

Influence of endurance running and recovery diet on intramyocellular lipid content in women: a ^1H NMR study

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Received 11 January 2001; accepted in final form 8 August 2001

Larson-Meyer, D. Enette, Bradley R. Newcomer, and Gary R. Hunter. Influence of endurance running and recovery diet on intramyocellular lipid content in women: a ^1H -NMR study. *Am J Physiol Endocrinol Metab* 282: E95–E106, 2002.—Using a randomly assigned crossover design, we evaluated the change in intramyocellular lipid stores (IMCL) from baseline after a 2-h treadmill run [67% of maximal oxygen uptake ($\dot{V}\text{O}_{2\text{max}}$)] and the recovery of IMCL in response to a postexercise very low-fat (10% of energy, LFAT) or moderate-fat (35% of energy, MFAT) recovery diet in seven female runners. IMCL was measured in soleus muscle by use of water-suppressed ^1H -NMR spectroscopic imaging before (baseline), after, and \sim 22 h and 70 h after the run. IMCL fell by \sim 25% ($P < 0.05$) during the endurance run and was dependent on dietary fat content for postexercise recovery ($P = 0.038$, diet \times time interaction). Consumption of the MFAT recovery diet allowed IMCL stores to return to baseline by 22 h and to overshoot (vs. baseline) by 70 h postexercise. In contrast, consumption of the LFAT recovery diet did not allow IMCL stores to return to baseline even by 70 h after the endurance run ($P < 0.01$ at 70 h). These results suggest that a certain quantity of dietary fat is required to replenish IMCL after endurance running.

serum triglycerides; proton magnetic resonance spectroscopy; insulin; leptin; dietary fat

UNDER TYPICAL DIETARY CONDITIONS, skeletal muscle contains significant quantities of lipid stored as triglyceride droplets within the muscle. In aerobically trained individuals, intramyocellular lipid stores (IMCL) are in contact with the mitochondria (14, 49) and are thought to serve as an important fuel source during exercise, particularly prolonged moderate-intensity exercise (8, 26, 38, 39). For instance, it has been estimated from isotope tracer studies in men and women that IMCL can contribute as much as 20–30% of energy expenditure during prolonged submaximal exercise (26, 38, 39). Indeed, complete blockade of muscle lipolysis in rats treated with propranolol (43) and in humans treated with the nonselective β -blocker nadolol (5) was

associated with decreased endurance and early fatigue. Findings from studies that have directly measured the change in IMCL before and after endurance exercise, however, have been equivocal. Some studies have found that IMCL are reduced by \sim 20–75% during moderate- to strenuous-endurance (2, 4–6, 8, 9, 17, 37) and ultra-endurance events (11, 32, 35, 45), whereas other studies have reported no change (1, 19, 23, 25, 44, 50). Unfortunately, previous studies have been conducted most frequently in active men, and less is known concerning IMCL utilization in active women.

In recent years, an increasing number of endurance athletes, particularly female athletes, have been adopting extremely low-fat diets (i.e., <10 –15% of energy from fat) in the belief that dietary fat consumption will increase adiposity and impair health and/or performance (36 and D. E. Larson-Meyer, unpublished observations). These extremely low-fat diets, however, may be unhealthy and actually compromise performance. For example, diets with a low composition of fat may be a factor contributing to exercise-induced amenorrhea (29), compromised immune function (48), and elevated serum triglycerides (3). Other studies have alluded to the possibility that extremely low-fat diets (i.e., 10–15% of total energy from fat) may be detrimental to performance in highly trained endurance athletes (14, 15, 33), possibly by compromising IMCL stores (33, 36). Similar to the concept of how adequate dietary carbohydrate influences muscle glycogen stores and performance, a certain quantity of dietary lipid may be crucial for supplying free fatty acids to exercising muscle via IMCL stores. Our current knowledge of the mechanisms by which IMCL are mobilized and contribute to fat metabolism during exercise has nicely been reviewed by Jeukendrup et al. (20–22). Although a few studies have investigated the effect of dietary fat content on IMCL stores at rest (24) and with exercise training (7, 14), even fewer (44) have examined the influence of diet composition on IMCL recovery after prolonged exercise.

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Most previous studies have assessed intramuscular triglyceride levels by use of needle biopsy methodology. This technique, however, is invasive, measures only a small sample that may not be reflective of the whole muscle, and does not allow for repetitive measurements in the same muscle. Recently, several investigators have developed the technology to noninvasively measure intracellular lipids in human muscle by $^1\text{H-NMR}$ spectroscopy on a 1.5 T system (2, 40, 46). Comparison of signals from skeletal muscle, adipose, and liver tissue has shown that muscle contains two compartments of triglycerides/fatty acids with a resonance frequency shift of 0.2 ppm, one that is associated with lipid within fat cells, or extramyocellular lipid (EMCL), and the other that is confined to the skeletal muscle cytoplasm, intramyocellular lipid (IMCL) (40, 46). Measurement of IMCL by NMR has recently been validated in *in vivo* animal and human models (46).

The purpose of the current study was twofold: 1) to ascertain the change in IMCL with endurance exercise in trained female runners and 2) to determine the pattern (time course) of IMCL replenishment (1 and 3 days postexercise) after an extremely low-fat (10% of energy from fat, LFAT) and a moderate-fat (35% of energy from fat, MFAT) recovery diet. We postulated that IMCL would decrease with exercise and that the level of IMCL would stabilize after endurance exercise faster after the MFAT compared with the LFAT diet.

METHODS

The study was a single-blind, randomly assigned crossover design with the investigators blinded to the diet treatment (LFAT or MFAT). Well-trained female runners completed two 7-day trials (see Fig. 1) during the follicular phase of their menstrual cycle (days 1–13) or, if the women were taking oral contraceptives, during the 7 days of inert pills. A 3- to 4-wk “washout” period (corresponding to the subject’s menstrual cycle) separated the two diet treatments.

Subjects. Nine premenopausal, well-trained endurance runners were recruited through newsletter and newspaper advertisements that specifically targeted well-trained endurance runners and triathletes. Inclusion criteria were regular endurance running (≥ 20 miles/wk), performance of at least two runs of ~ 2 h or more within the past 3 mo, negative past medical history assessed by a standard Health Status Questionnaire, normal iron status documented by a normal serum hemoglobin (12–15.2 g/dl), and normal eating habits assessed by a registered dietitian (D. E. Larson-Meyer). The study was approved by The University of Alabama at Birmingham (UAB) Institutional Review Board. All volunteers were briefed about the experimental protocol, and informed consent was obtained before testing.

Baseline fitness and body composition analysis. Before initiation of the experimental protocol, maximal oxygen uptake ($\dot{V}O_{2\text{max}}$) was determined during a running treadmill test session by use of either incremental speeds [starting at the subjects’ typical warm-up speed and increasing by 0.5 mph (13.4 m/min)] or grades (starting at 0°C and increasing by 2.5%) that increased every minute until exhaustion. The volumes of O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) were measured continuously on an open-circuit system. Respiratory gases were collected and analyzed continuously with a Beckman OM-11 oxygen analyzer and a Beckman LB-2 carbon dioxide medical gas analyzer. The

analyzers were calibrated before each test with Micro-Schoeller analyzed gas. Gas volumes were determined on a Rayfield Instruments dry gas meter. Data were collected and processed using a Rockwell International AIM 65 microcomputer. Heart rate was monitored by a Polar Vantage XL heart rate monitor (Polar Beat, Port Washington, NY). The highest $\dot{V}O_2$, respiratory exchange ratio (RER), and heart rate (HR) achieved over a 30-s period within the last 2 min of exercise were recorded as the maximum values, or $\dot{V}O_{2\text{max}}$, RER_{max} , and HR_{max} , respectively. For the test to be considered an acceptable measurement, two of the following criteria had to be met: 1) a leveling or plateauing of $\dot{V}O_2$ (defined as an increase in $\dot{V}O_2$ of $< 2 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ with increased workload), 2) $RER > 1.1$, and 3) HR_{max} within 10 beats of age-predicted maximum (41). An acceptable measurement of $\dot{V}O_{2\text{max}}$ was obtained from all subjects enrolled in the study. After completion of the $\dot{V}O_{2\text{max}}$ test, subjects rested for 20–30 min. A titration run was then performed to determine the treadmill speed (to the nearest 0.25 mph or 6.7 m/min) that would elicit a $\dot{V}O_2$ equal to 65% of $\dot{V}O_{2\text{max}}$. For descriptive purposes, body composition was also determined by dual-energy X-ray absorptiometry (12) by use of a total body scanner model DPX-L software version 3.2 (Lunar Radiation, Madison, WI).

