Intramyocellular triacylglycerol in prolonged cycling with high- and low-carbohydrate availability


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The effects of such dietary CHO-induced manipulations in IMCL content on subsequent exercise metabolism, in particular IMCL metabolism, have not been explored. A high-CHO diet is known to reduce the contribution of “whole body” lipid to metabolism during exercise when compared with a moderate- or low-CHO diet (16, 43). The reductions of IMCL previously observed by $^1$H-MRS during exercise occurred after high-cholesterol CHO intakes (18, 26, 28). Thus the contribution of IMCL to exercise metabolism in these studies may have been, in part, curbed by a high-CHO availability preceding exercise.

It was hypothesized that prior exercise followed by a low-CHO intake would 1) increase the storage of IMCL and 2) accentuate the reductions in IMCL and its contribution to exercise metabolism during a subsequent prolonged, self-paced strenuous cycling bout. To test these hypotheses, subjects were 1) exposed to a high- or very low-cholesterol CHO intake to potentially induce differences in IMCL content and 2) required to complete a bout of prolonged “time trial” cycling exercise under these conditions. The selected time trial protocol was designed to simulate competition conditions in highly trained endurance athletes, who have been suggested to have an enhanced ability to store and utilize IMCL. IMCL was measured before and after exercise by $^1$H-MRS.

METHODS

To assess the effect of high- vs. low-cholesterol CHO intake on resting vastus lateralis IMCL content and subsequent utilization during prolonged time trial cycling bouts, each subject completed a double-blind, randomized, crossover design of two experimental trials separated by at least 7 days.

Subjects

Six highly trained male cyclists (maximal $\dot{O}_2$ consumption ($V_{O2max}$) 72.5 ± 6.5 ml·kg$^{-1}$·min$^{-1}$, body mass 74.1 ± 6.0 kg, age 34 ± 5.4 yr) volunteered for this study. All participants were informed before the experiment that the purpose of the study was to compare endurance cycling performance after CHO loading with two different formulations of liquid CHO. Participants were informed of potential risks and provided written consent to participate. The study was approved by the Human Research Ethics Committee of The University of Sydney.

Assessment of Exercise Capacity

$V_{O2max}$ during cycling exercise was determined in each subject 1 wk before the subject’s first endurance exercise trial. All subjects performed this test on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands). The test required the subject to cycle at four submaximal “steady-state” power outputs, followed by an incremental increase in power (45 W/min) until volitional fatigue.Expired air was collected via a Hans Rudolph pneumotachograph to measure expired volume.Expired air entered a mixing chamber and was analyzed for expired fraction of $O_2$ and $CO_2$ by using a zirconium cell-based $O_2$ (Ametek S-3A/1, Pittsburgh, PA) and $CO_2$ (Ametek CD-3A) sensor. Gas analyzers and flow transducer were connected via an analog-to-digital converter (Exterstress). Both analyzers were calibrated immediately before each test by using gases of known composition. The flow transducer was calibrated immediately before each test with a 3-liter calibration syringe. Standard temperature pressure dry (STPD) values for minute ventilation were calculated according to corrected barometric pressure and temperature. $V_{O2max}$ was defined as the average $O_2$ consumption ($V_{O2}$) during the final 60 s of exercise, sampled each 10 s. All subjects experienced a plateau in $V_{O2}$ during the final 30 s.

