Low-fat diet alters intramuscular substrates and reduces lipolysis and fat oxidation during exercise

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Received 5 July 2000; accepted in final form 25 October 2000

Low-fat diet alters intramuscular substrates and reduces lipolysis and fat oxidation during exercise. Am J Physiol Endocrinol Metab 280: E391–E398, 2001.—We determined whether a low-fat diet reduces intramuscular triglyceride (IMTG) concentration, whole body lipolysis, total fat oxidation, and calculated nonplasma fatty acid (FA) oxidation during exercise. Seven endurance-trained cyclists were studied over a 3-wk period during which time they exercised 2 h/day at 70% of maximum O\textsubscript{2} uptake V\textsubscript{O\textsubscript{2}}\textsubscript{max} and consumed ~4,400 kcal/day. During the 1st wk, their fat intake provided 32% of energy. During the 2nd and 3rd wk, they were randomly assigned to eat 2 or 22% of energy from fat (2%FAT or 22%FAT). Compared with 22%FAT, 2%FAT lowered IMTG concentration and raised muscle glycogen concentration at rest (P < 0.05). Metabolism was studied during 1 h of exercise at 67% V\textsubscript{O\textsubscript{2}}\textsubscript{max} performed in the fasted state. 2%FAT resulted in a 27% reduction (P < 0.05) in total fat oxidation vs. 22%FAT without altering the stable isotopically determined rates of plasma free fatty acid or glucose disappearance. Therefore, 2%FAT reduced calculated nonplasma FA oxidation by 40% in association with a 19% reduction in whole body lipolysis while increasing calculated minimal nonplasma FA oxidation during exercise in the fasted state in association with a reduced concentration of intramuscular triglyceride.

Stable isotopes; lipid; carbohydrate; de novo lipogenesis; obesity

FAT AND CARBOHYDRATE, the substrates that power aerobic exercise, are stored directly within muscle fibers as intramuscular triglyceride (IMTG) and glycogen or are transported to them via the circulation, predominantly in the form of plasma free fatty acids (FFA), plasma glucose, and possibly plasma triglycerides (9, 27, 31). FFA is supplied largely from adipocyte triglyceride and plasma glucose from liver glycogen, albeit with some gluconeogenesis, in that plasma. Therefore, it is generally correct to think that the fat and carbohydrate oxidized during exercise are derived predominantly from triglyceride and glycogen stored within the body (27, 31). It is assumed that the fat oxidation during exercise that is not obtained from plasma FFA oxidation is derived predominantly from oxidation of intramyocellular triglyceride (27) as well as plasma triglyceride, although the quantitative importance of the latter is not clear.

It has been well established that active people who eat a diet low in carbohydrate have markedly reduced glycogen stores in muscle and liver, and they accordingly display impaired carbohydrate oxidation during exercise and a reduced ability to exercise intensely for prolonged periods (17). This is because dietary fat, and to a lesser extent dietary protein, have limited gluconeogenic ability and are thus incapable of maintaining glycogen stores (31). This classic understanding has led to the development of diets for active people that contain prescribed amounts of carbohydrate needed to optimally replenish glycogen stores and thus maintain carbohydrate oxidation and the ability to exercise intensely (4).

It is generally believed that fat oxidation during exercise is not influenced by body triglyceride storage in any tissue, because even lean individuals have a much greater amount of energy stored as triglyceride, predominantly in adipose tissue, compared with what can be expended during even prolonged exercise. To our knowledge, there have been no systematic studies of the minimal dietary fat intake needed to maintain fat oxidation during exercise and to maintain glycogen concentration or even whether there is a minimal need. These data might also be important in light of the controversy regarding the extent to which people who are weight stable can activate lipogenesis from dietary carbohydrate (2, 15, 16, 26). Although it is well known that people in positive caloric balance for several days exhibit marked lipogenesis from dietary carbohydrate (1), it has been demonstrated that weight-stable people who are in caloric balance display negligible lipogenesis from dietary carbohydrate and protein (16, 26). These observations support the concept that the turnovers (i.e., storage and oxidation) of body triglyceride

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and glycogen are generally independent of each other in weight-stable people (11).

