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Effect of Dietary Fat on Serum and Intramyocellular Lipids and Running Performance

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Abstract

Purpose—This study evaluated whether lowering IMCL stores via 3-d consumption of very-low-fat (LFAT) diet impairs endurance performance relative to a moderate-fat diet (MFAT), and whether such a diet unfavorably alters lipid profiles.

Methods—Twenty-one male and female endurance-trained runners followed a controlled diet and training regimen for 3 d prior to consuming either a LFAT (10% fat) or MFAT (35% fat) isoenergetic diet for another 3 d in random crossover fashion. On day 7, runners followed a glycogen normalization protocol (to equalize glycogen stores) and then underwent performance testing (90-min preload run at $62 \pm 1\%$ $\dot{V}O_{2\max}$ followed by a 10-km time trial) on the morning of day 8. Muscle biopsies obtained from vastus lateralis before and after performance testing were analyzed for IMCL (via electron microscopy) and glycogen content (via enzymatic methodology).

Results—Despite approximately 30% lower IMCL ($0.220 \pm 0.032\%$ LFAT, $0.316 \pm 0.049\%$ MFAT; $P = 0.045$) and approximately 22% higher muscle glycogen stores at the start of performance testing ($P = 0.10$), 10-km performance time was not significantly different following the two diet treatments (43.5 ± 1.4 min LFAT vs 43.7 ± 1.2 min MFAT). However, LFAT produced less favorable lipid profiles ($P < 0.01$) by increasing fasting triglycerides (baseline = 84.9 ± 8.6 ; LFAT = 118.7 ± 10.0 mg·dL⁻¹) and the total cholesterol:HDL cholesterol ratio (baseline = $3.42 \pm 0.13:1$; LFAT = $3.75 \pm 0.20:1$), whereas MFAT lowered triglycerides (baseline = 97.5 ± 12.2 ; MFAT = 70.9 ± 7.1 mg·dL⁻¹) and the total cholesterol:HDL cholesterol ratio (baseline = $3.47 \pm 0.18:1$; MFAT = $3.33 \pm 0.14:1$).

Conclusion—The results suggest that reducing IMCL via 3-d consumption of a LFAT diet does not impair running performance lasting a little over 2 h (compared with 3-d consumption of a MFAT diet plus 1-d glycogen normalization), but that even short-term consumption of a LFAT diet may unfavorably alter serum lipids, even in healthy, endurance-trained runners.

Keywords

SERUM TRIGLYCERIDES; CARDIOVASCULAR DISEASE RISK; TIME TRIAL; FEMALE RUNNERS

Lipid stored as triglyceride droplets within the myocyte (intramyocellular lipid (IMCL)) may influence lipid use and performance during endurance exercise. Under typical dietary conditions, skeletal muscle contains significant stores of IMCL (~7530–10,460 kJ (8,35)), which are thought to serve as important fuel during exercise, particularly moderate-intensity exercise lasting 90 min or longer (35). IMCL is found to be reduced by about 15–50% in active muscle following moderate to strenuous endurance running and cycling in both men (7,30,40) and women (7,17,31,40), but this is not consistently reported (16,29,39). By tracer methodology, IMCL has been reported to contribute as much as 20–40% of total energy expenditure during moderate-intensity exercise of about 55–65% $\dot{V}O_{2\max}$ (24,26,36) and about 10% or less during more intense efforts (> 75% $\dot{V}O_{2\max}$) (24,36). Interestingly, several studies have alluded to the possibility that very-low-fat diets (i.e., 10–15% fat) consumed in association with high-volume endurance training may be detrimental to performance in endurance athletes (12,13,21) and military recruits (10), possibly by compromising IMCL stores (21,23).

In recent years, a number of active individuals, particularly female endurance athletes, have adopted very-low-fat diets (2,23) with the belief that dietary fat increases adiposity and impairs health and/or performance. Instead, however, such low-fat diets may impair performance and have negative health consequences. For example, studies have reported associations between very-low-fat diets and exercise-associated amenorrhea (18), compromised immune function (37), and elevated serum triglycerides (4,34). Very-low-fat diets (~17% fat) selected by endurance runners also tend to be low in essential fatty acids, zinc, and energy compared with more moderate-fat diets (~31–44% fat) (13). On the other hand, very-high-fat diets (~70% fat) consumed as part of fat adaptation protocols (designed to enhance fat oxidation) do not enhance endurance performance, even when preceded by glycogen normalization (5,6), and may downregulate carbohydrate use and sprint performance (11). Therefore, more moderate-fat diets may be ideal for increasing IMCL stores while negating the negative effects on health and/or performance of higher fat diets.

To our knowledge, previous studies have not investigated whether compromised IMCL at the start of exercise will impair endurance performance. The primary objective of this study was to determine whether lowered IMCL as a result of very-low-fat (10%) diet impairs performance during a running time trial (90-min preload run at 65% $\dot{V}O_{2\max}$ followed by a 10-km time trial) compared with a moderate-fat diet (35% fat), and whether this effect would be different in men and women. A second aspect was to evaluate whether short-term consumption of such diets influences cardiovascular (CV) risk in healthy endurance-trained runners by altering lipid profiles. We hypothesized that compromised IMCL stores on LFAT would impair performance in the 10-km time trial by decreasing reliance on IMCL and increasing reliance on muscle glycogen during early submaximal effort (when IMCL may supply approximately 20–40% of total energy expenditure (24)). This shift would enhance glycogen depletion, result in less glycogen being available for use during the latter more intense effort of the time trial (when muscle glycogen serves as the predominant fuel source (24)) and, thereby, result in a slower overall 10-km time. We also hypothesized that the very-low-fat diet would result in a less favorable lipid profile compared with the moderate-fat diet, and that the impact of LFAT would be greater in women compared with men.

METHODS

Study volunteers

Twenty-one healthy, endurance-trained distance runners (11 men, 10 women) between the ages of 18 and 45 were recruited. To qualify, participants had to be running > 40 km·wk⁻¹, have completed at least two training runs of > 2 h within the past 3 months, and have a $\dot{V}O_{2\max} \geq 55$ mL·kg⁻¹·min⁻¹ for men and ≥ 50 mL·kg⁻¹·min⁻¹ for women. The study was

approved by the institutional review board of the Pennington Biomedical Research Center (PBRC). Volunteers were fully informed about the possible risks of all procedures before providing written informed consent.

Clinic screening

Prior to admission, volunteers were screened by a study physician and determined to be in good general health and to have normal fasting insulin and glucose concentrations and normal iron status. Volunteers also had to agree to consume all foods provided. Subjects were excluded if they smoked, demonstrated signs of a full or partial syndrome eating disorder (Body Shape Questionnaire cutoff score of 120), alcoholism, or other substance abuse problems, or were using prescription or over-the-counter medications or supplements (other than oral contraceptives) that could influence metabolism. For descriptive purposes, body composition was measured by dual-energy x-ray absorptiometry (DXA, Hologic QDR4500A).

