-CON vs. CON, ACP-HF vs. HF, $P < 0.05$). Similar to total carbohydrate oxidation, minimal glycogen oxidation was also lowered by the HF diet, from 128 ± 9 to 74 ± 7 mmol·kg$^{-1}$·min$^{-1}$ without acipimox (CON vs. HF, $P < 0.05$), and also with acipimox, from 147 ± 11 to 100 ± 7 mmol·kg$^{-1}$·min$^{-1}$ (ACP-CON vs. ACP-HF, $P < 0.05$; Table 1). Acipimox, with both the control and high-fat diets, significantly increased minimal glycogen oxidation during exercise (both $P < 0.05$).

Plasma glucose kinetics. During 10–60 min of exercise, $R_a$ glucose, $R_d$ glucose, and GCR were not affected by the high-fat diet in the normal conditions (CON vs. HF, all NS). However, under acipimox, the high-fat diet uniformly lowered $R_a$ glucose (19.3 ± 1.4 vs. 22.9 ± 2.4 mmol·kg$^{-1}$·min$^{-1}$; Fig. 5), $R_d$ glucose (18.8 ± 1.3 vs. 22.3 ± 2.1 mmol·kg$^{-1}$·min$^{-1}$; Fig. 5), and GCR (4.03 ± 0.34 vs. 4.74 ± 0.52 ml·kg$^{-1}$·min$^{-1}$) by 16, 16, and 15%, respectively (all ACP-HF vs. ACP-CON, $P < 0.05$). After the control diet, acipimox elevated $R_a$ glucose (22.9 ± 2.4 vs. 19.4 ± 1.5 mmol·kg$^{-1}$·min$^{-1}$, ACP-CON vs. CON; Fig. 5), $R_d$ glucose (22.3 ± 2.1 vs. 18.4 ± 1.5 mmol·kg$^{-1}$·min$^{-1}$, ACP-CON vs. CON; Fig. 5), and GCR (4.74 ± 0.52 vs. 3.81 ± 0.32 ml·kg$^{-1}$·min$^{-1}$, ACP-CON vs. CON) by 18, 21, and 24% during exercise, respectively (ACP-CON vs. CON, all $P < 0.05$). However, after the high-fat diet, acipimox did not increase $R_a$ glucose, $R_d$ glucose, or GCR during exercise (ACP-HF vs. HF, all $P > 0.05$). At rest, $R_a$ glucose was not significantly affected by diet or acipimox (12.4 ± 0.5, 12.1 ± 0.5, 13.6 ± 0.8, and 13.2 ± 0.7 mmol·kg$^{-1}$·min$^{-1}$ for CON, HF, ACP-CON, and ACP-HF, respectively).

Plasma glucose and insulin. At rest and during exercise, plasma glucose concentrations were similar during CON and HF and were not affected by acipimox (Fig. 6). At 60 min, plasma glucose concentrations in ACP-CON and ACP-HF were significantly below those of the respective resting values.
Table 1. Effects of diet (control or high-fat) and low plasma FFA concentration (acipimox) on total carbohydrate oxidation, glycogen oxidation, and plasma glucose disappearance during exercise

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total CHO Oxidation</th>
<th>Glycogen Oxidation</th>
<th>Rₜ Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>146±8</td>
<td>128±9</td>
<td>18.4±1.5</td>
</tr>
<tr>
<td>HF</td>
<td>90±7*</td>
<td>74±7*</td>
<td>17.3±1.0</td>
</tr>
<tr>
<td>ACP-CON</td>
<td>170±10†</td>
<td>147±11†</td>
<td>22.3±2.1†</td>
</tr>
<tr>
<td>ACP-HF</td>
<td>119±7†</td>
<td>100±7†</td>
<td>18.8±1.3</td>
</tr>
</tbody>
</table>

Total carbohydrate oxidation (Total CHO Oxidation), minimal glycogen oxidation (Glycogen Oxidation), and rate of plasma glucose disappearance (Rₜ Glucose) during 10–60 min of exercise at 50% of peak VO₂ consumption. Values are means ± SE expressed as μmol/kg·min⁻¹·min⁻¹. Trials (2 days): CON, control diet; HF, high-fat diet; ACP-CON, control diet with acipimox; ACP-HF, high-fat diet with acipimox. *HF significantly lower than CON, and ACP-HF significantly higher than HF. All P < 0.05.

(P < 0.05). There were no statistically significant effects of diet or acipimox at rest or during exercise on plasma insulin concentration. By 60 min of exercise, plasma insulin concentration was significantly below resting values in all trials (P < 0.05; Fig. 6).