The present investigation employed endurance-trained athletes who exercise intensely for 2 h/day and who thus normally oxidize a very large amount of triglyceride per day. Metabolism was studied during exercise in the fasted state after a week-long period on a eucaloric diet, during which time these athletes reduced their normal dietary fat intake (32% of calories) to levels of 22 and 2% of calories from fat. The main purpose of this study was to determine the extent to which a very low fat diet (2% of calories from fat) reduces fat oxidation during exercise and the possible mechanism of this effect. We hypothesized that the very low fat diet would reduce the resynthesis of IMTG stores, a significant source of fat oxidation during exercise (9, 27) and would subsequently reduce whole body lipolysis during exercise. By means of such low dietary fat intake in combination with high caloric expenditure, this study also provided the opportunity to study people during a period of prolonged negative fat balance, if indeed daily fat oxidation exceeded dietary intake. A secondary purpose was to determine whether a low-fat and high-carbohydrate diet produced signs of lipogenesis in weight-stable people who are physically very active.

METHODS

Subjects. Seven endurance-trained male cyclists participated in this experiment. Their peak oxygen consumption (V\textsubscript{O\textsubscript{2 peak}}) body weight, percent body fat, and age were 4.69 ± 0.24 L/min, 71.9 ± 4.1 kg, 13 ± 4%, and 25 ± 2 yr, respectively. Subjects were informed of the possible risks, and each signed a consent form approved by the Internal Review Board of the University of Texas at Austin.

Preliminary testing. V\textsubscript{O\textsubscript{2 peak}} was determined while subjects cycled on an ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) by means of an incremental protocol lasting 7–10 min. Furthermore, the exercise intensity and heart rate relative to the blood lactate threshold (LT, 1 mM) was determined, and exercise intensity and heart rate relative to the blood lactate threshold (LT, 1 mM) were determined.

Exercise training. During day 1 of each dietary period, subjects did not exercise; they adjusted to the diet and made final food selections by consuming an ad libitum amount of the diet provided. On days 2–6, the subjects cycled for 2 h in the morning after an overnight fast at a power output eliciting 10% below LT. After 30 min of exercise, subjects were provided with a 6% carbohydrate and electrolyte solution to drink. Cycling was supervised in the laboratory on days 2, 4, and 6, whereas cycling was usually performed outdoors on days 3 and 5, with intensity verified via heart rate monitoring. On day 7, metabolism was studied, as described below, during the 1st h of a 2-h bout of exercise.

Experimental procedure. Subjects arrived at the laboratory in the morning, and exercise began 10 h after the standard carbohydrate meal described above. Upon arrival, teflon catheters were inserted into an antecubital vein in each arm for determination of resting and blood sampling, respectively. A heating pad was affixed to the sampling forearm to obtain arterialized blood. After 60 min of resting isotope infusion (see Isotope infusion), subjects pedaled a cycle ergometer for 60 min at 67% V\textsubscript{O\textsubscript{2 peak}}, which corresponded to an intensity 10% of work rate below LT. Approximately 40 min before the start of exercise, a biopsy of the vastus lateralis muscle was obtained for determination of muscle glycogen (6) and muscle triglyceride concentration (12).

Isotope infusion. Upon catheterization, blood was sampled (6 ml) for determination of background isotopic enrichment. Then, a primed constant-rate infusion of [1,2,3,3,6\textsuperscript{2}H\textsubscript{5}]glycerol (prime = 3.7 \textup{mol/kg}; constant = 0.25 \textup{mol/kg} \cdot \text{min}^{-1}; Isotec, Miamisburg, OH) and [6,6-\textsuperscript{2}H\textsubscript{2}]glucose (0.39 \textup{mol/kg} \cdot \text{min}^{-1}; prime = 33 \textup{mol/kg}) was begun by use of calibrated syringe pumps (Harvard Apparatus, South Natick, MA). In addition, [1,13\textsuperscript{C}]palmitate (Cambridge Isotope Lab, Andover, MA) bound to 5% human albumin was infused (0.04 \textup{mol/kg} \cdot \text{min}^{-1}; no prime). These stable isotope infusions were delivered during 60 min of rest to achieve isotopic equilibrium and were maintained at their constant rate throughout exercise.