Pretrial Exercise and Dietary Manipulation

Before each experimental trial, subjects undertook a bout of strenuous exercise, similar to a previously reported cycling regime that had been shown to significantly reduce muscle glycogen stores (27). Subjects performed 2-min bouts at 100% $V_{O2max}$ and progressively decreased this intensity by 5% increments (95, 90, 85, 80, 75, and 70%) when a pedaling frequency of >60 rpm could not be maintained. Two-minute intervals were interspersed with 3-min “recovery” efforts at a power output of 50% $V_{O2max}$. The test was terminated when the subject could not maintain 2 min at 70% $V_{O2max}$. Subjects were supplied with water ad libitum during this bout. After this strenuous exercise bout, subjects were randomly assigned to a high-CHO (HC) (9 g·kg$^{-1}$·day$^{-1}$) or low-CHO (LC) (0.3 g·kg$^{-1}$·day$^{-1}$) treatment for the following 48 h. In both treatments, each subject performed 30 min of low-intensity cycling on the morning after (24 h) the pretrial exercise bout, and each was instructed to avoid all other forms of exercise. This 30-min bout was restricted to 60–70% of maximal heart rate. To adequately blind the subjects to the nature of their CHO status, in both treatments subjects were instructed to minimize dietary CHO but to consume fat and protein ad libitum during this time. To boost CHO intake in the HC treatment, subjects were given a tasteless maltodextrin powder (9 g·kg$^{-1}$·day$^{-1}$) dissolved in 7.5 liters of water. Subjects allocated to the LC arm were given the same volume of a sweetened (Nutrasweet) placebo beverage. The same amount of flavored protein powder was added to both beverages to ensure that their taste was identical. Beverage consumption commenced immediately after the pretrial strenuous exercise bout. Confirmation of subject compliance with this dietary regime, in addition to dietary fat and protein intake, was assessed by using weighed food diaries, which were subsequently analyzed via Foodworks (Xyris Software, Highgate Hill, Australia). Subjects were blinded to the study aim of investigating the effect of CHO manipulation on performance during exercise. This strategy was undertaken because many athletes are now aware of the potential performance benefits associated with a high-CHO intake (8), and it was believed that knowledge of lowered CHO status might reduce the expectation of optimal performance. Similarly, controlling for fat consumption by boosting fat intake in the LC condition could alert the subject to the nature of the dietary manipulation. Importantly, the study aimed to induce contrasts in IMCL content preceding exercise, and the weight of evidence suggests that this can be achieved by lowering CHO intake in contrast to a high-CHO diet, regardless of the level of fat intake. Subjects were instructed that the preexercise CHO manipulation was used to investigate the effect of the type of CHO drink being administered for CHO loading.

Experimental Time Trial Protocol

The experimental time trials were performed at the same time of day after 2 days of high- or low-cholesterol CHO intake, with no less than 10 h of overnight fasting. On presenting for the test, subjects voided and were weighed (model HW-
100K1, Berkel Avery, Sydney, Australia) to the nearest 10 g, while wearing only bicycle shorts. Postexercise weight was measured immediately on cessation of exercise. In both experimental conditions, after performing a 10-min warm-up restricted to <60% of maximal heart rate, each individual was required to complete a fixed amount of external work, equal to that required for the individual to perform 3 h cycling at 70% VO2 max (average 2,756 ± 294 kJ). This work requirement was the same for each individual in HC and LC conditions and was completed in the fastest time possible. The time trials were conducted on a wind-braked cycle ergometer, fitted with adjustable gearing (as on normal road bicycles) with power and kilojoules measured via a SRM system (Schoberer Radmesstechnik, Konigskamp, Germany). During both trials, subjects ingested a 7 g/100 ml glucose polymer solution at a rate of 15 ml·kg body mass⁻¹ ·h⁻¹ (∼1 g CHO·kg⁻¹·min⁻¹) (7) to minimize the possibility of hypoglycemia. Subjects remained fasted from exercise cessation until postexercise IMCL determination had been completed, during which time they were allowed to consume water ad libitum. Expired gas was collected continuously during the first 15 min and then for 5-min periods during exercise (30, 60, 90, 120, and 150 min) and the final 100 kJ of each trial, as per the previously outlined method. V02, CO2 production, and respiratory exchange ratio (RER) data were averaged and recorded every 30 s. Blood samples were collected via an indwelling venous catheter in the final minute of the expired gas sampling periods. At each sample time, 1 ml of collected blood was maintained on ice in a sodium fluoride Minicollect vacuette for plasma free fatty acid (FFA) and glycerol determination.