DISCUSSION

This study demonstrated that the higher fat oxidation during exercise after a short-term high-fat diet is associated with elevated IMTG concentration and whole body lipolysis. Furthermore, the increase in fat oxidation and lipolysis after the high-fat diet does not appear to depend on elevated adipose tissue lipolysis inhibition with acipimox did not attenuate the absolute increase in fat oxidation and lipolysis. This finding suggests that the major substrate responsible for elevated fat oxidation and lipolysis after a short-term high-fat diet is IMTG.

Although several studies have reported elevated concentrations of plasma glycerol after a high-fat diet (5, 26, 42), this is the first study, to our knowledge, that has directly measured whole body lipolysis after a high-fat diet. As expected, lipolysis was indeed elevated. However, an elevation of adipose tissue lipolysis during exercise was not necessary for the increase in whole body lipolysis and fat oxidation with the high-fat diet, as demonstrated when adipose tissue lipolysis was suppressed by acipimox. This suggests that a major source of increased lipolysis during exercise after the high-fat diet was IMTG. It appears that skeletal muscle is the tissue that responds to the high-fat diet used in this study by altering the amount of triglyceride and glycogen stores within its fibers. The high-fat diet significantly elevated IMTG concentration by 36%, and there was 8.8 ± 3.3 mmol/kg·dwt net breakdown of IMTG over the 60 min of exercise (P = 0.06). With the assumption of an active muscle mass of 10 kg during cycling exercise, and the wet-to-dry weight ratio being 4.31 (see METHODS), the increase in net IMTG breakdown during the exercise with the high-fat trial (8.8 vs. 4.5 mmol/kg·dwt) was, on average, 10 mmol triglyceride (10 kg × 4.3 mmol/kg × 1/4.31) and could account for about one-half of the increase in fat oxidation (i.e., 21 mmol triglyceride = 13.6 μmol fatty acid·kg⁻¹·min⁻¹ × 60 min × 76 kg × 1 μmol Tg/3 μmol fatty acid × 1 mmol/1,000 μmol) and lipolysis caused by the high-fat diet, because fat oxidation and lipolysis were elevated by 21 and 27 mmol triglyceride, respectively, over 60 min of exercise during the high-fat trial. Starling et al. (42) reported that, in as few as 24 h, resting IMTG concentration can be increased by 45% and fat oxidation during exercise can be significantly elevated by a high-fat diet. Helge et al. (20) also reported that 7 wk of a high-fat diet (65% fat) significantly elevated IMTG concentration by 73% and nonsignificantly increased IMTG breakdown (~7 mmol/kg·dwt) during 1 h of exercise at 68% VO₂peak. Although the 7 mmol/kg·dwt breakdown of IMTG during 1 h of exercise was not statistically significant in the study by Helge et al., this value is similar in magnitude to the present observation that IMTG breakdown was 8.8 mmol·kg·dwt⁻¹·60 min⁻¹ during HF (P = 0.06; Fig. 4). In addition, a recent report (26) using ¹H magnetic resonance spectroscopy to measure intramyocellular triglyceride net breakdown during 3 h of cycling at 70% VO₂peak demonstrated that 2 days of a high-fat diet resulted in an IMTG breakdown of ~9.5 mmol·kg·dwt⁻¹·60 min⁻¹ compared with
5.2 mmol·kg dw\(^{-1}\)·60 min\(^{-1}\) after a low-fat diet. Technical limitations in measuring IMTG concentration by biopsy due to heterogeneity of IMTG concentration introduce variability into this measure, as discussed by Watt et al. (47). However, the present findings seem to support previous reports of a positive correlation between IMTG concentration and net breakdown (41, 44) and fat oxidation (15) during exercise. It is possible that the elevated IMTG concentration was due to elevated triglyceride storage in adipocytes interlaced between muscle fibers, because our technique could not discern intracellular from extracellular lipid. However, a recent study (29) has demonstrated that the extracellular lipid is not affected by changes in dietary fat and carbohydrate intake over 48 h. Therefore, this increase in lipid storage in muscle samples is likely intracellular, although an increase in extracellular triglyceride storage cannot be completely ruled out. It is important to note that this rapid increase in IMTG concentration caused by the high-fat diet may have been due in part to the training history and the recent acute exercise bouts, as a previous study demonstrated that 3 days of a similar high-fat diet without exercise training did not significantly elevate intramyocellular lipid concentration in the soleus in healthy males but did significantly elevate tibialis anterior intramyocellular lipid concentration by 48% (1).