Blood sampling and analysis. For determination of resting blood glucose, glycerol, and palmitate kinetics, blood samples were withdrawn 5 min before and immediately before exer-

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<th>Table 1. Composition of the diets and body weight</th>
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*Values are means ± SE; n = 7. 32\% FAT, 22\% FAT, and 2\% FAT, diets with 32, 22, or 2% of energy from fat, respectively. All three diets were designed to be different from each other in the relative (%) and absolute (g/day) amounts of fat and carbohydrate.
cise. During exercise, blood samples (~14 ml) were collected at 20 and 30 min as well as at 50 and 60 min, and kinetics were calculated as the mean of a 25- and 55-min value to represent this 1-h period. After collection, blood samples were divided into three different prechilled tubes according to the constituents to be analyzed. For each tube, plasma was separated by centrifugation (3,000 rpm for 20 min at 4°C) and was immediately frozen at -70°C until analysis. Seven milliliters of each blood sample were placed into tubes containing 0.4 ml of EDTA (25 mg/ml) and analyzed for isotopic enrichment using gas chromatography-mass spectrometry (GC-MS; Hewlett Packard 5989) of the heptafluorobutyric anhydride (HFBA) derivative of glycerol (33), the aldinitol acetate derivative of [3]H]glucose (30), and the methyl ester derivative of palmitate (33). Five milliliters of plasma were placed in tubes containing 0.25 ml of EDTA (25 mg/ml) for determination of plasma glycerol (fluorometric assay) (8), lactate (14), free fatty acid (FFA, colorimetric assay), (25) and glucose (YSI 23a glucose autoanalyzer; Yellow Springs Instrument; Yellow Springs, OH). The final 2 ml of each blood sample were placed into a test tube containing 0.1 ml of EDTA (24 mg/ml)-aprotinin (0.5 TIU/ml) solution and analyzed for plasma insulin concentration (RIA; Linco Research, St. Charles, MO).  

Isotope enrichment sample preparation. Plasma samples (300 µl) were added to 3 ml of chloroform-methanol (3:1). Each tube was then shaken vigorously and centrifuged at 3,000 rpm for 10 min at 4°C. The supernatant was placed in clean tubes, and 3 ml of methanol and 1 ml of distilled water (pH 2) were added for lipid extraction. The tubes were then centrifuged again at 3,000 rpm for 10 min at 4°C. The top (aqueous) layer was removed, placed in separate tubes, and dried under nitrogen until further analysis for glucose and glycerol [2H] enrichment. The bottom part was also dried and stored at room temperature until analysis of [13C]palmitate enrichment.

The HFBA derivatives of glycerol and glucose were prepared by adding 200 µl of heptafluorobutyric acid (Supelco)-ethanol (1:3) to the tubes and incubating the tubes at 70°C for 10 min. Samples were then dried under nitrogen, and 100 µl of ethyl acetate were added before injection (1 µl) into the GC-MS for the measurement of glycerol enrichment. Samples were then dried again under nitrogen, and 150 µl of HFBA-ethyl acetate (1:3) were added, after which 1 µl was injected into the GC-MS for analysis of glucose enrichment.

The lipid extracts were derivatized by adding 250 µl of a iodomethane solution (500 µl iodomethane in 10 ml dichloromethane) and 250 µl 0.2 M tetrabutylammonium hydrogen sulfate. After shaking for 10 min and sonication for 30 min, 3 ml of hexane were added to each tube. The tubes were then vortexed and centrifuged at 3,000 rpm for 10 min at 4°C. The supernatant was passed through a silica gel solid-phase extraction column (2 g, Supelco), and the eluent was dried under nitrogen and reconstituted with 50 µl of heptane. One microliter was injected into the GC. Stable isotope enrichment was measured by electron impact GC-MS by selectively monitoring the mass-to-charge ratio of (m/z) molecular ions 270 and 271 for palmitate, 253 and 257 for glycerol, and 519 and 521 for glucose.

Measurements of gas exchange. At rest and periodically during exercise (20–30 and 50–60 min) subjects inhaled through a two-way Daniels valve while inspired air volume was measured with a Parkinson-Cowan CD4 dry gas meter (Rayfield Equipment, Waitsfield, VT). The expired gases were continuously sampled from a mixing chamber and analyzed for oxygen (Applied Electrochemistry, SA3, Ametek, Pittsburgh, PA) and carbon dioxide (Beckman LB2; Schiller Park, IL). These instruments were interfaced to a computer for calculation of V02 and VCO2.