Baseline testing

Approximately 2–3 wk before initiation of the experimental protocol, $\dot{V}O_{2\max}$ was determined on a motor-driven treadmill (MedTrack ST65, Quinton Industries, Inc, Bothell, WA), using a previously described protocol designed for endurance-trained runners (17,25). Briefly, following a 5-min warm-up, “workload” was increased by either speed (starting at the subject’s typical warm-up speed and increasing by 0.5 mph (13.4 m·min⁻¹)) or grade (starting at 0° and increasing by 2.5%) every minute until exhaustion. Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}O_2$) were measured using a metabolic cart (V-Max 29, SensorMedics, Yorba Linda, CA), and heart rate was monitored using a portable heart rate monitor (Polar S-610, Polar Beat, Port Washington, NY). The highest $\dot{V}O_2$, respiratory exchange ratio (RER), and heart rate achieved during a 20-s period within the last 2-min of exercise were recorded as the maximum values. Volunteers had to achieve two out of three of the following criteria: 1) a leveling or plateau of $\dot{V}O_2$ (defined as an increase of $\dot{V}O_2$ of < 2 mL·kg⁻¹·min⁻¹ with increased workload), 2) RER > 1.10, and 3) maximum heart rate within 10 beats of age-predicted maximum. $\dot{V}O_{2\max}$ was subsequently used to determine the running pace corresponding to 65% and 70% $\dot{V}O_{2\max}$.

After resting for 30 min, subjects who met the $\dot{V}O_{2\max}$ criteria performed a practice 90-min preload and 10-km time trial (described below). The purpose of the practice test was to familiarize subjects with the treadmill and the testing equipment, and to reduce a potential learning curve from the first to the second trials (25). Food and fluid intake were not controlled before the practice test and the results were not taken into consideration.

Overview of experimental protocol and scheduling

The study was a singly blind (with respect to the investigators), masked, randomly assigned crossover experiment. As outlined in Figure 1, participants completed two separate 8-d crossover experiments that were separated by approximately 3 wk in men and one menstrual cycle (3–5 wk) in women. Women were scheduled so that they could complete the 8-d experiment in the follicular phase of their menstrual cycle. Menstrual cycle phase was determined from the start of the subject’s menstrual bleeding and documented with measures of serum progesterone concentrations. Although the subjects were masked to their diet treatment, they were told that both experimental diets could potentially improve performance via different mechanisms. This was done to minimize a possible placebo effect on performance by subjects recognizing (or believing to recognize) one of the diet treatments and/or believing that one or the other diet might be ergogenic. To mimic free-living training conditions, subjects were asked to run on their own for 45 min at approximately 45 s per mile slower than their 10-km race pace (17), on days 1, 2, 5, and 6,

and they were also asked to maintain usual training between the two experimental phases. Exercise was not allowed on day 3 or on days 4, 7, or 8 when exercise sessions were scheduled in the laboratory.

Diet

The baseline and experimental diets were prepared using commercially available foods and beverages by the PBRC metabolic kitchen staff with precise control of both nutrient and caloric content. Composition and caloric content of these diets were verified by the PBRC food analysis laboratory. A baseline weight-maintaining diet (15% protein, 25% fat (6.9% saturated, 10.6% monounsaturated, 7.5% polyunsaturated, 60% carbohydrate) was provided for 3 d before the 2-h IMCL-depleting run (Fig. 1). Following the IMCL-depleting run, the experimental diets were provided for 3 d in random order and consisted of a very-low-fat diet (LFAT; 15% protein, 10% fat (3.5% saturated, 2.7% monounsaturated, 3.8% polyunsaturated), 75% carbohydrate) or an isoenergetic moderate-fat diet (MFAT; 15% protein; 35% fat (11.5% saturated, 14.1% monounsaturated, 9.4% polyunsaturated), 50% carbohydrate). The energy content of the diets was initially estimated based on our previous experiments (17), using the Harris-Benedict prediction equation for basal energy expenditure, multiplied by an activity factor of 1.8 for women and 2.0 for men for the baseline diet; and 2.0 and 2.2 for women and men, respectively, for the experimental (MFAT and LFAT) diets. Subjects ate dinner at the center and were given the following days breakfast, lunch and snacks to carry out. Subjects who were habitual coffee, tea, or caffeinated soft drink consumers were also provided a similar amount of caffeine (as measured instant coffee powder, instant tea powder or diet cola) with their meals. Subjects were asked to eat all food but to return anything not consumed to the research dietitian so it could be weighed and recorded. Body mass was obtained immediately before dinner, with the volunteers in street clothes with empty pockets and no shoes.

IMCL-depleting exercise

The IMCL-depleting run was performed on the morning of day 4 after an overnight fast in which the subjects ran on a motor-driven treadmill (LifeFitness TR 9500, Franklin Park, IL) for 2 h at 65% $\dot{V}O_{2\max}$ (17). An incline of 1% was assigned during the IMCL-depleting run as well as all other laboratory runs (with the possible exception of the 10-km time trial, as explained below) to simulate the oxygen cost of outside running. The purpose of the IMCL-depleting run was to lower IMCL stores and facilitate separation on the two experimental diets (17). Previously, we have reported that IMCL stores can be significantly separated using this protocol, because IMCL is not replenished even at 3 d after the endurance exercise (which lowers IMCL by an average 25%) when the 10% fat diet is consumed, and overshoots baseline stores with 3 d of a 35% fat diet (17). $\dot{V}O_2$ and $\dot{V}CO_2$ were measured for 10 min at the beginning (between 5 and 15 min after the start) and end (10 min before completion) of the run using a metabolic cart (Sensormedics Vmax 2900), with continuous monitoring of heart rate. During the first experiment only, pace was adjusted, if necessary, during the initial 5–15 min of the run to achieve an oxygen cost as close to 65% $\dot{V}O_{2\max}$ as possible, but it was not further adjusted.

Glycogen-loading protocol

A glycogen restoration (or glycogen normalization) protocol was completed on the last day of both experimental diets (day 7) to minimize potential differences in glycogen at the start of the performance test (the morning of day 8). This protocol was adapted from Burke et al. (5) and consisted of a 20-min run at 70% $\dot{V}O_{2\max}$ (treadmill grade = 1%), followed by a carbohydrate-loading diet. $\dot{V}O_2$ and $\dot{V}CO_2$ were monitored for 10 min at the beginning of the run, and heart rate was monitored continuously.