**IMCL Concentration Determination**

Approximately 1 h before the commencement of each experimental trial and between 60 and 80 min after exercise, vastus lateralis IMCL content was measured by using 1H-MRS. High-resolution longitudinal relaxation time (T1)-weighted imaging and image-guided, localized 1H-MRS were performed on a 1.5-T Gyroscan NT whole body system (Philips Medical Systems, Best, The Netherlands) by using a combination of whole-body and circular polarized standard extremity coils for radio frequency signal transmitting and receiving. The delayed time for postexercise assessment was selected to allow sufficient time for possible exercise effects on transverse relaxation times (T2) to be minimized while minimizing any time-related effects on further replenishment or degradation of the IMCL pool. Volumes of interest were centered within the vastus lateralis muscle at the level of midfemur, 3 cm anteromedial to the axillary line. This position was chosen to avoid vasculature, minimize inclusion of subcutaneous adipose depots, and ensure consistent orientation of the muscle fibers along the main magnetic field. Legs were fixed at the ankles, and the subjects were instructed to lie as still as possible to prevent movement artifacts. A vitamin E capsule was taped to the skin to identify the area of interest on the magnetic resonance images. During the initial scan, the leg was marked with indelible ink, and the mark was maintained until the second scan to ensure accurate repositioning of the 1H-MRS voxel to minimize systematic errors in IMCL determination between measures, within subject (21). Image-guided spectra were acquired by using the PRESS technique (repetition time = 5,000 ms, echo time = 32 ms, 32 measurements, 1,024 sample points, acquisition time 3 min). Fully automated shimming was carried out on a 5 × 1.5 × 1.5-cm voxel (5 × 2.0 × 1.5 cm in 1 sample) to ensure maximum field homogeneity. Excitation water suppression was used to suppress the water signal during data acquisition. The long repetition time was chosen to ensure a fully relaxed signal minimizing T1 saturation effects. Unsuppressed water spectra were also acquired for use as an internal standard.

**Spectral Data Processing**

Spectral data were transferred off-line for postprocessing with magnetic resonance user interface software jMRUI version 1.1, EU Project “Advanced Signal Processing for Medical Magnetic Resonance Imaging and Spectroscopy,” TMR, FMRX-CT97-0160 (32). After Fourier transformation and manual phasing of the spectra, the creatine-CH3 peak was identified and nominated at 3.02 parts/million (ppm). For the water-suppressed signal, the following steps were taken: 1) A 10-resonance model was used including IMCL (CH3: 0.88 ppm), EMCL (CH3: 1.19 ppm), IMCL -(CH2)n: 1.3 ppm), EMCL -(CH2)n: 1.51 ppm), 1-acetate (2.09 ppm), lipid (C2 methylene: 2.24 ppm), total creatine (CH3: 3.02 ppm), trimethylacetate group (3.2 ppm), taurine (N-CH3: 3.37 ppm), and residual water signal (4.69 ppm). 2) The signal amplitude was obtained in absolute units for each resonance by using AMARES, a nonlinear least squares quantitation algorithm. Prior knowledge applied incorporated known factors described in prior publications (34). Fitting of the lipid resonances at 2.24 ppm, 1-acetate at 2.09 ppm, and taurine at 3.37 ppm also allowed more reliable fitting of IMCL and EMCL peaks. The resonances were fitted assuming a Lorentzian line shape for creatine, trimethylacetate group, and taurine and a Gaussian line shape for all other resonances. The zero-order and first-order phase correction was manually estimated.

The unsuppressed water signal was calculated as follows: 1) the water resonance was identified at center frequency and nominated at 4.69 ppm, and 2) the signal amplitude was obtained in absolute units by using AMARES. The resonance was fitted to a Lorentzian line shape. As for the other resonances in the water-suppressed signal, zero-order and first-order phase correction was manually estimated.

**Absolute Quantification of Metabolites**

All signal amplitudes were corrected for coil loading and voxel volume. Signal amplitude of water, creatine-CH3, EMCL-(CH2)n, and IMCL-(CH2)n, were corrected by using appropriate T1 relaxation times available in the literature for leg skeletal muscle (6, 36) and the T2 relaxation times obtained under similar study conditions (39). The -(CH2)n resonance was used for measurement of the intramuscular triglyceride content given the higher signal intensity and narrower line width compared with the CH3 resonance (40). Absolute concentrations of IMCL and total creatine were also calculated in millimoles per kilogram wet weight by using the internal reference of muscle water concentration.

Total creatine was calculated as follows (5): 1) total creatine CH3 signal measured at 3.02 ppm resonance and unsuppressed water resonance measured at 4.69 ppm were corrected for T1 and T2 relaxation effects, 2) corrected signal amplitude was expressed as the ratio of total creatine to unsuppressed water, 3) the ratio was corrected for number of protons contributing to signal (3 for total creatine and 2 for water), and 4) this value was converted to units of millimoles per kilogram of wet weight assuming a water concentration of 55 mmol/kg wet weight and a tissue water fraction of 0.81 (40).