Calculations. Plasma glycerol, glucose, and palmitate kinetics were calculated using the one-pool model non-steady-state equations of Steele (29), modified for use with stable isotopes

$$R_a = (F - V_d(C_1 + (E_1 + E_2)/2)) \times (E_2 - E_1)/(t_2 - t_1))/V_d$$

$$R_d = [(C_2 - C_1)/(t_2 - t_1)]/(E_1 + E_2)/2$$

where F is the isotope infusion rate, V_d is the effective volume of distribution, C is the plasma concentration of the trace and (E_2 - E_1)/(t_2 - t_1) is the change in enrichment (i.e., E_tracer-to-tracer ratio) between two consecutive samples (t_2 - t_1 = 10 min). V_d was assumed to be 230 ml/kg for glycerol, 100 ml/kg for glucose and 40 ml/kg for palmitate, as discussed previously (27). The rate of appearance (R_a) of glycerol in plasma 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_d glycerol might potentially underestimate whole body lipolysis if glycerol is utilized by tissues instead of being released into plasma (19, 21). Glycerol kinase activity is low in adipose tissue (23); glycerol kinase activity is also low in mammalian skeletal muscle (20), although it appears sufficient to allow some glycerol to be used for IMTG resynthesis at rest (13). However, during exercise, it is unlikely that glycerol utilization takes place in human tissues to an extent that would meaningfully invalidate the measurement of lipolysis from blood glycerol kinetics.

R_a FFA was calculated by dividing R_a palmitate by the fractional contribution of palmitate to total FFA concentration as determined by gas chromatography (GC-FID; Varian 3400). Formulas for nonprotein respiratory quotient were applied to calculate fatty acid and carbohydrate oxidation (22). Finally, R_a glycerol was multiplied by 3 to account for the three fatty acids liberated from the complete hydrolysis of a triglyceride molecule. Nonplasma fatty acid (FA) oxidation was calculated as the difference between total FA oxidation and rate of disappearance (R_d) FFA, which assumes that all of R_a FFA is oxidized, whereas previous direct measures have reported 88% of R_d to be oxidized in the fasted state (7). Furthermore, it is assumed that nonplasma FA oxidation is derived predominantly from IMTG (7, 9, 27), although the quantitative importance of plasma triglyceride is not clear.

Statistical analysis. In that 32%FAT was always presented first and then 22%FAT and 2%FAT were subsequently randomized, this study was designed for planned comparisons with the use of mean contrasts of 2%FAT vs. 22%FAT. Treatments by time interactions were identified by means of analysis of variance with repeated measures in a complete within-subjects design (SuperAnova; Abacus, Berkeley, CA). Statistical significance was defined as P < 0.05. The results are presented as means ± SE.

RESULTS  

Diets, body weight, and body composition. The caloric contents of the diets were similar, and the body weights and percentages of body fat of the subjects did not vary from week to week (Table 1). The control diet (32%FAT) that was administered first contained a fat and carbohydrate content typical for these endurance athletes. The randomly administered subsequent diets contained <2% fat or 22% fat, with a relatively high
carbohydrate content that was significantly different (i.e., 901 ± 65 vs. 718 ± 34 g/day; Table 1).

**Muscle substrate concentrations.** Intramuscular triglyceride concentration with 2%FAT was significantly reduced (21%, \( P < 0.05 \)) compared with 22%FAT. Additionally, muscle glycogen concentration was higher (18%, \( P < 0.05 \)) with 2%FAT compared with 22%FAT. Figure 1 indicates that the amount of energy (kcal/kg dry) stored in the muscle as the sum of glycogen and IMTG was significantly lower (\( P < 0.05 \)) with 32%FAT compared with either 2%FAT or 22%FAT.

**Plasma substrate and insulin concentration.** Figure 2 indicates that plasma glucose and FFA concentrations were similar at rest and during 60 min of exercise with 2%FAT and 22%FAT. Preexercise plasma insulin was also similar at rest (4.6–5.6 μU/ml) and during exercise (2–4 μU/ml) with 2%FAT and 22%FAT; plasma lactate was also similar. However, plasma glycerol concentration during the 30–60 min period of exercise was reduced (\( P < 0.05 \)) with 2%FAT compared with 22%FAT, despite displaying identical levels at rest (Fig. 2).