Endurance performance test

On the morning of day 8, endurance performance was assessed on a motor-driven treadmill (LifeFitness) using a 90-min preloaded 10-km time trial as described previously (25) that was performed 3 h after a standardized breakfast (603 kcal for men and 470 kcal for women, 15% protein, 15% fat, 70% carbohydrate). Briefly, subjects completed a self-selected 5-min warm-up followed by the 90-min preload at 65% $\dot{V}O_{2\max}$ (an intensity that should rely on IMCL and spare glycogen (24)) and the 10-km time trial, with a 5-min break between the preload and time trial. Subjects were instructed to complete the 10-km time trial as fast as possible (i.e., to “race the 10-km”) and were allowed to adjust the treadmill speed as much as desired. Subjects were given feedback only at each mile mark (mile splits), and, hence, they only knew their time at each mile they had passed. Most subjects were assigned an incline of 1% (25); however, faster runners (who had completed a recent 10-km race in < 40 min) were given an incline of 2% for the time trial to account for treadmill limitations (maximal velocity = 10 mph or 267 m·min⁻¹). $\dot{V}O_2$ and $\dot{V}CO_2$ were measured for 10 min at the beginning (5–15 min after the start) and end (10 min before completion) of the preload to verify $\dot{V}O_2$ and to measure the RER. Gas-exchange data, however, were not measured during the time trial, so as not to interfere with performance, but heart rate was monitored continuously. During both the preload and time trial, water consumption was encouraged and allowed *ad libitum* (in weighed water bottles) during the first endurance test and that amount of water was then provided and encouraged during the second endurance test. Subjects were allowed to watch a movie or listen to music during the endurance test, but the entertainment selected for the first experiment was also used for the second (i.e., the same movie or music CD). The same researcher also monitored both preloaded time trials. The coefficient of variation for time to complete our 10-km time trial after the 90-min preload (when no muscle biopsies were obtained) is 0.53% for men and 1.26% for women (25).

Muscle biopsies and analysis

Muscle biopsies were obtained from the vastus lateralis before the 90-min preload and immediately after the 10-km time trial by the use of the percutaneous needle biopsy technique. Briefly, subjects rested in the supine position on a patient bed (where they were wheeled following the performance test). Adipose tissue and skeletal muscle fascia were anesthetized using about 5 mL of a 50/50 mixture of lidocaine and bupivacaine. The biopsy site was then dressed with ointment, a 2 × 2 gauze, and a clear adhesive dressing, and wrapped with an elastic wrap to facilitate hemostasis. For the preexercise biopsy, runners were encouraged to leave the elastic wrap on for at least the first 10–15 min of the run. The contralateral leg was biopsied (in random order) before and after exercise on both visits. A fraction of the tissue sample of each biopsy was processed for transmission electron microscopy (TEM) analysis of IMCL content and biochemical analysis of muscle glycogen concentration.

IMCL analysis

After excision, approximately 2 mm³ was cut, weighed, and immediately placed in chilled 2% glutaraldehyde and transported to the microscopy center. Approximately 3 h later, five randomly selected samples were fixed and embedded according to the methods of Sokolova et al. (28). Longitudinal, thin sections (~83 nm) were later cut from each of five blocks using an ultra-microtome (MT-XL, RMC Products, Boeckeler Instruments, Inc., Tucson, AZ), placed on Cu150 mesh grids and stained for 15 min in uranyl acetate (5% solution) followed by 4 min in a mixture of lead acetate, lead citrate, lead nitrate, and sodium citrate (CNA lead). Sections were then examined and photographed in a C-10 transmission electron microscopy (C. Zeiss, C-10, Germany). A total of two randomly selected fibers per block of undetermined type were photographed at ×3150 in a randomly selected location centered within the muscle fiber (two fibers per block, for a total of 10 fibers per biopsy). At ×3150,

this area comprised approximately 75% of the total two-dimensional fiber diameter. For each TEM session, a standard calibration grid (diffraction grating replicas no. 10000, 1134 lines per millimeter, Ernest F. Fullam, Inc, Latham, NY) was used to calibrate magnification and distance. The micrographs were scanned at 2400 dpi by a computer-linked scanner (Polaroid, SprintScan 45) using Hamrick and Vue Scan 8.2.15 software. The area of the lipid droplets was measured from the micrographs using an image analysis system (Image Pro Plus version 5.0, Meyer Instruments, Houston, TX) after calibration with the $\times 3150$ grid. After initial identification, lipid droplets were traced using the zoom function (which doubles the view in the window; Fig. 2), and the total volume area of the fiber shown in the micrograph was also determined. IMCL was expressed as a percentage of fiber area. Contact between mitochondria and lipid droplets, and mean intensity of lipid droplets, were also examined. Using Image Pro Plus, mean intensity is the value for all the pixels in the encircled lipid droplet (or identified area), where every pixel is assigned 1 of 256 intensity values ranging from 0 to 255, with “black” assigned a value of 0, and “white” a value of 255. A higher intensity value means that the encircled structure has transmitted more electrons and is therefore less electron dense and lighter in the image compared with a lower intensity value, which is more electron dense and darker on the image.

Muscle glycogen analysis

Following excision, a small sample of muscle (~15–20 mg) was dissected of visible blood and connective tissue, weighed, frozen in liquid nitrogen, and freeze dried (Labconco Freeze Dried System, Kansas City, MO) until analysis. Upon study completion, dried muscle samples were sent to McMaster University, and glycogen content was determined using previously described methodology (1). Briefly, samples were analyzed for glucosyl units after hexokinase digestion using the method of Passonneau and Lowry (22). Glucose content of samples was determined in duplicate fluorometrically at 340 nm (Hitachi F-2500, Hitachi Instruments, Tokyo, Japan).

Blood samples and analysis

Fasting blood samples were obtained on the morning of day 4 and day 7 for analysis of insulin, glucose, serum lipids, and lipoproteins (Fig. 1). On day 8, nonfasting blood samples were obtained immediately before and immediately after the preloaded time trial for analysis of insulin, glucose, lactate, glycerol, FFA, beta-hydroxybutyrate, cortisol, and growth hormone. In female runners, blood drawn before the start of the time trial was also analyzed for progesterone concentrations to document follicular status. Blood samples were analyzed according to standardized procedures. Briefly, insulin was analyzed by immunoassay, and progesterone was analyzed by radioimmunoassay using the DPC Immulite 2000 (Diagnostic Product Corporation, Los Angeles, CA). Glucose was analyzed via glucose oxidase electrode; lactate, glycerol, FFA, and beta-hydroxybutyrate were analyzed via enzymatic methods; cortisol and growth hormone were analyzed via immunoassay with fluorescence detection; total cholesterol was analyzed via the cholesterol esterase–oxidase–peroxidase method; and triglycerides were analyzed via the GPO–Trinder method, using the Beckman–Coulter Synchron CX7 (Brea, CA). HDL cholesterol concentrations were measured after precipitation of apolipoprotein (apo) B–containing lipoproteins with 50,000-molecular-weight dextran sulfate (DMA, Arlington, TX), and LDL cholesterol was calculated using the Friedewald equation.