Experimental protocol. The experimental protocol is outlined in Fig. 1. Baseline IMCL were measured after 3 days of a control diet (25% of energy from fat, 15% from protein, 60% from carbohydrate). Subjects were provided a standard 500-kcal breakfast of this same composition 1 h before the IMCL measurement and 2.5 h before the start of the 2-h run. Eight ounces of measured coffee were included in the breakfast meal if the subjects habitually drank coffee in their pretraining meals. Subjects then performed a 2-h run on a Quinton treadmill at 65% of $\dot{V}O_{2\text{max}}$ after a 5-min warm-up walk/run at a self-selected speed and a 5-min stretching routine. The treadmill was calibrated before each run. A portable HR

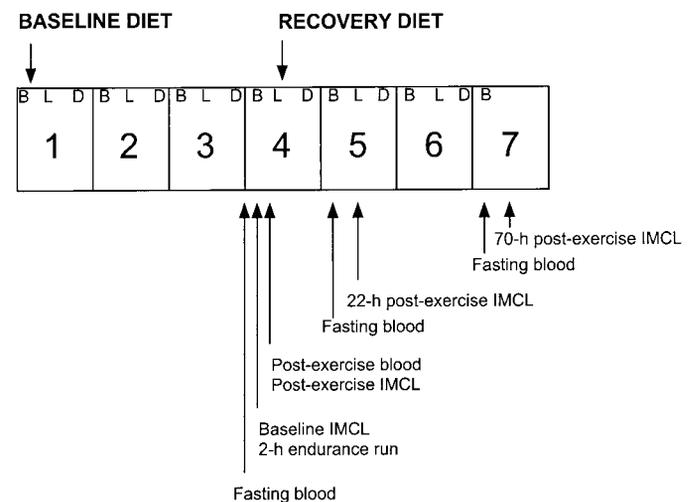


Fig. 1. Experimental protocol. Intramyocellular lipid (IMCL) content was determined by ^1H NMR spectroscopic imaging at baseline (preexercise), immediately after a 2-h endurance run (postexercise), and 1 day and 3 days after initiation of the randomly assigned experimental (very low-fat or moderate-fat) diet that began with the post-run lunch meal. Fasting blood samples were obtained for analysis of insulin, glucose, free fatty acids, triglycerides, and leptin before the breakfast meal on the days of the ^1H NMR measurements. The same protocol was performed in the crossover trial, except that subjects received the alternate experimental diet. Each box represents 1 day; B, breakfast; L, lunch; D, dinner.

monitor was used to continuously monitor the subjects' HR (Polar Vantage XL, Polar Beat). $\dot{V}O_2$ and $\dot{V}CO_2$ were monitored for 3 min at the beginning (between 5 and 10 min after the start), middle (1 h into), and end (10 min before completion) of the 2-h run. After completion of the midpoint respiratory gas collection (after 1 h and 5 min of running), subjects were given a 5-min break. After voiding, body mass was obtained on a balance scale immediately before exercise, during the midpoint break, and immediately after exercise. Subjects were encouraged to remain well hydrated by drinking enough water to maintain body mass. Water was provided ad libitum during the 2 h. IMCL were then measured within 1 h of the completion of the run (postexercise). After postexercise testing, subjects were randomly assigned to the LFAT (10% of energy from fat, 15% from protein, and 75% from carbohydrate) or MFAT diet (35% of energy from fat, 15% from protein, and 50% from carbohydrate) for 3 days, starting with the lunch meal on *day 4* and ending with the breakfast meal on *day 7*. IMCL were measured 22 h (Rec-1) and 70 h (Rec-3) postexercise, i.e., after the subject had consumed three full meals (lunch, dinner, and breakfast) of the experimental recovery diet. As outlined in Fig. 1, blood samples for analysis of insulin, glucose, free fatty acids, triglyceride, and leptin concentrations were also drawn from the antecubital vein before each IMCL measurement. Specifically, samples were obtained before breakfast (fasting) on *days 4* (preexercise), *5* (Rec-1), and *7* (Rec-3) and within 10 min after completion of the 2-h run on *day 4* (postexercise).

Dietary manipulation. Baseline and experimental diets were prepared and administered by the General Clinical Research Center (GCRC) at UAB. Each morning of the baseline and experimental diet treatments, subjects reported to the GCRC in the fasting condition. After voiding, body weight was recorded to the nearest 0.1 kg (Toledo electronic scale, Worthington, OH), with subjects dressed in a hospital gown. Subjects were then given their meals and snacks for the day, which consisted of real food and beverages, and were allowed to consume them in free-living style. Eucaloric requirements were estimated from the Harris Benedict prediction equation for estimating basal energy expenditure (BEE) (13), multiplied by an activity factor, and rounded to the nearest 100 kcal. For all subjects, an activity factor of 1.85 times BEE was used for the baseline diet, and a factor of 2.0 times BEE was used for the LFAT and MFAT experimental recovery diets. The higher activity factor and thus higher energy content of the experimental recovery diets were assigned to offset the energy cost of completing the 2-h run (>1,000 kcal), i.e., to prevent several days of negative energy balance. During all diet treatments, subjects were requested to consume all food and beverages provided. They were asked not to consume anything (food, beverages, sports nutrition products, etc.) in addition to those provided but were encouraged to consume plain water ad libitum. The subjects were also asked to report honestly to the GCRC dietitian any deviations from the diet and to return any food not eaten to the GCRC the following morning. The dietary fat contents of the experimental recovery diets are similar to those used by Muoio et al. (33) and were selected to provide high to moderate amounts of carbohydrate and not be impractical.

Training runs. To mimic free-living training conditions, subjects performed 45-min training runs at a self-selected pace (between ~70 and 85% of $\dot{V}O_{2\max}$) on *days 1, 2, 5, and 6*. HR, monitored with a portable HR monitor (Polar Beat), rate of perceived exertion (RPE, modified Borg Scale), running route, and time of day were recorded. In the crossover treatment, subjects were asked to perform similar training runs (i.e., on the same course, at the same time of day, with the