IMCL and EMCL concentrations were similarly calculated by using muscle water as the internal reference with the
added considerations of 1) IMCL structure similar to trioleate (61.0 mmol of \(^{1}H/m\)l triglyceride) and 2) tissue water fraction of 0.81 and tissue density of 1.05 (40).

Although prior knowledge in the use of the MRUI peak-picking routine is intended to separate IMCL-(CH\(_2\))\(_n\) and EMCL-(CH\(_2\)\(_n\)) content, the presence of EMCL within the volume of interest can cross-contaminate the IMCL signal, reducing the precision of IMCL estimation. Careful placement of the voxel entirely within the muscle ensured exclusion of subcutaneous adipose tissue.

IMCL values were then expressed as the ratio of IMCL to water (IMCL/H\(_2\)O), IMCL concentration, and the ratio of IMCL to creatine concentration.

**Blood Analysis**

Plasma FFA concentration was determined by using a Wako NEFA C test kit (Wako Chemical), scaled down for the microplate. Whole blood glycerol determination was performed as described by Boobis and Maughan (4). All blood biochemical measurements were made in duplicate.

**Statistics**

Average values for VO\(_2\) and whole body lipid utilization assuming negligible protein contribution were calculated from the area under the VO\(_2\) and RER curves of each individual's measurements over time (14). These values, along with pretrial exercise time, time trial time, nutritional intake, relative reduction in IMCL/H\(_2\)O between conditions, pre- and postexercise IMCL/H\(_2\)O, and absolute concentration of IMCL pre- and postexercise were compared by paired Student's \(t\)-tests. Differences in FFA and glycerol during exercise were compared via two-way repeated-measures analysis of variance (diet \(\times\) time), with significance at each time point confirmed by paired Student's \(t\)-tests. Pearson's correlation coefficients were used to test the simple relationship between absolute resting IMCL/H\(_2\)O content and dietary fat and CHO intake. Statistical significance was accepted at \(P < 0.05\). All calculations were performed by using SPSS for Windows version 10. All values are expressed as means \(\pm\) SD.

**RESULTS**

**Pretrial Exercise And Dietary Manipulation**

On average, subjects performed 98.2 \(\pm\) 16.8 and 100.2 \(\pm\) 12.6 min of cycling when undertaking the strenuous exercise regime before the HC and LC trials, respectively. No difference in cycling time during these bouts was evident between conditions (\(P > 0.05\)). Food diary analysis showed that subjects had ingested minimal CHO (excluding the HC CHO-supplemented treatment) during the 48 h preceding both performance trials (Table 1). Energy derived from CHO, fat, and protein comprised 63.0, 19.0, and 17.3% vs. 5.7, 56.3, and 37.0% in the HC and LC treatments, respectively.

CHO intake in the HC treatment was significantly greater than the LC treatment. Although large individual differences were evident, dietary fat consumption was significantly higher in the LC compared with the HC treatment. Although tending to be higher in the LC vs. HC treatment, no significant difference existed in daily protein consumption (\(P > 0.05\)). Similarly, daily energy intake tended to be lower in the LC vs. HC treatment, but this did not reach statistical significance (\(P > 0.05\)).

**Time Trial Cycling Time**

Average time to complete the time trial was significantly shorter in the HC trial (177.5 \(\pm\) 11.1 min) compared with the LC trial (200.1 \(\pm\) 21.7 min; \(P < 0.05\)). Three subjects were able to maintain similar performance in both conditions, performing on average 6 min faster in the HC compared with LC condition. The remaining three subjects performed substantially faster (average 39 min) in the HC compared with LC trial.