Statistical procedures

Statistical analyses were performed using SPSS analysis software (SPSS 13.0 for windows, Chicago, IL). Repeated-measures ANOVA was used to test for diet $\times$ time (day 4 vs day 7) or diet $\times$ exercise (day 8, pre- vs postexercise) interactions or main effects, and, if appropriate, *post hoc* pairwise comparisons were made using Bonferroni-adjusted *t*-test

comparisons. In all appropriate analysis, sex was included as a between-subject factor. One-way ANOVA was used to test the differences between baseline characteristics of male and female runners as well as the characteristics of runners responding to versus not responding to our hypothesized performance improvement on the two diets. Alpha (before Bonferroni corrections) was set at 0.05.

A sample size analysis conducted with variance estimates based on previous replicated time trial data from our laboratory (variance = 23 s (25)) and an alpha = 0.05 determined that a sample size of $N = 10$ was sufficient to detect, with 80% power, a minimal difference of approximately 30 s in 10-km performance between the two experimental diets (SigmaStat 3.1, Systat Software, Inc., San Jose, CA). Using actual sample size ($N = 21$) and these same assumptions, a power analysis determined that a 30- to 60-s difference in 10-km performance between diets could be detected, with close to 100% power.

RESULTS

All 21 volunteers enrolled in the study successfully completed the study. However, some data are missing for several variables, including fasting lipids, fluid intake, body mass change during exercise, and IMCL content. Specifically, three participants missed a fasting blood draw on either day 4 or day 7, two splashed water on themselves during the time trial (rather than consuming it), two had missing gas-exchange data during the time trial preload due to scheduling conflicts with the metabolic cart, three had incomplete heart rate data during the time trial due to monitor interference, and one had muscle biopsy samples not suitable for TEM analysis (due to twisted fibers). Data for these variables were analyzed with $N < 21$.

The characteristics for the 21 well-trained endurance runners (11 men and 10 women) are shown in Table 1. Most of the participants regularly competed in local road races including 5-km, 10-km, and marathon distances; 5 of the 21 (four men and one woman) were Division I college runners (studied in the offseason) at the time of the study. As expected, male runners were heavier and leaner with higher maximal oxygen uptakes (expressed per kilogram of body mass) than female runners, but they were relatively closely matched to females for age, $\dot{V}O_{2\max}$ (expressed relative to fat-free mass), and reported average training distance.

Dietary control and controlled running

All participants completed the dietary and controlled pre-time trial running required of both treatments. As shown in Table 2, energy and protein intakes at baseline (days 1–3) and on the experimental diets (days 4–7) were similar between LFAT and MFAT treatments. Energy and macronutrients were also similar between treatments on the carbohydrate-loading diet during glycogen normalization (day 7).

The 2-h IMCL-depletion run (morning of day 4) was completed at $3.12 \pm 0.07 \text{ m}\cdot\text{s}^{-1}$ ($3.31 \pm 0.08 \text{ m}\cdot\text{s}^{-1}$ for men, $2.91 \pm 0.07 \text{ m}\cdot\text{s}^{-1}$ for women) and elicited a similar oxygen cost and heart rate response of $62.0 \pm 1.2\%$ and $63.8 \pm 1.2\% \dot{V}O_{2\max}$ and 150 ± 3 and 151 ± 3 bpm on LFAT and MFAT, respectively. The 20-min glycogen normalization run that preceded the carbohydrate-loading diet (day 7) was performed at $3.71 \pm 0.08 \text{ m}\cdot\text{s}^{-1}$ ($3.93 \pm 0.05 \text{ m}\cdot\text{s}^{-1}$ for men and $3.49 \pm 0.11 \text{ m}\cdot\text{s}^{-1}$ for women). The oxygen cost and heart rate response of the 20-min runs were similar (72.4 ± 2.0 and $74.8 \pm 0.8\% \dot{V}O_{2\max}$ and 163 ± 3 and 165 ± 3 bpm on LFAT and MFAT, respectively). RER was significantly higher (0.95 ± 0.01) after LFAT compared with MFAT (0.93 ± 0.01) ($P = 0.04$) but was not significantly influenced by sex ($P < 0.05$).

Preexercise intramyocellular lipid and muscle glycogen

IMCL was about 30% lower at rest (morning of day 8) with LFAT ($0.220 \pm 0.032\%$) compared with MFAT ($0.316 \pm 0.049\%$) ($P = 0.045$, main diet effect, $N = 20$). Muscle glycogen stores, on the other hand, were about 23% higher with LFAT (360 ± 43 mmol glucosyl units per kilogram of dry mass) compared with MFAT (293 ± 25 mmol glucosyl units per kilogram of dry mass), but this difference was not statistically significant ($P = 0.10$, diet main effect, $N = 21$). Figure 3 illustrates the differences in IMCL and muscle glycogen by sex. IMCL appeared to be higher in women at the start of the time trial with MFAT but not LFAT, but this difference was not statistically significant ($P = 0.531$, diet $\times$ sex). However, muscle glycogen was higher at the start of LFAT in women and higher at the start of MFAT in men ($P = 0.004$, diet $\times$ sex). Pre-exercise IMCL was significantly correlated between the two diets ($r = 0.45$, $P = 0.04$), but preexercise IMCL was not correlated with preexercise muscle glycogen on either diet ($r = -0.02$, $P = 0.67$ for LFAT; and $r = -0.10$, $P = 0.67$ for MFAT).

Time trial preload

The 90-min preload was completed at an average pace of 3.12 ± 0.07 m·s⁻¹, which elicited an average $\dot{V}O_2$ of 60.6 ± 1.0 and $62.8 \pm 1.1\%$ $\dot{V}O_{2\max}$ for LFAT and MFAT, respectively. Metabolic measurements obtained at the beginning and end of the preload run, and the fluid consumed and body mass change are summarized in Table 3. A diet $\times$ exercise effect or interaction with sex was not found for any of the measurements, indicating that the metabolic effect of performing the preload was similar for both trials and both sexes. A statistically significant main effect for exercise ($P < 0.05$), however, was noted for heart rate ($N = 18$) and RER ($N = 19$).