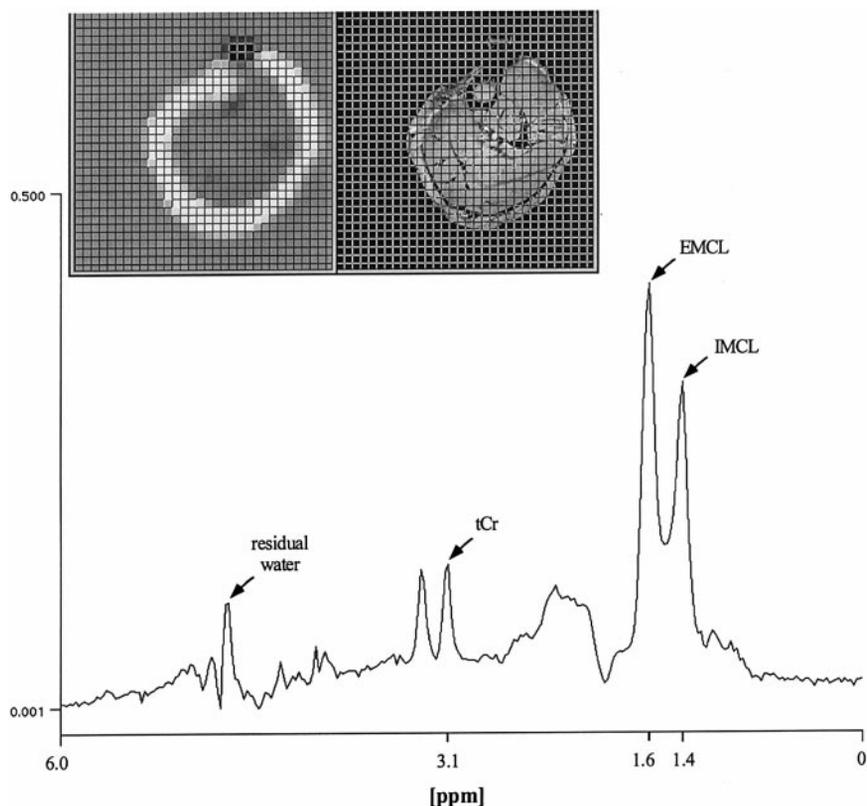
same rate of perceived exertion, and at a similar HR). No exercise was performed on *day 3*, the day before the 2-h experimental run.

IMCL. IMCL were measured in the soleus muscle by 1H NMR spectroscopic imaging on a 4.1 T whole body imaging and spectroscopy system (Bruker Instruments, Billerica, MA). Measurements were obtained from the right calf, with the subject lying in the supine position within the spectrometer. The subject's leg was positioned inside a single-tuned 1H birdcage coil, with the knee in extension and the ankle in a neutral position. A lab-constructed foot holder was used to stabilize the heel, and rolled pads were positioned inside the coil to prevent movement of the leg during imaging. To ensure a similar slice selection, an external reference (phantom) was secured to the anterior surface of the midtibia, at the maximum circumference of the calf. During the baseline IMCL measurement, the position of the phantom was marked on the skin with a nontoxic waterproof marker (Marks-A-Lot, Office Products, Brea, CA), so it could be repositioned during subsequent measurements within the same experimental visit. The exact slice of the gastrocnemius-soleus muscle complex containing the phantom was positioned within the homogeneous volume of the magnet. The orientation of the leg in reference to the magnetic field and the coil position was selected in previous work (unpublished observations) to provide optimal splitting of the IMCL and EMCL resonances in the soleus muscle. A standard water-suppressed spectroscopic image (SI) was obtained using a slice-selective 2D sequence [repetition time (TR) = 1,000 ms, echo time (TE) = 24 ms, 16×16 -cm field of view (FOV), 32×32 -cm matrix, 10-mm slice thickness]. This allowed for determination of myocellular (Fig. 2) and bone marrow lipid peaks within selected regions of the calf (i.e., soleus and tibia, respectively). Although it is possible with SI to obtain myocellular lipid spectra from other muscles of the calf, our experiments were designed to optimize the spectra obtained from the soleus because of its fiber type (predominantly slow-twitch fiber) and involvement during running (8). Thus data from other muscles will not be reported, because locating spectra from the gastrocnemius, tibialis anterior, and tibialis posterior muscles that were free from subcutaneous fat artifacts and that produced adequate splitting between the EMCL and IMCL peaks was difficult. The magnetic field was shimmed on the slice on which the SI was acquired.

After the SI was acquired, a set of high-resolution axial scout images was obtained on the same slice selection. The high-resolution images were used to coregister with the SI so the muscle group of each pixel could be identified (Fig. 2). These images were collected with a protocol of TR = 703 ms, TE = 14.3 ms, 16×16 -cm FOV, 256×256 -mm matrix, and 10-mm slice thickness with a slice separation of 15 mm.

IMCL and EMCL content was determined from the sum of nine contiguous pixels (usually forming a 3×3 box) from an area of the soleus muscle that was free from marbling (Fig. 2). This box of nine pixels represents a volume of muscle equal to 2.5 ml. The approximate location of the box was kept constant for each subject during each experimental trial. This box size was selected from an earlier analysis, which determined that IMCL content measured from a summed 3×3 box was both reproducible [within-subject coefficient of variation (CV) = 6.4%, Table 1] and feasible in various-sized soleus muscles (unpublished observations). Measurement of EMCL content in our experiments and in those of others (2, 46) by use of NMR technology, however, is considerably less (within-subject CV = 18.3%, Table 1). Peak positions and areas of interest [extramuscular methylene signal $[(CH_2)_n]$,

Fig. 2. ^1H NMR spectra from a single pixel obtained from a water-suppressed spectroscopic image (*left inset*) of resting calf muscle in a representative subject. A high-resolution scout image (*right inset*) was used to coregister with the spectroscopic image so the muscle group of each pixel could be identified. IMCL content was determined from the sum of 9 contiguous pixels (usually forming a 3×3 box) from an area of the soleus muscle that was free from marbling. See text for collection protocol and further detail. Spectra are unaltered except for Fourier transformation and zero-order phasing. tCr, total creatine; EMCL, extramyocellular lipid; IMCL, intramyocellular lipid.



intramuscular $(\text{CH}_2)_n$, and creatine (46)) were determined by time domain fitting by Fitmasters (Phillips Medical Systems, Shelton, CT) and a set of prior knowledge files. The EMCL $(\text{CH}_2)_n$ peak was assigned to 1.6 ppm, and the other peak positions, IMCL $(\text{CH}_2)_n$ and creatine, were allowed to float in reference to the EMCL peak. This put the IMCL consistently at ~ 1.4 ppm, the creatine peak at ~ 3.1 ppm, and the residual water peak at 4.7 ppm. The line widths (LW) of all peaks were allowed to float. The position and LW of all peaks were later checked for fit accuracy. An example of a raw ^1H NMR spectrum, its associated fit, and resulting difference spectrum (i.e., after subtracting fitted curves from raw data) of a representative subject are shown in Fig. 3. In the spectra

that did not have good fit, peak position and/or LW were fixed. In the latter case, LW were fixed only if the fit was not improved by position fixing and were achieved by using the ratio of the EMCL LW to creatine LW, and IMCL LW to creatine LW, of that subject's baseline (preexercise) or recovery (Rec-3) spectrum. In all cases, the spectra with a poor initial fit had either inadequate splitting between EMCL and IMCL $(\text{CH}_2)_n$ ($<15\%$ of IMCL peak amplitude) or a severely asymmetrical EMCL peak. Fixing spectra on the basis of LW ratios was determined in pilot work from our laboratory and in this study was performed in $\sim 16\%$ of the spectra analyzed.

Because the intensity of the water peak resonance, often used as an internal reference standard to roughly "quantify"

Table 1. *Reproducibility of bone marrow and myocellular lipid content measured in 10 subjects*

Subject No.	BLM Peak		EMCL Relative to BML		IMCL Relative to BML	
	Time 1	Time 2	Time 1	Time 2	Time 1	Time 2
1	330.5	322.2	0.27	0.20	0.24	0.24
2	394.6	374.6	0.29	0.25	0.18	0.16
3	427.4	371.5	0.08	0.05	0.04	0.05
4	596.2	545.6	0.32	0.40	0.29	0.31
5	460.1	447.7	0.06	0.16	0.08	0.08
6	661.8	682.9	0.27	0.23	0.10	0.11
7	493.0	537.4	0.20	0.19	0.21	0.18
8	1006.0	1093	0.15	0.14	0.07	0.08
9	483.1	531.5	0.38	0.33	0.21	0.22
10	949.3	922.0	0.24	0.17	0.14	0.13
Mean \pm SD	580.2 \pm 218.2	582.8 \pm 237.8	0.23 \pm 0.10	0.21 \pm 0.09	0.16 \pm 0.08	0.16 \pm 0.08
CV _b , %		41.0		42.0		52.2
CV _w , %		5.4		18.3		6.4

BLM, bone marrow lipid; EMCL, extramyocellular lipid; IMCL, intramyocellular lipid; CV_b, coefficient of variation between subjects; CV_w, coefficient of variation within subjects. The CV_w for IMCL was improved by use of BML peak as the internal reference standard. CV_w = 16.9% for EMCL and 8.2% for IMCL, respectively (raw data not shown).