Because calculated IMTG utilization appears to account for one-half of the elevated fat oxidation, another source of fatty acid is also probably involved in the increase in fat oxidation caused by the high-fat diet. The absolute increase in fat oxidation after the high-fat diet apparently did not require an increase in plasma FFA concentration, because it also occurred and was approximately the same magnitude of increase (i.e., 13–14 \(\mu\)mol·kg\(^{-1}\)·min\(^{-1}\)) under the low prevailing plasma FFA concentration of \(\sim 0.1\) mM created by acipimox. In agreement with the findings of this investigation, a recent study reported that nonplasma FFA oxidation during rest and exercise, determined by \(^{13}\)C-palmitate infusion and indirect calorimetry, increased after 7 days of a high-fat (60% fat, 25% carbohydrate) diet compared with a typical control diet (30% fat, 55% carbohydrate) (39). Although plasma FFA oxidation was not directly determined in the present study, it is expected that plasma FFA concentration provides a general reflection of turnover, since plasma FFA uptake appears proportional to plasma FFA concentration, in the range of 0.1–0.4 mM observed in this study (45). Important for the comparison between control and high-fat diets, plasma FFA clearance seems unaffected by short-term (24, 39) and long-term (20) high-fat diets. This suggests that a major factor driving oxidation of plasma FFA, as well as IMTG, is its concentration. Another source of triglyceride that may contribute to the increase in fat oxidation and lipolysis after the high-fat diet may be VLDL-triglyceride, as the uptake of this substrate has been reported to be increased after 7 wk on a high-fat diet (20). The recent finding that nicotinic acid, an analog of acipimox, almost halts VLDL synthesis (46), combined with the present finding that acipimox did not attenuate the increase in fat oxidation, suggests that the VLDL-triglyceride turnover was not critical for the increase in fat oxidation caused by the high-fat diet. Nevertheless, whether or not 2 days of a high-fat diet can increase leg uptake of VLDL-triglyceride is unknown and should be examined as a potentially significant source of increased fat oxidation and lipolysis after a short-term high-fat diet.

In contrast to the findings of the present study and others (39), there are many reports of elevated plasma FFA concentrations (20, 24, 26, 42) and uptake (20, 24) after high-fat diets. These findings of increased plasma FFA concentration and uptake may have been due not just to the cumulative effects of the high-fat diet over days or weeks but more as a consequence of the carbohydrate content of the final meal eaten in the hours preceding exercise. In previous studies (20, 24, 26, 42), the last meal preceding exercise in the high-fat trial was low in carbohydrate, whereas the meal preceding exercise after the control diet was relatively high in carbohydrate. The carbohydrate content of a meal has profound effects on elevating insulin, inhibiting lipolysis, and lowering subsequent plasma FFA concentrations for 12–16 h (30, 38). Therefore, the commonly reported elevation of plasma FFA concentration and uptake should be considered an effect of the final meal and not a
reflection of the cumulative effects of the high-fat diet over longer periods of a day to weeks. The final meal before all four trials of the present study, given 12 h before exercise, was identical and contained 0.5 g/kg of carbohydrate to avoid any confounding effects of the final meal. This resulted in similar plasma FFA concentrations between CON and HF trials at rest and throughout the first 40 min of exercise, with a small increase in plasma FFA after 50–60 min of exercise with HF (0.46 ± 0.11 vs. 0.34 ± 0.04 mM, P < 0.05). Therefore, it seems that provision of an identical last meal containing carbohydrate causes a high-fat diet to elevate plasma FFA concentration only slightly and only after ~1 h of moderate intensity exercise. Furthermore, the acipimox trials ensured that plasma FFA was very low and similar between the control and high-fat diets during exercise, and it was further verified that the effects of the high-fat diet in raising fat oxidation and lipolysis were independent of plasma FFA.

The marked reduction in muscle glycogen concentration may also have been an important factor responsible for the increased fat oxidation, because low muscle glycogen concentration reduces glycogenolysis (21, 37) and carbohydrate oxidation. However, Burke et al. (5) reported that elevated fat oxidation and reduced glycogenolysis after 5 days on a high-fat diet were still maintained over a control diet, even after muscle glycogen was restored to control levels with a 1-day high-carbohydrate diet (5). Whether these results from a 5-day high-fat diet apply to the present 2-day high-fat diet is unknown, as IMTG concentration was not measured by Burke et al. However, associated with this attenuation of glycogen utilization by a high-fat diet was the elevation of plasma glycerol concentration, suggesting elevated whole body lipolysis (5). Because artificially elevating lipolysis with Intralipid and heparin infusion can reduce muscle glycogenolysis somewhat (19, 22), elevated lipolysis and fat oxidation from IMTG after a high-fat diet may have suppressed muscle glycogenolysis.