Time trial performance

Overall, men performed the 10-km time trial faster than women ($P = 0.03$, sex main effect). Performance time during the 10-km time trial did not differ by diet treatment ($P = 0.95$, $N = 21$, Fig. 4) or sex ($P = 0.57$, diet $\times$ sex) and was $43:30 \pm 1:23$ min:s for men (range = 37:12–49:27) and $48:29 \pm 1:42$ min:s for women (range = 40:24–59:02) for LFAT, and $43:44 \pm 1:17$ min:s for men (range = 37:41–49:01) and $48:16 \pm 1:32$ min:s (range = 39:13–58:00) for MFAT, respectively. Notably, however, the variation of individual performance times between LFAT and MFAT treatments was much greater (by as much as a 5-min difference; Fig. 4) than was previously noted during reliability testing of our preloaded time trial on a controlled low-fat diet when muscle biopsies were not obtained (17). Specifically, 12 subjects had slower 10-km times after LFAT (nine with more than a 30-s impairment), as hypothesized; on the other hand, nine subjects had slower times after MFAT (six with more than a 30-s impairment). Interestingly, those who were more likely to experience impairments with LFAT were runners with higher percentages of body fat ($P = 0.03$), lower aerobic fitness relative to body weight ($P = 0.018$) and fat-free mass ($P = 0.06$), and longer average performance times ($P = 0.03$) than those who experienced improvements with LFAT, even though sex was not a significant factor. Preexercise IMCL and glycogen stores were not found to be important determinants.

Intramyocellular lipid and muscle glycogen in response to exercise

As shown in Figure 3, IMCL was not lowered following the time trial on either LFAT or MFAT ($P = 0.17$, diet $\times$ exercise) in male or female runners ($P > 0.05$, all possible sex interactions and main sex effects). However, there were trends for lipid droplet number per square area to be higher in the postexercise (LFAT, 0.012 ± 0.001 ; MFAT, 0.013 ± 0.002) compared with the preexercise samples (LFAT, 0.010 ± 0.001 ; MFAT 0.013 ± 0.001) ($P = 0.08$) and for the mean intensity of the lipid droplets to increase following exercise ($P =$

0.05, diet $\times$ exercise; $P = 0.06$ main exercise effect) from 107.6 ± 1.6 to 114.4 ± 2.4 on LFAT and from 110.1 ± 1.7 to 110.6 ± 3.0 on MFAT. There was no diet $\times$ time interaction or main effect for percentage of lipid droplets touching mitochondria (73.8 ± 2.0 and 74.5 ± 2.4 before and after LFAT, and 72.1 ± 3.3 and 74.4 ± 2.7 before and after MFAT), but this differed by sex, with men showing an increase (preexercise, 73.0 ± 3.3 ; postexercise, 77.9 ± 2.4) and women showing a decrease (preexercise, 73.9 ± 3.6 ; postexercise, 70.2 ± 2.8) in the percentage of lipid droplets in contact with the mitochondria following the time trial ($P = 0.039$, exercise $\times$ sex). In contrast, muscle glycogen fell significantly after both time trials ($P < 0.001$, exercise main effect) and was reduced by 198 mmol glucosyl units per kilogram of dry mass on LFAT and 165 mmol glucosyl units per kilogram of dry mass on MFAT (or 56.3% and 55.0% of preexercise on LFAT and MFAT, respectively), with no significant effect of diet ($P = 0.17$, diet $\times$ exercise) or sex ($P = 0.56$, exercise $\times$ sex). Preexercise muscle glycogen concentrations predicted greater absolute muscle glycogen use during both the LFAT ($r = 0.89$, $P < 0.001$) and MFAT ($r = 0.78$, $P < 0.001$) experiments, and preexercise IMCL content predicted greater absolute ($r = 0.41$, $P = 0.07$, LFAT; $r = 0.59$, $P = 0.007$, MFAT) and relative ($r = 0.60$, $P = 0.006$, LFAT; $r = 0.34$, $P = \text{NS}$, MFAT) use on both experiments. During LFAT only, higher preexercise muscle glycogen predicted a smaller fall in IMCL ($r = -0.43$, $P = 0.06$).

Blood metabolite and hormone response to exercise

As summarized in Table 4, a diet $\times$ exercise effect ($P < 0.01$, $N = 21$) was found for glycerol and serum cortisol concentrations, which were not as elevated following the time trial with LFAT versus MFAT. Main effects for diet and exercise were also noted for beta-hydroxybutyrate, which was lower both at rest and following exercise with LFAT versus MFAT. Glucose, lactate, free fatty acids, and growth hormone were not affected by diet but were significantly increased following exercise ($P < 0.001$, main effect for exercise, $N = 21$). None of the blood metabolites or hormone concentrations at rest or following exercise were influenced by sex.

Fasting insulin, glucose, cholesterol, and triglycerides

Fasting plasma glucose and insulin did not differ between trials or by time (day 4 versus day 7) (Table 5). However, serum cholesterol and triglyceride concentrations were significantly influenced by diet ($P < 0.001$, diet $\times$ time, $N = 18$). As summarized in Table 5, fasting triglyceride concentrations were significantly elevated, and total cholesterol, HDL cholesterol, and LDL cholesterol concentrations were significantly lowered, relative to baseline concentrations with LFAT; triglyceride concentration and the total cholesterol:HDL cholesterol ratio were significantly lowered below baseline with MFAT. Women had significantly higher total cholesterol concentrations overall, and their HDL cholesterol concentrations were found to respond more drastically to the different diets ($P = 0.04$, diet $\times$ sex). There was no sex effect for any of the other fasting blood concentrations. The number of runners with serum cholesterol or triglyceride concentrations above or below the desired range (9) at baseline and following the LFAT and MFAT diets is shown in Table 6.

DISCUSSION

The purpose of the present study was to investigate whether a very-low-fat diet would impair endurance performance when compared with a normal, moderate-fat diet due to reduced IMCL stores, and to determine whether short-term consumption of such a diet would unfavorably alter the lipid profile. We had hypothesized that reduced IMCL stores would impair performance in our 10-km time trial (relative to the moderate-fat diet) by decreasing reliance on IMCL fuel during early submaximal effort (when IMCL may supply approximately 20–40% of total fuel (24)), and increasing reliance on muscle glycogen. This

shift would then accelerate muscle glycogen depletion and result in less glycogen being available for use during the latter more intense effort of the time trial. In partial contrast to our hypotheses, we found that lowering IMCL stores by approximately 30% relative to the moderate-fat diet did not alter substrate oxidation or impair performance in either male or female runners. Unfortunately, we also found that the 1-d glycogen normalization protocol previously demonstrated to minimize potential differences in glycogen stores in male cyclists (5) did not “work” as expected in runners and may have interfered with our ability to adequately test this hypothesis. In agreement with our hypothesis, we did find that short-term consumption of a very-low-fat diet produced less favorable lipid profiles that may affect endurance-trained women more than men.