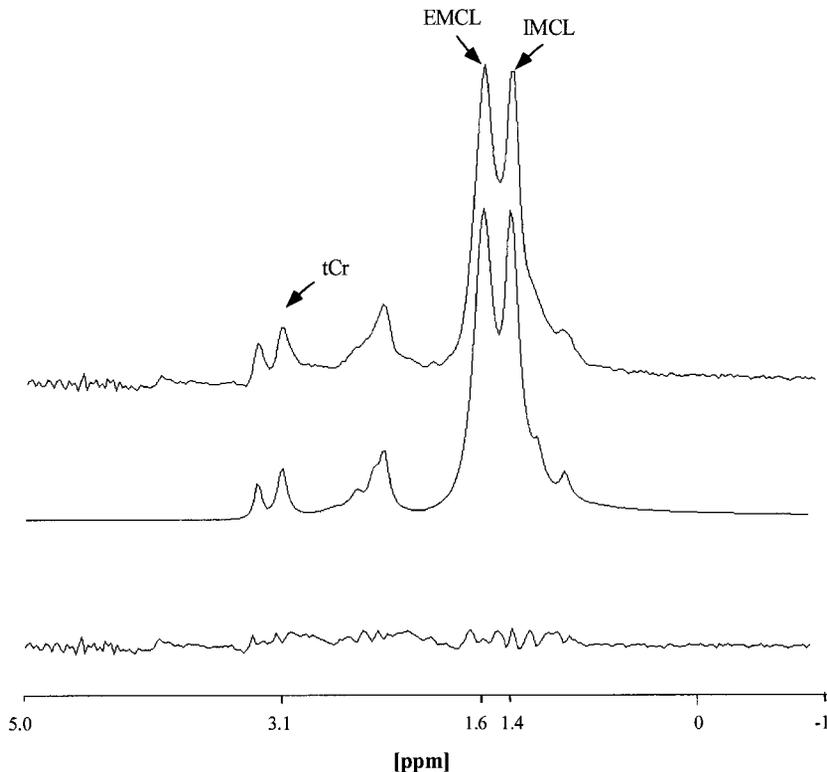


Fig. 3. An example of a raw ^1H NMR spectrum (middle), its associated time domain fit (far back or top), and the resulting different (front or bottom) spectrum for a representative subject. Peak positions and areas of total creatine (tCr), extramyocellular methylene signals $[(\text{CH}_2)_n]$ (EMCL), and intramyocellular $(\text{CH}_2)_n$ (IMCL) were determined by time domain fitting using Fit Masters (Phillips Medical Systems, Shelton, CT) and a set of prior knowledge files.

IMCL (2, 27, 28), is not an appropriate reference during exercise because of possible intra- and extracellular fluid shifts during and after exercise, the peak amplitude of the tibia's bone marrow lipid (BML) was used as the reference standard. The voxel that corresponded to the maximum BML area was used as the BML spectrum. These voxels were always within the bone marrow of the tibia, so no partial volume effects were noted for the BML spectrum. The peak position and area of the BML resonance were determined by time domain fitting, as described above, so that both the position and LW were allowed to float. We have previously determined that use of BML peak area to normalize area under the IMCL and EMCL curves for day-to-day variations in system performance was more reliable than either use of the creatine peak or use of no internal reference (unpublished observations). For example, when the methods in the current paper were used, the CV for IMCL was 6.4% when BML was used as the internal reference standard, 16.9% when creatine was used, and 8.2% when no internal reference was used.

Blood analysis. Glucose was measured in 10 μl of sera with an Ektachem DT II System (Johnson and Johnson Clinical Diagnostics). This analysis has a mean intra-assay CV of 0.61% and a mean interassay CV of 1.45%. Insulin was assayed using reagents obtained from Linco Research (St. Charles, MO). For insulin, the mean intra- and interassay CV values were 4.5 and 2.3%, respectively, and assay sensitivity (90% bound) was 17.16 pmol/l. Free fatty acids were assayed with NEFA-C reagents obtained from Wako Diagnostics (Richmond, VA). The assay was modified to accommodate a reduced sample volume (10 μl) and use of a microplate. Triglycerides were measured with the Ektachem DT II System. Control sera of low and high substrate concentration are analyzed with each group of samples, and values for these controls must fall within accepted ranges before samples are analyzed. The DT II is calibrated every 6 mo with

reagents supplied by the manufacturer. Serum leptin was measured in duplicate 100- μl aliquots by use of a double-antibody RIA (Linco Research). This assay has a sensitivity of 0.4 ng/ml, a mean intra-assay CV of 5%, and a mean interassay CV of 6%.

Statistical analysis. All statistics were performed using SPSS analysis software (SPSS 10.0 for Windows, Chicago, IL). Values shown in the text, tables, and figures refer to means \pm SD unless otherwise indicated. Doubly repeated-measures ANOVA was used to test for diet \times time interaction and, if appropriate, all main effects or simple effects. Bonferroni adjustments were made for all subsequent post hoc pairwise comparisons. Pearson correlation coefficients were used to test the simple relationship between IMCL depletion during exercise and whole body measurements. Alpha was set at 0.05.

RESULTS

Subjects. Of the nine subjects enrolled in the study, one became pregnant between the dietary treatments and was unable to complete her crossover treatment; one had soleus spectra in both dietary trials that were not usable, i.e., there was no splitting between the EMCL and IMCL peaks in the SIs obtained in any of her eight visits (4 visits each trial). Interestingly, on further questioning, we learned that this subject had a history of pain and cramping deep in her right calf muscle that was often induced by vigorous exercise outdoors. Data analysis, therefore, could be performed on only seven subjects. The physical characteristics, body composition, and $\dot{V}\text{O}_{2\text{max}}$ of the seven subjects completing the study are shown in Table 2. Of these seven subjects, four were first assigned to the MFAT diet and three to the LFAT diet. Four had regular

Table 2. *Physical characteristics of female endurance-trained runners*

Characteristic	Mean \pm SD	Range
Age, yr	35.3 \pm 8.1	23–49
Body mass, kg	55.8 \pm 5.5	48.4–64.5
Body mass index, kg/m ²	20.8 \pm 1.3	19.6–23.0
Body fat, %	23.4 \pm 5.8	16.7–32.4
$\dot{V}O_{2\max}$, ml·kg ⁻¹ ·min ⁻¹	52.4 \pm 6.2	40.0–57.2

Values are means \pm SD of 7 subjects. $\dot{V}O_{2\max}$, maximal oxygen uptake.

menstrual cycles, and three were taking oral contraceptives. Only one habitually consumed coffee in her prerun meal.