**Substrate oxidation during exercise.** There were no treatment-by-time interactions, thus values for 60 min of exercise are reported as the means of the 20- to 30- and 50- to 60-min values. The most striking effect of 2%FAT compared with 22%FAT was that, during exercise, it reduced whole body glycerol appearance by 19% (\( P < 0.05 \); i.e., \( R_a \) glycerol) while also reducing total fat oxidation by 27% (\( P < 0.05 \); Table 3), despite displaying similar \( R_a \) glycerol at rest before exercise. However, plasma FFA kinetics (\( R_n \) or \( R_d \) FFA) were not different with these two diets, either at rest or during exercise. Therefore, the minimal oxidation of nonplasma FA during exercise appeared to be reduced by 40% (\( P < 0.05 \)) with 2%FAT compared with 22%FAT (Table 3).

Plasma glucose kinetics were not different at rest or during exercise; yet total carbohydrate oxidation was 17% higher (\( P < 0.05 \)) with 2%FAT compared with 22%FAT (Table 3). Thus calculated muscle glycogen oxidation was also elevated 19% (\( P < 0.05 \)) with 2%FAT compared with 22%FAT.

**Composition of plasma FA.** After the week-long control dietary period on the 32% fat diet, palmitate comprised 21.9 ± 3.5% of the total fatty acids in plasma. After 22%FAT and 2%FAT, the percentage of fatty acids that was palmitate was increased to 26.0 ± 4.8% and 28.8 ± 2.4%, respectively.
DISCUSSION

The week-long dietary regimen employed in this study attempted to place the subjects in negative fat balance by having them cycle for 2 hours/day while restricting fat intake to <2% of calories (2%FAT) in the form of just 9 g/day of essential fatty acids. This was compared with a diet of 22% fat (105 g/day), which in itself is somewhat lower than a more typical diet, containing 32% fat (149 g/day), eaten during week 1 of the study (CONTROL). A general effect of 2%FAT compared with 22%FAT was that it lowered IMTG concentration 21% \((P < 0.05)\) and raised muscle glycogen concentration 18% \((P < 0.05; \text{Table 1})\) without influencing the \(R_{d}\) or \(R_{a}\) of plasma glucose or FFA or their concentrations. Thus the effects on substrate utilization seemed to be localized to the exercising musculature and not to plasma substrate delivery or uptake. The first major finding of this study was that the reduction of whole body fat oxidation in 2%FAT vs. 22%FAT appeared to be due to reduced oxidation of nonplasma FA, which presumably reflected reduced IMTG oxidation. This was also associated with a significant 19% reduction in the independent measure of whole body lipolysis (i.e., \(R_{d}\) glycerol) during exercise in 2%FAT vs. 22%FAT. This response would be expected if IMTG lipolysis and nonplasma FA oxidation were indeed reduced. Together, these observations suggest that a diet containing 2% fat, as opposed to 22% fat, exerts its primary effect on muscle substrate concentration by lowering IMTG and possibly by raising glycogen and that, during subsequent exercise, the oxidation of these intramuscular substrates was altered in general accordance with their altered concentration.

Elevated muscle glycogen during exercise typically results in increased glycogenolysis and thus increased carbohydrate oxidation with reduced fat oxidation (4, 6, 17). A possible mechanism for the reduction in fat oxidation with increased glycolytic flux might relate to inhibition of fatty acid transport into the mitochondria via carnitine palmitoyltransferase (7). Therefore, we cannot discount the possibility that the presently observed reduction in total fat oxidation and nonplasma FA oxidation with 2%FAT compared with 22%FAT was somewhat related to elevations in glycogen concentration and glycolytic flux separate from or in addition to the effects of the 2%FAT on reducing IMTG concentration.