The present study confirmed previous observations that the fat content of the diet influences IMCL stores (12,15, 17,38). In fact, the 30% lower IMCL with the LFAT compared with MFAT was in line with our earlier study in similarly trained female runners, which assessed IMCL noninvasively using proton magnetic resonance spectroscopy (32% lower on LFAT vs MFAT) (17). Two previous studies using TEM and slightly more drastic dietary fat regimens found that IMCL was 39–55% lower in trained male runners and duathletes after 4–6 wk of a 16.5–18% fat diet compared with a 52–55% fat diet (12,38). However, the differences in IMCL between the lower- and higher-fat diets did not reach statistical significance due to large interindividual variances. The mechanism whereby lower-fat, higher-carbohydrate diets lead to reduced IMCL is thought to be related to carbohydrate-induced suppression of lipoprotein lipase (LPL), which catalyzes the uptake of triglyceride-rich lipoproteins in the capillary endothelium of skeletal muscle (17). Neither the present nor the previously published studies (12) found that the fat content of the diet impacts the amount of IMCL in physical contact with the mitochondria.

We were somewhat surprised that IMCL was not lowered following our time trial, which lasted between 2 and 2.5 h. Using a variety of methodologies, previous studies have reported conflicting results concerning the reduction in IMCL during exercise. While the majority report approximately 15–50% reductions in muscle lipid following moderate to strenuous endurance running and cycling in both men (7,30,40) and women (7,17,31,40), others have found either no change (16,29,39) or an increase in IMCL following exercise (3), most likely because of methodological error. Such methodological error, rather than lack of IMCL use (16) and/or IMCL synthesis (27) during the time trial, most likely accounts for our findings. In the current study, we employed TEM because it allows for visualization of IMCL droplet size and number, as well as assessment of droplet contact with mitochondria, but this technique also has some inherent problems that include its variability (12,38) and altered appearance of IMCL following exhaustive exercise (14). For example, Howald et al. have noted that lipid droplets resemble “dense material of onion-peel appearance” following exhaustive exercise (14) rather than appearing as the typical spherical droplets that contain slightly shaded (or “creamy”-colored) material surrounded by a thin borderline. In support of these methodological concerns, we found that the mean intensity of the lipid droplets was significantly higher (or less electron dense) after exercise compared to before exercise (i.e., less creamy and more like an onion peel), and that the number of lipid droplets per square area was higher on average in the post-exercise sample. The former suggests that the postexercise IMCL droplets were less tightly packed with triglyceride, which, if valid, would result in the tendency for TEM to overestimate IMCL content after exercise and, thereby, underestimate exercise-associated IMCL depletion. The latter suggests a possible sampling bias that resulted in the inclusion of more IMCL-rich type I fibers after exercise, which may have been systematic (i.e., perhaps due to obtaining of slightly deeper biopsies after exercise, when blood flow to the muscle is increased).

Nevertheless, an important finding of the current study is that running performance remained nearly identical under both dietary regiments. This finding, along with our gas-exchange data (which were different before but not after glycogen normalization), suggests that small differences in muscle fuel storage (30% lower IMCL and 23% elevated muscle glycogen on LFAT compared with MFAT) did not impair or enhance performance. Although further investigation using longer exercise bouts or more extreme IMCL-loading protocols undertaken in the fasting state may be of interest to ultimately determine the importance of IMCL as a fuel during endurance exercise, previous studies subjecting trained male athletes to more extreme protocols also did not detect differences in various types of performance, including a half-marathon run (38) and a 1-h time trial undertaken after 4 h of cycling (6). Furthermore, previous results did not seem to be influenced by whether glycogen normalization was performed (6) or not performed (12,14) before performance testing. Nevertheless, it is important to mention that several factors may have interfered with our being able to detect impaired performance on LFAT (due to greater glycogen use during the preload and pace slowing during the 10-km run), which include the unexpected differences in preexercise glycogen stores between the diets (as well as between the sexes), the provision of a high-carbohydrate preevent breakfast, the obtainment of preexercise muscle biopsies, and the intersubject time trial differences (which ranged from approximately 2 to 2.5 h). Particularly concerning was the higher preexercise glycogen stores on LFAT, which would have provided additional carbohydrate substrate, and which was, not surprisingly, found to negatively predict IMCL use during the LFAT time trial.

The present study also evaluated the effect of sex on diet-induced alterations in IMCL storage and use during the time trial. While we found higher average IMCL stores in female compared with male runners on MFAT, but not LFAT, these differences were not statistically significant and did not result in altered substrate-use patterns during the time trial. Previous studies investigating sex differences in IMCL storage and use during exercise have found conflicting results. Some have found that women store (7,32) and use (31) IMCL to a greater extent than do men, but others have not found such differences (33,40). While the intent of the current study was to determine whether LFAT would have a greater impact on compromising IMCL stores and decreasing performance in women compared with men, our findings were once again complicated by the glycogen normalization protocol, which resulted in greater muscle glycogen storage in women with LFAT and in men with MFAT. Nevertheless, we were intrigued by the finding that those runners who had impaired performance with LFAT (as hypothesized) had significantly higher body fat percentages, lower $\text{VO}_{2\text{max}}$ values, and slower 10-km times, and were more likely to be women (although there was not a significant sex effect) than the runners who experienced improved performance with LFAT. These results suggest that well-trained runners may respond differently to different diets depending on factors that are related to but not directly due to sex.

A second objective of our study was to evaluate whether short-term consumption of a very-low-fat diet would influence CV disease risk specifically by altering serum lipid profiles compared to a baseline 25% fat diet and the 35% moderate-fat diet. While a few previous studies in endurance-trained athletes observed that fat-restricted diets elevate triglyceride concentrations by as much as 50% (4,17, 34) and generally alter lipid profiles unfavorably (4,19,34), these studies were longer in duration and did not assess potential individual variability on CV disease risk. One study in male distance runners found that consumption of a low-fat (17% fat), high-carbohydrate diet for 14 d reduced HDL cholesterol by 9% (which was primarily due to a fall in the HDL₂ fraction) and increased triglycerides by 30% relative to a “normal,” 32% fat diet (34). Another study in male and female runners found that 4 wk of consuming a low-fat (16% fat) diet lowered HDL cholesterol and apo A-1, and raised the total cholesterol:HDL cholesterol ratio compared with a 42% fat diet. While the

present study confirms these findings, it also suggests that these aberrations, which include a 40% elevation in fasting triglycerides, a 10.8% reduction in HDL cholesterol, and a significantly increased total cholesterol:HDL cholesterol ratio, are present after 3 d of the diet. The moderate-fat diet, in contrast, resulted in a 27% reduction in fasting triglycerides and significantly lowered the total cholesterol:HDL cholesterol ratio relative to baseline. The mechanism whereby low-fat, high-carbohydrate diets unfavorably alter triglycerides and HDL cholesterol is most likely a function of carbohydrate suppression of LPL, which also catalyzes the hydrolysis of chylomicrons and very-low-density lipoproteins in the capillary endothelium of tissues (including skeletal muscle) and aids in the subsequent transfer of surface remnants of these triglyceride-rich lipoproteins to HDL (20,34). Because suppression of LPL activity has been shown to occur after 3 d of a low-fat diet (20), reduced LPL activity may be at least partially responsible for the increased triglycerides and reduced HDL-cholesterol concentrations (as well as the mechanism responsible for reduced IMCL storage) (15).