Endurance run. Subjects completed the 2-h treadmill run at 168.4 \pm 30.9 m/min, which elicited an average $\dot{V}O_2$ of 67.7 \pm 3.5 and 67.2 \pm 2.4% of $\dot{V}O_{2\max}$ during the LFAT and MFAT trials, respectively. Subjects' body mass and metabolic measurements obtained at the beginning, middle, and end of the 2-h run are shown in Table 3. A diet \times time interaction was not found for any of the measurements, indicating that the metabolic effect of performing the 2-h runs was similar before each recovery diet treatment. A statistically significant main effect for time ($P < 0.05$), however, was noted for $\dot{V}O_2$, RER, and HR but not for body mass. During the run, $\dot{V}O_2$ fell significantly between the beginning and middle measurements ($P = 0.025$). RER fell continuously and was statistically significant between the beginning and end of exercise ($P = 0.009$). HR continued to drift upward, being statistically significant at all time points ($P < 0.05$). Despite constant encouragement to remain adequately hydrated, body mass tended to drop; however, a statistically significant main effect for time was not noted ($P = 0.06$).

Dietary manipulation. Average intake on the baseline diet was 2,457.1 \pm 222.5 kcal/day, which provided $\sim 6.6 \pm 0.7$ g carbohydrate/kg body wt and 1.2 ± 0.1 g fat/kg body wt, with an average fiber content of 21.0 \pm 5.0 and an unsaturated-to-saturated fatty acid ratio of 1.8. The energy, protein, fat, and carbohydrate contents of the experimental LFAT and MFAT diets are shown in Table 4. For the group, a significant diet \times time interaction was not found for body mass across the 7 days. Although body mass remained relatively consistent during both trials, a statistically significant main effect for time was noted. This time effect ap-

Table 4. *Energy and macronutrient content provided by the eucaloric LFAT and MFAT recovery diets*

	LFAT (10% fat)	MFAT (35% fat)
Energy, kcal	2,643 \pm 251	2,643 \pm 251
Protein, g	99.1 \pm 9.4	99.1 \pm 9.4
Protein, g/kg body wt	1.8 \pm 0.2	1.8 \pm 0.2
Fat, g	29.4 \pm 2.8	102.8 \pm 9.8
Fat, g/kg body wt	0.5 \pm 0.1	1.8 \pm 0.2
Unsaturated/saturated fat, ratio	2.27	1.72
Carbohydrate, g	495.5 \pm 47.0	330.4 \pm 31.3
Carbohydrate, g/kg body wt	8.9 \pm 0.9	5.9 \pm 0.6
Fiber, g	25.4 \pm 4.9	20.9 \pm 1.5

Values are means \pm SD.

peared to be due to ~ 0.5 kg lower body weight on the morning after the 2-h run, but this drop was not statistically significant by the Bonferonni corrected post hoc analysis.

Training runs. A significant diet \times time interaction or main effect for time was not found for run time, RPE, or average HR during the 45-min training runs on days 1, 2, 5, and 6. These data are summarized in Table 5 and indicate that differences noted in IMCL concentration were not due to differences in the intensity or duration of the training runs.

BML measurements. Simple (diet \times time) and main effects were determined for BML peak intensity, because it was used (rather than water peak intensity) to normalize IMCL and EMCL content for day-to-day variations in system performance. BML values measured before exercise, after exercise, at Rec-1, and at Rec-3 were 496.5 \pm 105.3, 519.0 \pm 145.3, 486.3 \pm 128.4, and 443.9 \pm 102.0 for the LFAT treatment and 473.8 \pm 167.6, 449.2 \pm 114.3, 489.6 \pm 166.0, and 508.0 \pm 190.0 for the MFAT treatments, respectively. Although a significant diet \times time interaction for BML peak was found ($P = 0.04$), the post hoc pairwise comparisons indicated that this interaction was due to a statistically significant difference in BML peak between the trials only after exercise, i.e., when the subjects were following the baseline diet and had not started the LFAT or MFAT recovery diets. Pairwise comparisons indicated that BML peak intensity was not different between the LFAT and MFAT diets at Rec-1 ($P = 0.94$) or Rec-3 ($P = 0.18$), suggesting that diet treatment did not influence the reference peak.

Table 3. *Metabolic measurements obtained at the beginning (5–10 min after the start), middle (1 h into), and end (10 min before completion) of the 2-h run*

	LFAT Trial			MFAT Trial		
	Beginning	Middle	End	Beginning	Middle	End
Body mass, kg	56.6 \pm 5.2	56.3 \pm 5.4	56.2 \pm 5.6	56.6 \pm 5.2	56.2 \pm 5.5	56.0 \pm 5.5
$\dot{V}O_2$, ml·kg ⁻¹ ·min ⁻¹	35.4 \pm 4.3	34.7 \pm 4.4	35.1 \pm 4.3	35.5 \pm 4.5	34.6 \pm 4.5	34.8 \pm 3.6 ^a
RER, ratio	0.94 \pm 0.02	0.90 \pm 0.03	0.87 \pm 0.03	0.92 \pm 0.05	0.92 \pm 0.03	0.89 \pm 0.05 ^{a,b}
HR, beats/min	154.6 \pm 10.3	159.8 \pm 12.1	164.5 \pm 14.0	157.2 \pm 11.8	162.5 \pm 13.9	167.4 \pm 10.6 ^{a,b}

Values are means \pm SD. LFAT, low-fat diet; MFAT, moderate-fat diet; $\dot{V}O_2$, oxygen uptake; RER, respiratory exchange ratio; HR, heart rate. Significant time effect, ^a $P < 0.05$; ^b $P < 0.05$ beginning vs. middle; ^c $P < 0.05$ beginning vs. end; ^d $P < 0.05$ middle vs. end.

Table 5. Recorded running time, RPE, and average HR of 45-min training runs performed at a subject-selected pace

	LFAT Trial			MFAT Trial		
	Time, min	RPE	HR, beats/min	Time, min	RPE	HR, beats/min
Day 1	45.0 ± 0.5	13.4 ± 1.5	166.8 ± 9.8	44.9 ± 0.3	13.6 ± 1.5	168.4 ± 12.3
Day 2	44.8 ± 0.4	13.3 ± 0.5	164.8 ± 9.8	45.2 ± 0.3	13.6 ± 1.4	165.9 ± 14.0
Day 5	44.9 ± 0.7	14.7 ± 2.4	166.4 ± 9.0	44.9 ± 1.1	14.3 ± 2.0	171.7 ± 6.6
Day 6	44.8 ± 0.3	13.9 ± 1.4	166.6 ± 8.0	44.3 ± 0.7	13.1 ± 2.6	166.4 ± 9.0

Values are means ± SD. RPE, rate of perceived exertion. There was no significant main effect for either diet × time or time.

IMCL measurements. Figure 4 shows IMCL content relative to BML peak intensity (*top*) before (Pre), immediately after (Post), and ~1 day (Rec-1) and 3 days (Rec-3) after the 2-h run. A significant diet × time interaction was found for IMCL content ($P = 0.038$), which recovered much faster on the MFAT than on the LFAT diet after exercise (Fig. 4). Post hoc pairwise comparisons found that the difference in IMCL content tended to be higher in the MFAT vs. LFAT trial at Rec-1 ($P = 0.16$) and was significantly greater at Rec-3 ($P = 0.01$). As expected, IMCL content was not different pre- and postexercise when all subjects were following the same baseline diet. It should be mentioned, however, that the slightly higher preexercise IMCL mean on the LFAT trial was due to one subject who, interestingly, was assigned to the MFAT diet on her first visit and retrospectively admitted to adding more fat to her regular diet between the MFAT and the LFAT trials (because she enjoyed the food on the MFAT diet). Figure 4 also illustrates that IMCL content fell significantly ($P = 0.003$, main effect for time) in response to the 2-h exercise regimen.

The same trends in the data were also found in the raw IMCL signal amplitude, i.e., when IMCL peak area was not expressed relative to BML. The unadjusted raw IMCL content values measured before and after exercise and at Rec-1 and Rec-3 were 64.3 ± 37.5 , 43.8 ± 25.7 , 46.4 ± 23.8 , and 46.2 ± 20.1 for the LFAT treatment and 50.2 ± 23.4 , 38.0 ± 17.0 , 50.1 ± 30.1 , and 67.8 ± 39.8 for the MFAT treatments, respectively. The diet × time interaction approached statistical significance ($P = 0.078$; not shown), and the main effect for time, due mainly to the fall in IMCL with exercise, was statistically significant ($P = 0.008$).