**Water signal.** Mean corrected unsuppressed water signal did not change significantly during exercise in the HC condition (5.85 \(\times\) 10\(^{-2}\) \(\pm\) 1.24 \(\times\) 10\(^{-2}\) vs. 5.27 \(\times\) 10\(^{-2}\) \(\pm\) 1.15 \(\times\) 10\(^{-2}\) pre- and postexercise, respectively; \(P = 0.18\) ) or in the LC condition (6.07 \(\times\) 10\(^{-2}\) \(\pm\) 1.53 \(\times\) 10\(^{-2}\) vs. 5.85 \(\times\) 10\(^{-2}\) \(\pm\) 1.43 \(\times\) 10\(^{-2}\) pre- and postexercise, respectively; \(P = 0.20\) ). There was no significant difference in mean corrected unsuppressed water signal between conditions preexercise (\(P = 0.71\) ) and postexercise (\(P = 0.30\) ). Similarly, no significant change was evident in creatine during exercise in the HC condition (20.92 \(\pm\) 11.76 vs. 29.41 \(\pm\) 9.38 mmol/kg wet wt pre- and postexercise, respectively; \(P = 0.14\) ) or in the LC condition (25.94 \(\pm\) 6.94 vs. 24.85 \(\pm\) 11.01 mmol/kg wet wt pre- and postexercise, respectively; \(P = 0.79\) ). This ensured a valid basis for IMCL concentration calculation because there was no demonstrable change in water concentration during all phases of the study.

**IMCL.** Vastus lateralis IMCL concentration before exercise was significantly higher in the LC vs. HC condition (\(P < 0.01\) ). Calculated absolute IMCL content was 10.71 \(\pm\) 3.06 and 7.02 \(\pm\) 2.87 mmol/kg wet wt in the LC and HC conditions, respectively (\(P < 0.01\) ). IMCL concentration was significantly reduced in both conditions postexercise (\(P < 0.01\) ). No significant difference in IMCL concentration was evident between conditions postexercise (\(P = 0.41\); Fig. 1). Thus mean IMCL degradation during exercise was significantly greater in the LC vs. HC condition (6.63 \(\pm\) 1.08 and 3.63 \(\pm\) 1.80 mmol/kg wet wt, respectively). IMCL concentration had decreased after exercise by \(\sim\)64 and \(\sim\)57% in the LC and HC conditions, respectively. The relative reduction in IMCL concentration was not different between conditions (\(P = 0.51\) ). Four subjects

### Table 1. Dietary intake during 48 h before experimental trials

<table>
<thead>
<tr>
<th></th>
<th>CHO, g kg(^{-1}) day(^{-1})</th>
<th>Fat, g kg(^{-1}) day(^{-1})</th>
<th>Protein, g kg(^{-1}) day(^{-1})</th>
<th>Energy, kJ kg(^{-1}) day(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>9.52 (\pm) 0.08</td>
<td>1.24 (\pm) 0.47</td>
<td>2.47 (\pm) 0.49</td>
<td>239.15 (\pm) 24.75</td>
</tr>
<tr>
<td>LC</td>
<td>0.59 (\pm) 0.21*</td>
<td>2.90 (\pm) 1.76†</td>
<td>3.49 (\pm) 1.57</td>
<td>179.98 (\pm) 92.00</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SD for 6 subjects. CHO, carbohydrate; HC, high CHO; LC, low CHO. *Significantly different vs. HC diet, \(P < 0.01\). †Significantly different vs. HC diet, \(P < 0.05\).
showed reductions of IMCL/H₂O in excess of 70% during exercise.

**Respiratory Gas Analysis**

Subjects averaged 71 ± 6 and 66 ± 8% VO₂max in the HC and LC trials, respectively. However, this difference did not reach significance (P = 0.06). RER was significantly lower at all points in time during exercise in the LC vs. HC condition (P < 0.01) (Fig. 2). Average rates of nonprotein whole body lipid metabolism were significantly higher in the LC (1.59 g/min) vs. HC (0.93 g/min) condition (P < 0.01).

**Blood Analysis**

Plasma FFA and glycerol kinetics during exercise are presented for five subjects because all samples could not be obtained from one subject. Plasma FFA content was significantly higher at rest and throughout exercise in the LC vs. HC condition (P < 0.01) (Fig. 3). After a significant decrease in both trials from rest to 15 and 30 min of exercise, FFA concentration increased continually in both conditions, becoming similar to resting level at 60 min. Although increasing thereafter, FFA concentration was not significantly greater than rest at the end of exercise in both trials (P > 0.05). Whole blood glycerol concentration was not different between conditions at rest. Glycerol levels increased significantly in both conditions during exercise (P < 0.01) and were higher during the first 150 min in the LC vs. HC condition. After 2 h of exercise in the LC condition, there was a decrease in glycerol concentration that was not evident in the HC condition (P < 0.05). This decline reflected the reduction in exercise intensity after ~2 h of three of six subjects who were substantially slower in the LC compared with HC condition.