Overall, our results indicate that even endurance-trained athletes should take caution about following fat-restricted diet, particularly if they have a personal (or family) history of altered lipids. Nevertheless, the actual extent of the problem and the specific driving mechanisms need to be further explored in larger groups of athletes. In a previous study, Thompson and colleagues (34) noted that low-fat-diet-induced aberrations were less severe in male distance runners training $16 \text{ km} \cdot \text{d}^{-1}$ than in sedentary individuals, but they did not report individual variability in these changes with respect to CV risk. In our study of a mixed group of 21 male and female endurance runners, we were surprised to find that five (~25%) had elevated cholesterol concentrations, three had lower-than-optimal HDL cholesterol concentrations, and two had elevated triglycerides at baseline despite running $\geq 40 \text{ km} \cdot \text{wk}^{-1}$ (Table 6). Even more notable, the number of athletes with less-than-optimal profiles was increased on the low-fat diet and decreased on the moderate-fat diet. These findings are important for sports nutritionists and other professionals working with athletes, and they may implicate that moderate-fat diets (in the upper range recommended by Dietary Guidelines and the National Cholesterol Education Program) may be beneficial for those with altered lipid profiles. In accordance with the recommendations, moderate-fat diets should emphasize foods high in mono-unsaturated fats and should deemphasize foods containing saturated and trans fats.

The current study provides novel findings suggesting that lowering IMCL with a very-low-fat diet does not produce a significant performance disadvantage during endurance running lasting between 2 and 2.5 h. However, even short-term consumption of such very-low-fat diets can significantly impact triglycerides and the lipid profile, which may increase cardiovascular disease risk in some athletes. Further studies are needed to determine whether low-fat regimens would impair performance during longer endurance or ultraendurance events, or under conditions in which endogenous carbohydrates are not readily available.

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FIGURE 2.
TEM micrograph of a longitudinal section of the vastus lateralis muscle in a male runner on the MFAT diet.

FIGURE 3.

Intramyocellular lipid (IMCL) (*upper panel*) and muscle glycogen (*lower panel*) in male and female runners before (pre) and after (post) the 90-min preloaded 10-km time trial on the very-low-fat (LFAT) and moderate-fat (MFAT) trials. * Statistically different preexercise between LFAT and MFAT ($P = 0.045$, main effect of diet); t statistically different preexercise vs postexercise; § statistically significant diet $\times$ sex interaction ($P = 0.004$, diet $\times$ sex).

FIGURE 4.

Time to complete the 10-km time trial on the low-fat and moderate-fat trial diets for the male (*upper panel*) and female (*lower panel*) runners. Trials were completed after a 90-min preload run at approximately 62% $\text{VO}_{2\text{max}}$.

TABLE 1

Characteristics of male and female runners.

	Men (N = 11)		Women (N = 10)		Effect
	Average Value	Range	Average Value	Range	
Age (yr)	27 ± 3	19–45	29 ± 2	20–44	
Height (cm)	177.1 ± 2.2	163.6–185.6	163.5 ± 1.3	156.7–169.3	*
Weight (kg)	69.8 ± 1.5	59.2–74.2	56.2 ± 1.5	46.6–62.0	*
Body fat (%)	12.8 ± 0.7	10.0–18.3	20.6 ± 0.7	16.6–25.2	*
VO _{2max} (mL·kg ⁻¹ ·min ⁻¹)	63.7 ± 1.9	54.8–77.3	53.2 ± 1.7	47.5–63.3	*
VO _{2max} (mL·kg ⁻¹ FFM·min ⁻¹)	72.1 ± 2.1	64.8–88.2	66.5 ± 2.2	58.0–78.2	
Maximum heart rate (bpm)	191 ± 3	178–217	192 ± 4	182–211	
Average training distance (km·wk ⁻¹)	49.4 ± 6.2	42.2–56.7	56.0 ± 3.3	41.9–70.0	

Average values are means ± SEM. VO_{2max}, maximal oxygen uptake; FFM, fat-free mass.

* Significantly different ($P < 0.05$, men vs women).

TABLE 2

Average daily energy, macronutrient composition, and macronutrients provided by the baseline diet, the very-low-fat (LFAT) and moderate-fat (MFAT) experimental diets, and the carbohydrate-loading (glycogen normalization) diet.

		LFAT Experiment				MFAT Experiment			
		Energy (kJ·d ⁻¹)	Pro (g·kg ⁻¹)	Fat (g·kg ⁻¹)	CHO (g·kg ⁻¹)	Energy (kJ·d ⁻¹)	Pro (g·kg ⁻¹)	Fat (g·kg ⁻¹)	CHO (g·kg ⁻¹)
Baseline (Days 1-3)	Men	13,799 ± 264 (16% pro)	1.9	1.4	7	13,799 ± 264 (16% pro)	1.9	1.4	7
		27% fat, 57% CHO				27% fat, 57% CHO			
	Women	10,029 ± 280 (16% pro)	1.6	1.3	6	10,042 ± 272 (16% pro)	1.6	1.3	6
		27% fat, 57% CHO				27% fat, 57% CHO			
Experimental Diet (Days 4-6)	Men	14,154 ± 389 (16% pro)	1.9	0.5	9	14,874 ± 402 (15% pro)	1.9	2.1	7
		10% fat, 74% CHO				36% fat, 49% CHO			
	Women	10,033 ± 247 (16% pro)	1.7	0.5	8	10,694 ± 247 (15% pro)	1.6	1.9	6
		10% fat, 74% CHO				37% fat, 48% CHO			
Glycogen Repletion Diet (Day 7)	Men	13,891 ± 448 (16% pro)	1.9	0.5	9	13,891 ± 448 (16% pro)	1.9	0.5	9
		10% fat, 74% CHO				10% fat, 74% CHO			
	Women	10,364 ± 238 (16% pro)	1.7	0.5	9	10,293 ± 255 (16% pro)	1.7	0.6	8.5
		10% fat, 74% CHO				13% fat, 71% CHO			

Values are means ± SEM. Percentage of energy from the protein (pro), fat, and carbohydrate are rounded and may not add up to 100.