EMCL measurements. Figure 4 shows EMCL content relative to BML peak intensity (*bottom*) at the same time points before, immediately after, and at 1 and 3 days postexercise. In contrast to the IMCL measurements, a diet × time interaction was not found for EMCL. A main effect for time, however, was noted, which was due to the elevated EMCL in Rec-1 and was statistically significant only between the postexercise and Rec-1 time points. A diet × time ($P = 0.14$) or time effect ($P = 0.09$) was not noted in the raw EMCL data.

Serum hormones and metabolites. Figure 5 illustrates the change in serum hormone and metabolite concentrations throughout the experimental trial. A significant diet × time effect was found only for serum

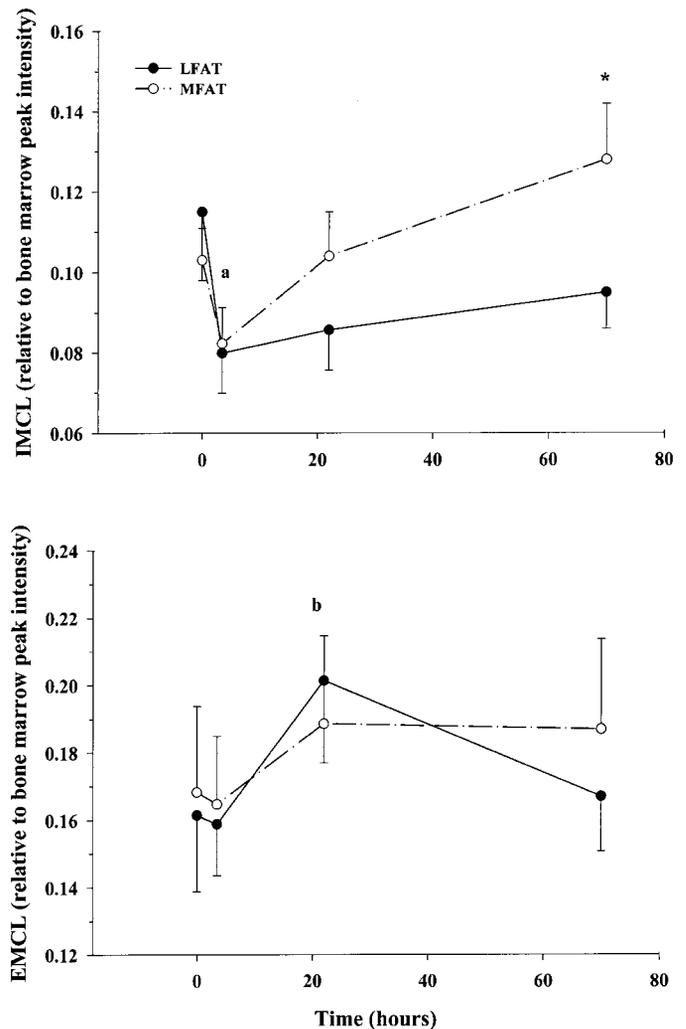


Fig. 4. Myocellular lipid content measured by ^1H NMR spectroscopic imaging before, immediately after, and ~1 day (22 h) and 3 days (70 h) after a 2-h treadmill run. IMCL (*top*) and EMCL (*bottom*) are expressed relative to bone marrow peak. A significant diet × time interaction was found for IMCL content ($P = 0.038$); $*P < 0.05$ by Bonferroni-corrected post hoc pairwise comparisons. A statistically significant effect for time was found for the change in IMCL content with exercise ($^aP < 0.05$ by Bonferroni-corrected post hoc comparisons of time pre- vs. postexercise) and for the rise in EMCL on day 1 of recovery exercise ($^bP < 0.05$ by Bonferroni-corrected post hoc comparisons of time, postexercise vs. 22 h). LFAT, low-fat diet; MFAT, moderate-fat diet.

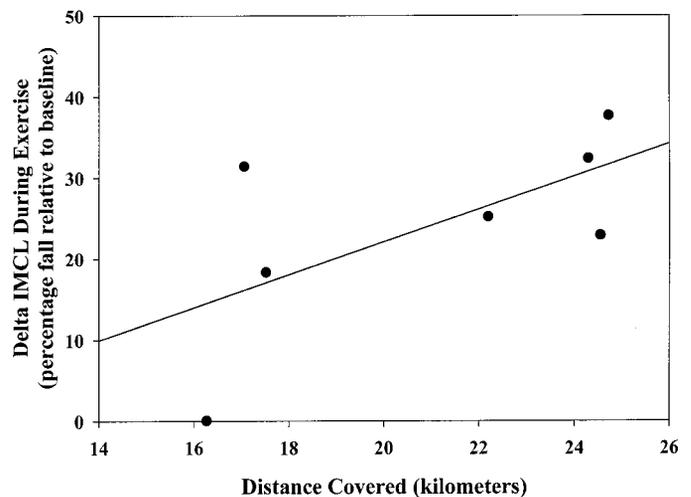
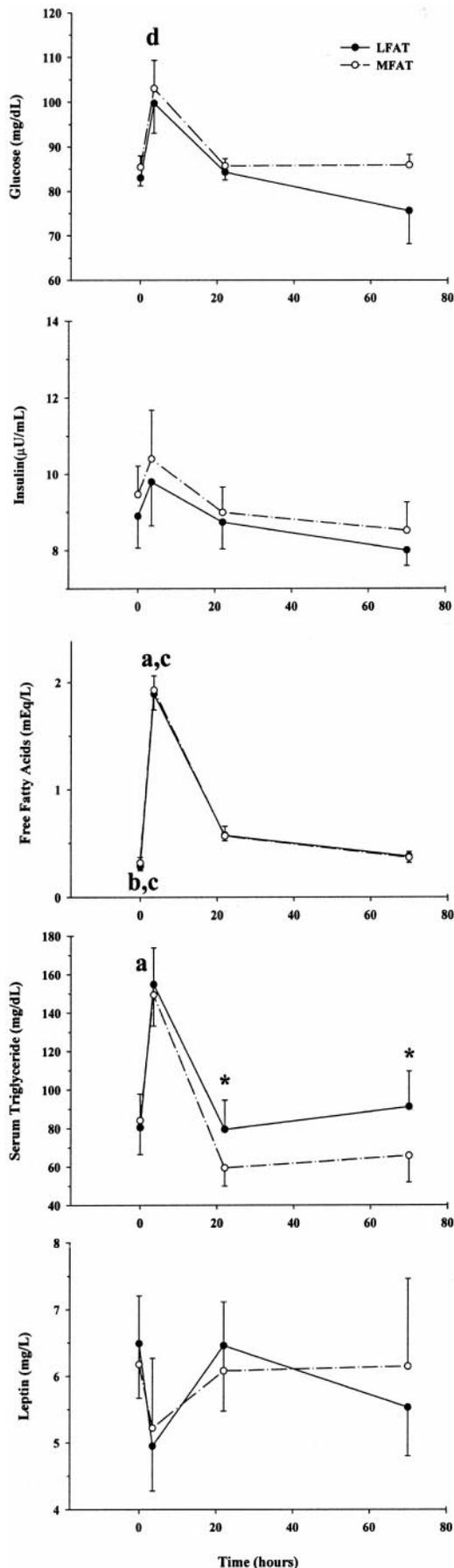


Fig. 6. Relationship between average fall in IMCL and distance covered during the 2-h run at 67% maximal oxygen uptake ($\dot{V}O_{2\max}$) ($P < 0.05$, $r = 0.62$).

triglycerides ($P = 0.04$), which, according to the Bonferroni-corrected post hoc pairwise comparisons, was due to significantly elevated triglyceride concentrations on the LFAT vs. the MFAT recovery diets at Rec-1 ($P = 0.028$) and Rec-2 ($P = 0.024$). A significant effect for time was found for serum glucose, triglyceride, and free fatty acid concentrations, which were all elevated (above baseline) postexercise.