**Fluid Replacement and Body Weight**

Average weight loss during both trials accounted for <1% of preexercise body weight, indicating that the fluid replacement regime during the trials was successful. Body weight was significantly higher in the HC vs. LC condition before exercise (P < 0.05), but no difference was evident between these conditions after exercise (P > 0.05). No difference in weight loss relative to preexercise weight was evident during exercise between conditions (P > 0.05).

**DISCUSSION**

Howald et al. (21) have recently demonstrated a high correlation (r = 0.93) between IMCL content derived by 1H-MRS and electron microscopic morphometry after 2 h of exercise, supporting the validity of this methodology for use in the design of the present study. Significantly, the results reported in the present study accurately reflect the contrasts between treatment groups and with exercise because the major findings...
were an ~83% increase in IMCL/H2O after low-dietary CHO intake and no significant difference between the LC and HC conditions after exercise. Thus these results, and therefore the greater net IMCL utilization evident in the LC condition, exist independent of any potential confounding changes in T2 with exercise, because they are expressed between conditions before and after exercise bouts of identical external work. Furthermore, these results were not significantly different from those obtained with creatine as an internal reference to IMCL (IMCL/creatine were therefore not reported).

It is unclear whether the ~1-h elapse of time between exercise cessation and IMCL determination in the present study may have affected the observed changes in IMCL. However, this time was believed necessary to ensure minimal effects of exercise on T2. Reports on the kinetics of IMCL after exercise are conflicting; however, no study has reported significant changes in IMCL from cessation to 60–80 min postexercise. Although Krassak et al. (26) showed partial replenishment of IMCL after 5 h of fasting after exercise, the magnitude of change in IMCL during this ~1-h period in the present study, if any, is likely to be negligible relative to the changes in IMCL noted during exercise.

The present study showed that when dietary CHO intake was curtailed during the 48 h after a strenuous exercise bout, resting vastus lateralis IMCL content of highly trained endurance athletes was significantly elevated above levels obtained with a CHO-loaded diet. Significant reductions of IMCL during time trial cycling in both HC and LC conditions suggested that lipid derived from IMCL stores contributed to whole body lipid metabolism during exercise in conditions of both high- and low-CHO availability. The substantially greater absolute quantity of IMCL degraded during exercise in the LC compared with the HC condition suggested that the higher rates of whole body lipid utilization in this condition were in part attributable to a greater IMCL utilization. To our knowledge, this is the first study to report near depletion (73–85% in 4 subjects) of vastus lateralis IMCL during prolonged cycling exercise. This suggests that intramuscular lipid stores, presumably the triacylglycerol component, may, like glycogen, be exhausted by prolonged strenuous exercise. This finding may largely reflect depletion of the more oxidative muscle fibers, because little or no change in triacylglycerol content of less oxidative fibers is evident in rodents (38) and humans (29) as a result of prolonged exercise. The observation of near depletion of IMCL in the present study may be explained by the training status of the subjects recruited and/or the prolonged, intense exercise protocol employed. We considered exercise of this nature to best simulate competition conditions.

IMCL was elevated in each subject after low- vs. high-dietary CHO. This contrast was evident despite the ad libitum dietary fat intake employed and the subsequent large individual variations in fat consumption. No correlation was evident between dietary fat intake and preexercise vastus lateralis IMCL content in either experimental condition. Elevation of FFA concentration, whether a function of a high-fat diet (12) or fasting (26, 39), results in replenishment or elevations of IMCL content. Thus CHO intake rather than fat intake may largely dictate the replenishment of IMCL. As previously noted by other researchers (3, 13, 31), large individual differences were evident in IMCL content. That is, preexercise IMCL was highly correlated within individuals between conditions (r = 0.88; P = 0.02), such that individuals who displayed the highest IMCL content in the HC condition also did so in the LC condition, largely irrespective of dietary fat intake. Thus there appears to be some inherent factor that potentiates IMCL accumulation, possibly muscle fiber type. Indeed, Essen et al. (13) observed greater concentrations of IMCL in more oxidative muscle fibers by needle biopsy. These results suggest that dietary CHO availability may largely dictate the replenishment of IMCL after exercise, but inherent factors perhaps determine the potential storage capacity of this substrate. However, the ad libitum dietary fat intake employed in the present study to blind the subjects to the nature of their CHO status, coupled with the small sample size, may reduce the ability to explain these variations. Further research controlling for dietary fat intake is required to confirm this finding.