TABLE 3

Oxygen consumption, respiratory exchange ratio (RER), heart rate (HR), water consumption, and body mass during the 90-min preload run (day 8) on the very-low-fat (LFAT) and moderate-fat (MFAT) experiments.

	LFAT Experiment		MFAT Experiment		Effect
	Beginning	End	Beginning	End	
$\dot{V}O_2$ (mL·min ⁻¹) (N = 19)	2205 ± 85	2224 ± 77	2268 ± 87	2292 ± 89	
RER (N = 19)	0.94 ± 0.01	0.89 ± 0.01	0.93 ± 0.01	0.89 ± 0.01	*
Heart rate (bpm) (N = 18)	151 ± 13	155 ± 11	149 ± 13	153 ± 11	*
Water consumed (mL) (N = 19)	682 ± 83		660 ± 95		
Body mass change (kg) (N = 18)	-0.7 ± 0.1		-0.9 ± 0.2		

* Significant main effect for exercise.

No diet × exercise or main effects for diet were observed.

TABLE 4

Serum hormones and metabolites before and after the 90-min preloaded 10-km time trial on the very-low-fat (LFAT) and moderate-fat (MFAT) experiments.

	LFAT Experiment		MFAT Experiment		Effect
	Preexercise	Postexercise	Preexercise	Postexercise	
Insulin ($\mu\text{U}\cdot\text{mL}^{-1}$)	16.5 $\pm$ 2.9	11.4 $\pm$ 1.7	14.3 $\pm$ 1.8	11.9 $\pm$ 1.8	
Cortisol ($\mu\text{g}\cdot\text{dL}^{-1}$)	19.7 $\pm$ 1.4	28.3 $\pm$ 1.6 ^b	18.7 $\pm$ 1.4	30.7 $\pm$ 1.7 ^b	*
Growth hormone ($\mu\text{g}\cdot\text{mL}^{-1}$)	2.7 $\pm$ 1.3	23.8 $\pm$ 3.7	2.9 $\pm$ 0.9	27.3 $\pm$ 3.7	[†]
Glucose ($\text{mg}\cdot\text{dL}^{-1}$)	83.1 $\pm$ 2.4	135.4 $\pm$ 6.3	85.0 $\pm$ 3.1	140.2 $\pm$ 6.8	[†]
Lactate (mM)	1.62 $\pm$ 0.09	5.73 $\pm$ 0.43	1.65 $\pm$ 0.12	5.62 $\pm$ 0.45	[†]
FFA (μM)	55 $\pm$ 13	1271 $\pm$ 85	62 $\pm$ 13	1377 $\pm$ 105	[†]
Glycerol (μM)	43 $\pm$ 6	353 $\pm$ 33 ^b	31 $\pm$ 4	388 $\pm$ 35 ^b	*
Beta-hydroxybutyrate (μM)	48 $\pm$ 6	174 $\pm$ 19 ^b	56 $\pm$ 8	190 $\pm$ 18 ^b	^{††}

* Significant diet $\times$ exercise effect ($P < 0.05$);

[†] significant main effect for exercise ($P < 0.05$);

[‡] significant main effect for diet ($P < 0.05$).

^a Significantly different between preexercise LFAT vs MFAT;

^b significantly different between preexercise and postexercise;

^c significantly different postexercise LFAT vs MFAT by Bonferroni corrected *post hoc* pairwise comparisons ($P < 0.0125$).

TABLE 5

Fasting glucose, insulin, cholesterol, and triglyceride concentrations at baseline and after 3 d of very-low-fat (LFAT) and moderate-fat (MFAT) isoenergetic diets.

	LFAT Experiment		MFAT Experiment	
	Baseline	LFAT	Baseline	MFAT Effect
Insulin ($\mu\text{U}\cdot\text{mL}^{-1}$)	7.5 $\pm$ 7.5	6.4 $\pm$ 3.3	5.8 $\pm$ 2.5	5.6 $\pm$ 1.7
Glucose ($\text{mg}\cdot\text{dL}^{-1}$)	92.5 $\pm$ 1.5	92.1 $\pm$ 2.0	91.1 $\pm$ 1.5	91.7 $\pm$ 2.1
Total cholesterol ($\text{mg}\cdot\text{dL}^{-1}$)	181.6 $\pm$ 7.7	173.6 $\pm$ 7.3 ^b	183.9 $\pm$ 7.9	186.4 $\pm$ 7.6 * ^c
HDL ($\text{mg}\cdot\text{dL}^{-1}$)	54.4 $\pm$ 3.0	48.5 $\pm$ 3.1 ^b	55.0 $\pm$ 3.1	57.4 $\pm$ 3.0 * ^c
Chol:HDL (ratio)	3.42 $\pm$ 0.13:1	3.75 $\pm$ 0.20:1	3.47 $\pm$ 0.18:1	3.33 $\pm$ 0.14:1 ^b * ^c
LDL ($\text{mg}\cdot\text{dL}^{-1}$)	110.6 $\pm$ 4.8	101.5 $\pm$ 5.5 ^b	109.8 $\pm$ 6.2	115.3 $\pm$ 5.5 * ^c
Triglycerides ($\text{mg}\cdot\text{dL}^{-1}$)	84.9 $\pm$ 8.6	118.7 $\pm$ 10.0 ^b	97.5 $\pm$ 12.2	70.9 $\pm$ 7.1 ^b * ^c

Average values are means $\pm$ SEM.

* Significant diet $\times$ time effect, $P < 0.001$.

^b Significantly different between baseline (day 4) and the experimental diet (day 7) via Bonferroni corrected *post hoc* pairwise comparisons ($P < 0.017$);

^c significantly different between low-fat and moderate-fat treatments (day 7) via Bonferroni corrected *post hoc* pairwise comparisons ($P < 0.017$).

TABLE 6

Number of runners with serum cholesterol or triglyceride concentrations above or below desired range at baseline and after 3 d of very-low-fat (LFAT) and moderate-fat (MFAT) isoennergic diets. Based on the third report of the Expert panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III).

	Optimal Range	LFAT Trial		MFAT Trial	
		Baseline (Day 4)	LFAT (Day 7)	Baseline (Day 4)	MFAT (Day 7)
Cholesterol	<200 mg·dL ⁻¹	5	3	5	7
HDL					
Women	>50 mg·dL ⁻¹	2	4	1	1
Men	>40 mg·dL ⁻¹	1	4	0	0
LDL	<100 mg·dL ⁻¹	12	10	13	12
Triglyceride	<130 mg·dL ⁻¹	3	2	3	6
	<150 mg·dL ⁻¹	2	6	1	1
CHO:HDL ratio	<3.5:1	9	11	8	6