Relationship between IMCL depletion and whole body measurements. In this small sample size, the percentage fall of IMCL during exercise was not significantly correlated with RER and the other metabolic measurements made at the beginning, middle, or end of the 2-h endurance run during either of the experimental trials, or with the postexercise blood hormone and metabolite concentrations obtained during both trials. The average percentage fall of IMCL, however, was significantly correlated with $\dot{V}O_{2\max}$ ($r = 0.786$) and the distance covered during the 2-h run at 67% $\dot{V}O_{2\max}$ ($r = 0.62$, Fig. 6) (and thus the assigned running velocity), but not with body fat percentage ($r = 0.22$).

DISCUSSION

The current investigation evaluated noninvasively by use of ^1H NMR techniques the change in IMCL from baseline after a 2-h treadmill run at 67% of $\dot{V}O_{2\max}$ and the recovery of IMCL in response to a postexercise LFAT (10% of energy as fat) or MFAT (35% of energy

Fig. 5. Change in serum hormone and metabolite concentrations throughout the experimental trial. A significant diet \times time effect was found for serum triglycerides ($P = 0.04$), which by Bonferroni-corrected post hoc pairwise comparisons was due to significantly elevated triglyceride concentrations at Rec-1 ($*P = 0.028$) and Rec-2 ($*P = 0.024$) of the LFAT vs. the MFAT recovery diet. A significant effect for time was found for serum glucose, triglyceride, and free fatty acid concentrations, which were all elevated (above baseline) postexercise. ^a $P < 0.05$ vs. baseline; ^b $P < 0.05$ vs. postexercise; ^c $P < 0.05$ vs. 22 h postexercise; ^d $P < 0.05$ vs. 70 h postexercise by Bonferroni-corrected post hoc pairwise comparisons.

as fat) recovery diet. In this study of well trained female recreational runners, we found that IMCL was reduced by ~25% in soleus muscle after the endurance run and was dependent on dietary fat content for adequate recovery in the 3 days after the 2-h run. Consumption of the MFAT recovery diet allowed IMCL stores to return to baseline by 22 h (1 day) and to overshoot (vs. baseline) by 70 h (3 days) postexercise despite the continuation of a controlled training regimen. In contrast, consumption of a LFAT recovery diet did not allow IMCL stores to return to baseline, even by 70 h after the endurance run. In contrast, EMCL stores were not affected by either the 2-h run or the recovery diet.

With use of biochemical or stereological analysis of biopsy samples, previous studies have reported conflicting results concerning the use (and therefore reduction) in IMCL during endurance exercise. A number of studies have found ~20–40% reductions in IMCL during prolonged exercise at intensities between ~55 and 75% of $\dot{V}O_{2\max}$ (2, 4, 5, 8, 9, 17, 37) and even greater reductions of ~42–75% during marathon and ultraendurance skiing and running competitions (11, 32, 35, 45). Other studies, however, have not noted statistically significant reductions in IMCL content with endurance exercise lasting between 25 and 120 min (1, 19, 23, 25, 44, 50). The lack of an exercise-induced reduction in IMCL in the latter studies, however, may be related to the shorter exercise duration [1 h or less (1, 19)], exercise mode [one-legged exercises (23)], or exercise intensity [intervals to exhaustion (25)]. In all previous studies, the difficulty of accurately measuring IMCL from single biopsy samples, however, is extremely problematic (50). Although it is established that single biopsies provide reliable estimations of muscle glycogen content (50), glycogen is homogeneously distributed as small granules in the muscle fibers, and resting concentration in type I and II fibers differs by only 11–27% (42). IMCL, on the other hand, are stored in droplets that are not homogeneously distributed within the muscle fiber (10) and also vary considerably between type I and II fibers (8, 10, 32). Wendling et al. (50) recently reported that the CV for IMCL between multiple biopsies of the same site was quite high: ~20% at rest and ~26% after 90 min of cycling. Thus detection of significant exercise-induced differences in IMCL would be difficult, given that the error of the measurement is on the same order as the expected exercise-induced change. In addition, contamination by extramuscular and subcutaneous adipose tissue (which may be difficult to completely remove despite careful precautions) also creates error in IMCL content measured by biochemical analysis of biopsy samples (50). In the present article, we present an alternative method of measuring IMCL that is noninvasive, allows measurement of a larger area of active muscle (3 × 3 pixel box representing 2.25 ml of soleus muscle), and is considerably more reliable than standard biopsy procedures; i.e., the intraclass CV of our NMR SI technique is <7%, which may explain our ability to detect a significant exercise-induced change

on the order of 25%. Our results are in agreement with recent work by Krssak et al. (28), who found a significant (33.5%) reduction in IMCL measured by ^1H NMR spectroscopy in subjects running at 65–70% of $\dot{V}O_{2\max}$ until exhaustion.

Although the current study is one of the first to measure IMCL in response to exercise and recovery in women, our findings are in agreement with previous investigations in male runners. For example, in nine male distance runners, Costill et al. (6) found that IMCL concentration of the vastus lateralis was lowered by an average 31% after a 30-km cross-country race that was completed in 123–171 min. In 10 male marathoners, Staron et al. (45) found that the IMCL concentration of the lateral head of the gastrocnemius dropped by an average of 42% after completion of a marathon race. In the aforementioned NMR study, Krssak et al. (28) found a 33.5% reduction in IMCL measured in the soleus muscle of nine trained subjects performing 2–3 h of treadmill running at 65–70% of $\dot{V}O_{2\max}$. Our female runners completed an average distance of 20 km (15.7–23.8 km) during the 2-h treadmill run at a controlled pace and experienced an average 25% drop in IMCL content of soleus muscle.

Of interest in the current study (but not commonly reported in previous studies) was that the variability in the exercise-induced depletion in IMCL, which ranged from no change (one subject both trials) to almost a 40% fall from baseline, was correlated with both $\dot{V}O_{2\max}$ and work performed, even in this group of well trained runners ($r^2 = 0.62$). The relationship with $\dot{V}O_{2\max}$, however, may be explained by the one “nonresponder,” who had a lower than expected $\dot{V}O_{2\max}$ (40 ml·kg⁻¹·min⁻¹) despite years of endurance training and a decent weekly training schedule (>40 km/wk). This relationship would be expected in a more heterogeneously trained group, because endurance training is thought to increase IMCL utilization during prolonged exercise (17, 37). On the other hand, it is possible that slower, “back of the pack” endurance-trained runners also have higher proportions of type II fibers, which have a 2–3 times lower concentration of IMCL than type I fibers (8) and/or use running biomechanics, which does not recruit as much soleus muscle. In agreement with the former possibility, the IMCL content measured in this runner at all time points (except postexercise) was considerably lower than that of the other runners. On the other hand, the relationship between IMCL depletion and work (distance performed in 2-h) (Fig. 5), although somewhat dependent on $\dot{V}O_{2\max}$ (i.e., runners with higher $\dot{V}O_{2\max}$ are more likely to have higher running velocities at 65% $\dot{V}O_{2\max}$ and thus cover more distance in 2 h), is important and in support of the validity of our methods. Certainly, differences in aerobic power, training, muscle fiber composition, and running mechanics may explain some of the variation in IMCL depletion with endurance exercise and would be of interest in future investigations.