We are not aware of any previous studies that have systematically altered IMTG concentration within a given population and determined whether this influences its use during exercise. Standl et al. (28) reported that insulin-dependent diabetics deprived of insulin, compared with normal subjects, display increased IMTG stores and increased IMTG degradation that was associated with larger increases in plasma glycerol concentration during exercise. Furthermore, Essén-Gustavsson and Tesch (10) observed that, in bodybuilders performing heavy-resistance exercise, the reduction in IMTG concentration was significantly related to initial concentration. Therefore, these cross-sectional observations in these unique populations support the idea that IMTG use during exercise might be related to initial concentration. It should be kept in mind that our present approach of limiting fat intake with 2%FAT was extreme; yet it did indicate that too little dietary fat can indeed reduce IMTG concentration and its subsequent oxidation. However, this study was not optimally designed to determine whether alterations in percentage of dietary energy within the range of 22–32% fat influences IMTG concentration, because the control diet containing 32% fat was administered first. Given that limitation and thus the need for further study, it is interesting to note that raising dietary fat intake from 22 to 32% did not appear to increase IMTG concentration (Fig. 1).

The observation that whole body lipolysis during exercise was reduced with 2%FAT, despite normal rates of lipolysis at rest, supports the idea that, during exercise, IMTG lipolysis is reduced when its concentration is significantly reduced. \(R_{d}\) FFA was not different at rest or during exercise when 2%FAT and 22%FAT were compared, indicating that adipose tissue metabolism was similar. This was expected given the fact that the last meals eaten before exercise were identical (24). Although it was not possible to distinguish lipolysis of IMTG from that of adipose tissue triglyceride, these observations support the idea that adipose tissue lipolysis was not altered and that the reduction in whole body lipolysis was due primarily to reduced IMTG lipolysis during exercise with 2%FAT. Whereas increased muscle glycogen concentration and glycogenolysis might contribute directly to reduced fat oxidation, as discussed above, little is known regarding mechanisms by which increased glycogenolysis might directly reduce IMTG lipolysis. However, it is reasonable to expect that IMTG lipolysis is reduced when its concentration is significantly reduced, as presently observed.

It is not surprising that plasma glucose and FFA kinetics were similar on the two diets, given the fact that the last meal was identical (i.e., 1.5 kg/kg of carbohydrate consumed 10 h before exercise), leading to a similar insulin action on adipose tissue, liver, and muscle (24). Therefore, the two diets differed primarily in chronic fat content and secondarily in chronic carbohydrate content that most likely led to respective differences in IMTG and muscle glycogen concentration. It is interesting that the increase in daily carbohydrate intake from 718 g/day with 22%FAT to 901 g/day with 2%FAT resulted in an 18% increase in muscle glycogen concentration. Previous studies performed over 24 h are unclear as to whether increasing carbohydrate intake above already high levels of 500–700 g/day would show a further increase in muscle glycogen concentration (5, 6). The present diets were administered for 6 days, which may have had a cumulative effect on raising muscle glycogen with only 2% fat and ~900 g/day of carbohydrate. Figure 1 demonstrates that the total substrate energy, expressed in kilocalories per kilogram and stored as the sum of glycogen and triglyceride within the muscle fibers, was...
intake of 9–105 g/day in these endurance-trained cyclists. It is believed that synthesis of triglyceride from carbohydrate occurs to a minimal extent in people who are weight stable (16, 26). However, Hudgins et al. (15) have observed that normal volunteers consuming a eucaloric diet for 25 days and thus who are weight stable, display stimulated fatty acid synthesis and increases in percent palmitate when dietary fat is reduced to 10% and carbohydrate is increased to 75% of energy, amounting to probably ∼450 g/day, in the form of glucose polymers. It seems that the stimulation of lipogenesis by low-fat, high-carbohydrate diets is accentuated when carbohydrate is taken mostly in the form of sugars and glucose polymers (16). Although the type of carbohydrate eaten by the subjects in the present study was not standardized, part of the increase in dietary carbohydrate above control was derived from increased consumption of sugars and high-glycemic carbohydrates. This might have accentuated the lipogenic effects of the 2%FAT and 22%FAT diets (16).

Furthermore, these eucaloric endurance athletes needed to consume 718–901 g/day of carbohydrate during the 22%FAT and 2%FAT trials, and that extremely large amount of carbohydrate intake might stimulate lipogenesis. Given the observation that muscle glycogen concentration was also extremely high with 2%FAT and 22%FAT and thus was limited as a site for further substrate storage, it seems reasonable to suspect that lipogenesis would be stimulated. These observations do not prove that lipogenesis was stimulated but rather provide an interesting paradigm for future consideration.