Potential mechanisms for elevated fat oxidation may be related to changes in malonyl-CoA and/or AMP-activated protein kinase (AMPK) after the high-fat diet. Recent reports have indicated that elevated AMPK activity is associated with lower muscle glycogen in human muscle (50). An elevated AMPK activity associated with this high-fat diet and low muscle glycogen may have activated fatty acid oxidation, as it has been reported that activation of AMPK can increase fatty acid oxidation, by reducing malonyl-CoA via acetyl-CoA carboxylase inhibition (49). However, Kaushik et al. (27) reported that AMPK activation in overnight-fasted rat soleus does not lower already low malonyl-CoA concentrations or increase fat oxidation. In addition, it appears that elevation of malonyl-CoA in human muscle requires marked elevation in plasma insulin (2). Because the subjects in the present studies were overnight fasted, and insulin concentrations were similarly low between diets at rest (4–5 µU/ml) and during exercise (2–4 µU/ml), it is likely that malonyl-CoA concentrations were already very low after both diets.

The lowering of muscle glycogen concentration with the high-fat diet might be expected to increase R_d glucose, as low muscle glycogen has sometimes been associated with elevated muscle glucose uptake in rats (37) and humans (16). However, this effect of low glycogen on increasing glucose uptake has not been consistently observed in exercising humans (18, 24, 48). Along these lines, without acipimox, the high-fat diet did not alter plasma glucose kinetics despite a 50% lower muscle glycogen at the start of exercise. We interpret the literature to suggest that increased glucose uptake, associated with low muscle glycogen, may occur when there are very low muscle glycogen concentrations and high relative exercise intensity and under conditions when fat oxidation cannot increase sufficiently to compensate for reduced muscle glycogen oxidation (31). Indeed, after the control diet, the lowering of plasma FFA and fat oxidation with acipimox elicited an increase in plasma glucose R_s and R_d, agreeing with our interpretation of the literature. However, under conditions of high-fat diet and the low plasma FFA concentrations created by acipimox, we observed decreased R_d glucose, R_s glucose, and GCR by 16, 16, and 15%, respectively, despite the lowering of muscle glycogen concentration. This observation does not agree fully with our interpretation stated above. An explanation for the decrease in R_s glucose with combined high-fat diet and acipimox administration may be based on a heavy reliance of R_s glucose on gluconeogenesis in the face of decreased hepatic glycogen stores (32) after a high-fat diet (36). Because of the reported dependence of gluconeogenesis on plasma FFA availability in 16-h-fasted individuals at rest (7), the suppression of plasma FFA may have compromised R_s glucose. In addition, suppression of glycerol production, an important gluconeogenic precursor, with nicotinic acid, an analog of acipimox, has been reported to impair R_s and R_d glucose at rest after a 3.5-day fast, a time when R_s glucose is very dependent on gluconeogenesis (23). Therefore, suppression of glycerol production, as well as plasma FFA mobilization, when the liver is dependent on plasma FFA for gluconeogenesis and on glycerol as a gluconeogenic precursor, may have compromised the ability of R_s glucose to increase. It is interesting that R_d glucose and GCR were also reduced by the high-fat diet under acipimox without any lowering of plasma glucose concentrations. Although it is known that hepatic portal glucose concentrations can positively affect muscle glucose uptake independently of changes in peripheral plasma glucose concentrations (4), whether or not hepatic glycogen also exerts effects on R_d glucose and GCR is unknown, and further research into this area is warranted.

In conclusion, elevated fat oxidation during exercise after a 2-day high-fat diet was associated with an increase in preexercise IMTG concentration and whole body lipolysis during exercise. Additionally, the magnitude of elevation in fat oxidation and lipolysis did not appear to depend on elevated adipose tissue lipolysis and plasma FFA concentration during exercise. These data indicate that IMTG lipolysis and oxidation together are a major source of elevated fat oxidation and lipolysis after a high-fat diet when the confounding influence of the last meal is controlled. Therefore, skeletal muscle appears to be the tissue that is primarily responsible for increased fat oxidation during exercise after a short-term high-fat diet as a result of altered intrinsic substrate storage.

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