Although a number of studies have investigated the effect of exercise on IMCL concentrations (as previ-

ously discussed), very few have focused on the recovery of IMCL after exercise. Using stereological methods, Staron et al. (45) found that the volume percentage of lipid in the gastrocnemius, which was depleted by 41% during a marathon race, was not replenished even 7 days after the marathon run when subjects consumed a high-carbohydrate (50–60% carbohydrate, 23–30% fat) recovery diet. Interestingly, IMCL was even further depleted at 1 and 3 days postmarathon and was ~35% lower than baseline at 7 days postmarathon. Although Kiens and Richter (25) did not find that IMCL was reduced by glycogen-depleting bicycle exercise, they found that IMCL was reduced in the recovery period when subjects were fed a high carbohydrate recovery diet. IMCL was reduced 3 h postexercise and reached nadir level (22% lower than baseline) at 18 h postexercise (the time when muscle glycogen concentrations were restored close to baseline). In contrast, two recent NMR spectroscopy studies, by Boesch et al. (2) and Krssak et al. (28), found that IMCL content was recovered by 24–40 h postexercise. Boesch et al. (2) reported that the time constant of IMCL recovery was ~40 h in the tibialis posterior of one male subject who experienced a 40% fall in IMCL induced by 3 h of bicycle training. The diet composition, however, was self selected and not reported. A more controlled study by Krssak et al. (28) found that IMCL was nearly recovered 20 h after 2–3 h of exhaustive treadmill running at 67% $\dot{V}O_{2\max}$ and elicited a 34% decrease in IMCL content (from 1.37 to 0.91% of water resonance peak intensity). One discrepancy between these two studies, however, was that Boesch et al. (2) found that the recovery of IMCL was not initiated until the post-exercise meal was consumed, whereas Krssak et al. noted that IMCL recovery was initiated by 4 h postexercise, while their subjects were still fasting. The potential fluid shifts and muscle swelling that can occur after exercise make it difficult to interpret these results, because IMCL content in both studies was “quantified” or normalized in reference to the water peak.

In the current study, we used BML as the internal reference peak rather than water, because intra- and extracellular water compartments are likely to change during exercise and in recovery. BML could be measured simultaneously in our SIs and, at 1.5 Tesla, the T1 and T2 relaxation times of BML methylenes are found to be comparable to those of EMCL methylenes (40). Our results, however, are not dependent on the use of BML as the internal reference. We got exactly the same results when the raw (unnormalized) peak areas were analyzed (see METHODS). Although we did not directly measure whether the T1 and T2 relaxation times of BML were influenced by diet or exercise, our finding that bone marrow peak area within a subject did not vary considerably across all measurement points obtained under different exercise and dietary conditions suggests that any potential changes in T1 or T2 values are very small and inconsequential to the use of BML as the internal reference.

To our knowledge, the current study is one of only two that have investigated the importance of dietary fat in the postexercise recovery period. Using biopsy procedures, Starling et al. (44) found that IMCL concentration of the vastus lateralis was significantly higher 24 h postexercise when trained male cyclists consumed a high-fat (68% of energy from fat) vs. a very low-fat (5% of energy from fat) postexercise diet, even though IMCL concentration was only depleted by 6–11% after 2 h of cycling at 65% $\dot{V}O_{2\max}$. Similar to our findings, IMCL concentration was slightly lower than the postexercise measurement on the very low-fat diet and overshot baseline IMCL concentration when the high-fat diet was consumed (44). However, despite the replenishment of IMCL content, the high-fat diet compromised glycogen replacement and subsequent performance during a cycling-time trial 24 h later. In the current study, we found that IMCL returned to baseline ($99.9 \pm 5.3\%$ of baseline) when female runners were given a diet that contained more reasonable amounts of fat (35% of energy from fat), and overshot preexercise levels 3 days postexercise ($122.1 \pm 6.5\%$ baseline). In contrast, IMCL content did not recover even by 3 days postexercise ($87.5 \pm 6.6\%$ of baseline) when the runners consumed a recovery diet providing 10% of energy from fat. Our findings, however, are not in support of the conclusion of Kiens and Richter (25) that IMCL are important for providing fuel for muscle metabolism in the postexercise recovery period. Possible reasons for this difference may be the exercise protocol, which in our study was not designed to be glycogen depleting as it was in the Kiens and Richter study, and the gender of the subjects.

The ability to increase muscle triglyceride concentrations after a higher-fat diet in both our study and that of Starling et al. (44) may be linked to activity of lipoprotein lipase (LPL), which catalyzes the hydrolysis of triglyceride-rich lipoproteins in the capillary endothelium of both adipose tissue and skeletal muscle. Several investigators have demonstrated that LPL activity is increased by endurance activity (32, 47) and may be inhibited by consumption of a high carbohydrate, low-fat diet (18, 24, 30, 31). Increases in skeletal muscle LPL correlate with a higher fractional removal rate of triglycerides (31), reduced very low density lipoproteins, and serum triglycerides (30, 31) and elevated muscle triglyceride concentrations (24). The specific mechanism for these findings, however, is not known but thought to be related to differences in postprandial (but not necessarily fasting) insulin concentrations (31). Thus possible differences in LPL activity could also explain, at least in part, why serum triglycerides were increased on the LFAT diet (from 84.1 ± 46.5 to 65.7 ± 36.6 mg/dl) and decreased in the MFAT diet (from 80.4 ± 45.9 to 91.1 ± 48.7 mg/dl) compared with the baseline diet. An alternative explanation for the elevated triglycerides is that synthesis of free fatty acid and triglyceride in liver is increased on the high-carbohydrate low-fat diet.

Finally, our results with respect to EMCL may be of interest to the question of whether EMCL can serve as

a source of lipid oxidation during exercise. Whereas our data seem to suggest that EMCL are not mobilized, the variability of EMCL measurements by NMR has been shown to be quite high (>19%) (unpublished observations, 46), which may be due to both physiological variation (inhomogeneous storage of bulk fat adiposites) and methodological error (EMCL stored in fat layers are sensitive to bulk magnetic susceptibility) (2). In light of the error associated with NMR measurement of EMCL, many investigators do not report EMCL data (28) or mention it only in passing (2). Because of this, our finding that EMCL was significantly higher 22 h after exercise needs to be interpreted with caution.

Results from the current study provide evidence that a certain quantity of dietary fat will facilitate replacement of IMCL after endurance running and may therefore be important to endurance athletes in heavy training. These findings are of interest in light of the observation that more and more athletes, particularly female athletes, are attempting to follow extremely low-fat diets with the belief that consumption of even a small amount of fat leads to decreased performance or body weight gain. However, because our studies were conducted under eucaloric conditions, the time course of IMCL recovery, especially on the low-fat diet, may be expected to be even slower than that reported in the current study, because male and female runners on low-fat ad libitum diets often consume inadequate energy (16). The significance of compromised IMCL stores as a result of heavy endurance training or consumption of a very low-fat diet needs to be determined. The current study provides additional evidence that diets too low in fat are probably not ideal for endurance athletes. In particular, our results suggest that if IMCL are to be replenished after endurance running, diets containing more moderate (but not unreasonable) amounts of fat should be consumed. Future studies should focus on determining the diet composition that optimizes both muscle glycogen and muscle triglyceride stores after endurance exercise.

We thank Nancy Davis and Katja Lerew for their invaluable assistance with data collection, Betty Darnel and the metabolic kitchen staff of the University of Alabama at Birmingham General Clinical Research Center (GCRC) for assistance with the baseline and experimental diets, and Dr. Barbra Gower and Kangmei Ren of the GCRC Core Laboratory for hormone and blood lipid analyses. We also thank the volunteers.

This research was supported in part by GCRC no. M01-RR-00032, National Center for Research Resources no. PO1-RR-11811, a pilot grant from the University of Alabama at Birmingham Center for Nuclear Imaging Research and Development, and unrestricted funds from Bristol-Meyers-Squibb.

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