The extreme dietary manipulations utilized in the present study induced marked contrasts in whole body substrate utilization during exercise. The HC treatment induced a predominant reliance on CHO during subsequent exercise, in contrast to the predominance of lipid metabolism after the LC treatment. The approximately twofold greater IMCL utilization during exercise in the LC condition compared with the HC condition is suggestive of a major contribution of this substrate to the elevated whole body lipid utilization observed in this trial. However, because of reesterification of fatty acids that may occur within the muscle cell during exercise, it is not possible to partition the relative contributions of blood-borne FFAs and IMCL to this substrate mix. Lysis of a triglyceride molecule results in the extrication of three fatty acid chains for every glycerol molecule. Because glycerol released from adipose tissue lipolysis cannot be reesterified within adipose tissue, the plasma concentration of glycerol is believed to be an indicator of lipolytic rate within the adipose tissue (23), whereas the resulting plasma concentration of FFAs represents the net effect of fatty acid uptake and lipolysis by active tissue. The elevated blood FFA and glycerol concentrations during exercise reported in the present study suggest an increased rate of whole body lipolysis in the LC vs. HC condition.

We suggested that reductions in IMCL observed during exercise with the use of 1H-MRS may have been curbed by the high-dietary CHO intake used before exercise in these studies (18, 26, 28). Accordingly, in the present study, low-CHO availability resulted in a significantly greater IMCL utilization during exercise than high-CHO availability. However, IMCL was utilized in both conditions, such that the relative
exercise-induced decrease in IMCL was not statistically different between conditions. A slight but insignificant disparity in relative IMCL degradation between conditions may reflect the uncontrolled exercise time employed in the present study. Such variability in cycling time is an inherent confounder of studies using self-paced performance cycling, which requires a fixed volume of work to be completed in the fastest time possible.

The factors regulating the use of IMCL during exercise are not well understood. Metabolism of lipid during exercise is believed to be affected by its availability, glycogen availability, and hormonal milieu (19). Rodent studies have demonstrated glycogen mobilization preceding muscle triglyceride utilization during exercise (38), with the authors speculating that a "shortage of available carbohyd-rates" was the major determinant of increased muscle triglyceride use. However, in the present study, similar relative reductions in IMCL were observed in the LC and HC conditions, with a significantly higher absolute IMCL utilization in the LC vs. HC condition. Exogenous CHO was equal at all times during both conditions. Although muscle glycogen content was not measured, it is likely that the exercise and dietary manipulations resulted in a significant contrast in preexercise muscle glycogen availability between conditions. The pretrial exercise protocol employed in the present study has been shown to reduce vastus lateralis glycogen content to low levels (27). Furthermore, it has been demonstrated that low-CHO intake after such strenuous exercise attenuates glycogen repletion after 48 h, when compared with an unrestricted (41) or high-CHO intake (16, 43). Therefore, the findings of the present study suggest that IMCL degradation may largely be a function of exercise dose and IMCL availability. Indeed, Standl (37) reported a significant, albeit modest, correlation (r = 0.68) between resting vastus lateralis triglyceride, as measured by needle biopsy, and its degradation during exercise in control and diabetic subjects. Although the present study has allowed an understanding of the role of IMCL in prolonged time trial cycling, further investigation, using a controlled exercise dose, is required to determine the effect of IMCL content on its utilization during subsequent exercise in highly trained athletes.

In conclusion, prior exercise and a subsequent low-dietary CHO intake allowed a greater storage and absolute degradation of IMCL during prolonged time trial cycling than did a high-CHO diet. Near depletion of IMCL during exercise was evident in some athletes, indicating that intramuscular triacylglycerol stores may be exhausted by prolonged strenuous cycling exercise.

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