The primary precursors for resynthesis of IMTG are plasma FFA and plasma triglycerides. Plasma triglycerides in the form of dietary chylomicron triglyceride

| Table 2. Preexercise muscle substrate concentration with the various diets |
|------------------|------------------|------------------|
| Muscle glycogen, μmol/g | 32%FAT | 22%FAT | 2%FAT |
| dry wt | 533 ± 48 | 699 ± 68 | 824 ± 47 |
| Intramuscular triglyceride, μmol/g dry wt | 28.3 ± 3.6 | 29.2 ± 4.8 | 23.0 ± 4.4 |

Values are means ± SE; n = 7. *2%FAT significantly different from 32%FAT, P < 0.05; †control (32%FAT) significantly lower than 22%FAT and 2%FAT, P < 0.05.

The only direct data suggestive of lipogenesis were obtained by analysis of the percentage of plasma fatty acids that were made of palmitate, a saturated fatty acid preferentially formed by mammalian fatty acid synthase and indicative of de novo lipogenesis (18). The significant increase in percent palmitate above control levels with both 22%FAT and 2%FAT suggests a stimulation of lipogenesis when carbohydrate intake was increased to 718–901 g/day with a low dietary fat intake of 9–105 g/day in these endurance-trained cyclists. It is believed that synthesis of triglyceride from carbohydrate occurs to a minimal extent in people who are weight stable (16, 26). However, Hudgins et al. (15) have observed that normal volunteers consuming a eucaloric diet for 25 days and thus who are weight stable, display stimulated fatty acid synthesis and increases in percent palmitate when dietary fat is reduced to 10% and carbohydrate is increased to 75% of energy, amounting to probably ∼450 g/day, in the form of glucose polymers. It seems that the stimulation of lipogenesis by low-fat, high-carbohydrate diets is accentuated when carbohydrate is taken mostly in the form of sugars and glucose polymers (16). Although the type of carbohydrate eaten by the subjects in the present study was not standardized, part of the increase in dietary carbohydrate above control was derived from increased consumption of sugars and high-glycemic carbohydrates. This might have accentuated the lipogenic effects of the 2%FAT and 22%FAT diets (16).

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The primary precursors for resynthesis of IMTG are plasma FFA and plasma triglycerides. Plasma triglycerides in the form of dietary chylomicron triglyceride
would be low with 2%FAT vs. 22%FAT, whereas very low density lipoprotein (VLDL) triglycerides might be elevated from lipogenesis in the liver (15). Our present observation that IMTG concentration was reduced but not depleted with 2%FAT suggests that VLDL triglycerides and plasma FFA were able to partially, but not fully, compensate for the near absence of dietary fat and thus chylomicron triglycerides. Indications of stimulated lipogenesis, obtained from elevated percent palmitate were observed with 2%FAT compared with control. It is also interesting that 22%FAT did not lower IMTG concentration compared with control despite the reduction in dietary fat intake. However, 22%FAT, compared with control, also displayed evidence for increased lipogenesis with elevated percent palmitate. Therefore, it is possible that IMTG resynthesis during 22%FAT was maintained at control levels by increased lipogenesis compensating for reductions in dietary fat.

In summary, the present study indicates that an extremely low fat (i.e., 2% of energy) and high-carbohydrate diet lowers total fat oxidation during exercise by reducing whole body lipolysis in association with reduced IMTG concentration and calculated non-plasma FA oxidation. Chronic manipulation of dietary fat and carbohydrate, when the acute effects of the last meal before exercise are standardized, appears to exert its primary effect on substrate oxidation during exercise by altering substrate stores of IMTG and muscle glycogen. Low-fat diets that result in negative fat balance might stimulate lipogenesis in weight-stable endurance-trained men, which seems to compensate for reduced dietary fat and serves to partially restore IMTG concentration.

We appreciate the assistance from Dr. Lauri Byerley, Melissa Dominic-Ruthven, Jeannine Payne, and the participants of this study. This study was supported in part by a grant from Mars Inc.

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