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No attenuation of gastric distress or benefit to performance with adaptation to octanoate-rich esterified oils in female cyclists

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Running title: MCFA adaptation and performance in females

Abstract

We investigated the effects of modifying a normal dietary fatty-acid composition and the ingestion of high-fat exercise supplements on gastrointestinal distress, substrate oxidation, and endurance cycling performance. Five female cyclists completed a randomized triple-crossover comprising a two-week diet high in octanoate-rich esterified oil (MCFA), or twice, long-chain fatty acids (LCFA). Following the diets subjects performed 3-h of cycling at 50% peak power followed by ten maximal sprints while ingesting either a) a carbohydrate (CHO)+MCFA-rich oil emulsion after the two-week MCFA-rich dietary condition (MC-MC, *Intervention*) and b) after one LCFA-rich dietary condition (LC-MC, *Placebo*), or c) CHO only following a second LCFA-rich diet (LC-CHO, *Control*). Ingestion of the CHO+MCFA-rich oil emulsion during 3-h steady-state exercise substantially reduced endogenous-fat oxidation by 88% (90% CI: 59–117%) and 74% (40–108%) in the MC-MC and LC-MC conditions, respectively, relative to LC-CHO, but there was no effect of MCFA adaptation. Adaptation (MC-MC) also had no clear effect on endogenous CHO (11%, -10–33%) or exogenous octanoic-acid oxidation rates (-2%, -20–16%), relative to the placebo (LC-MC). Ingestion/oxidation efficiency of octanoic acid was high (0.98), but the CHO+MCFA-rich oil emulsion caused mild-moderate gastrointestinal distress during exercise and there was no attenuating effect of MCFA adaptation evident, nor any clear benefit of the oil emulsion or adaptation to performance. Plasma triacylglycerol concentrations were lower by 17% (23– -4%) and 15% (20– -3%; reduction likely) in the LC-MC and MC-MC conditions, respectively, relative to LC-CHO. Total plasma free-fatty acid concentrations were 20% lower (39–3%) in the LC-MC condition, relative to LC-CHO. Plasma glycerol concentrations trended lower in the LC-

MC and MC-MC conditions, but uncertainty meant these effects were qualified unclear. There was no clear effect of MCFA adaptation on total and ^{13}C-enriched plasma octanoic-acid concentrations, nor were there any detectable elongation products (^{13}C-enriched fatty acids >8:0) seen in the plasma in either the MC-MC or LC-MC conditions To conclude, despite high efficiency of exogenous-MCFA oxidation, no clear benefit to prolonged-endurance performance was observed with the ingestion of a CHO+MCFA-rich esterified oil emulsion or following adaptation to a MCFA-rich diet in female cyclists.

Key words: medium-chain fatty acids, structured triacylglycerols, dietary adaptation, supplementation

Introduction

The finite availability of endogenous glycogen as an energy substrate contributes to the development of fatigue, and is therefore a limiting factor in athletic performance during prolonged strenuous exercise (Kiens and Helge 1998). An increase in plasma free fatty acid (FFA) and fat oxidation has been shown to spare endogenous glycogen, most reliably via fat infusion studies (Vukovich, Costill et al. 1993). However, fat infusion during racing conditions is not feasible and long chain triglyceride ingestion before or during exercise has not been shown to enhance performance (Rowlands and Hopkins 2002). More recently, interest has focused on the ingestion of medium-chain triglycerides (MCTs). Medium-chain fatty acids (MCFA) can be absorbed more rapidly and directly from the intestinal lumen, and their entry into mitochondria does not appear to be rate-limited by the acyl-carnitine transfer system to the extent of long chain fatty acids (LCFA) during high-intensity exercise (Greenberger, Rodgers et al. 1966; Sidossis, Gastaldelli et al. 1997). Therefore, ingestion of MCFAs during exercise has the potential to enhance performance by elevating plasma FFA concentrations and sparing muscle glycogen (Van Zyl, Lambert et al. 1996; VanZyl, Lambert et al. 1996; Vistisen, Nybo et al. 2003).

Previously, studies have focused on the effects of ingesting MCFA-rich exercise supplements on performance in males only. In trials feeding 25-30 g MCT suspensions, the oxidation of MCFA reached 30% of the ingested quantity compared with 70% oxidation when co-ingested with carbohydrate (CHO). Plasma FFA and ketone concentrations were elevated, but no overall change in total lipid or CHO oxidation was observed (Jeukendrup, Saris et al. 1995; Jeukendrup, Saris et al. 1996). In contrast, 86 g MCT co-ingested with CHO has resulted in elevation of serum FFA concentration, reduced reliance on

endogenous CHO, and performance enhancement (2.5% decrease in 40-km time-trial time) relative to CHO-only ingestion with no reports of gastrointestinal distress (Vistisen, Nybo et al. 2003). Conversely, Jeukendrup et al. (1998) and Goedecke et al. (2005); (1999) did not observe performance benefits when feeding up to 85 g of MCT co-ingested with CHO during endurance and ultra-endurance exercise, which was related to the development of moderate-to-severe gastrointestinal distress. Additionally, no clear differences between fat and CHO oxidation with or without MCT ingestion were observed in these later studies.

Chronic dietary MCT adaptation (Misell, Lagomarcino et al. 2001; Misell, Lagomarcino et al. 2001; Oopik, Timpmann et al. 2001) and ingestion of structured triacylglycerols (Vistisen, Nybo et al. 2003) have been investigated as ways to attenuate gastric distress when ingesting MCTs during exercise. Though gastric distress was alleviated in these studies, no performance benefit was observed when ingesting MCFA supplements during exercise. We recently investigated whether combining a dietary adaptation period (2-weeks) plus ingestion of a structured triacylglycerol (randomized-structured MCFA oil+CHO) emulsion during exercise would further attenuate gastric distress, and thereby allow a performance benefit to show through due to greater exogenous-energy supply (Thorburn, Vistisen et al. 2006). Gastric distress in response to the MCFA-rich exercise supplement was attenuated during the performance test following the 2-week MCFA adaptation period. In addition, almost 3-fold greater (0.43 $g \cdot min^{-1}$) MCFA oxidation was observed in comparison to that previously reported (0.15 $g \cdot min^{-1}$) (Jeukendrup, Saris et al. 1996). However, sprint performance remained substantially impaired in comparison to CHO-only.

The aim of the present study, therefore, was to investigate the effects of a 2-week MCFA-rich dietary adaptation period and ingestion of MCFA-rich exercise supplements on gastrointestinal distress, MCFA oxidation, and performance in a cohort of trained female cyclist. The majority, but not all, of well controlled studies have shown lower RER in females than males during submaximal endurance exercise (reviewed by (Tarnopolsky 2000) indicating the possibility of proportionately higher fat oxidation. Therefore, females could potentially make better use of exogenous MCFA supplements than their male counterparts, which may translate into performance benefits.

Materials and Methods

Subjects

Five well-trained female cyclists 31 years (SD 3) and 65 kg (2) completed the study. Three additional subjects withdrew due to illness not related to the study interventions or to other commitments. All cyclists had been training for at least 8-10 h per week and riding competitively for at least 6 months. Mean VO_2max and peak power output were 3.4 $L \cdot min^{-1}$ (0.2) and 243 W (9) respectively. Before beginning experimentation, all subjects read the study information sheet, were informed of their rights, screened for precluding health conditions, and signed a consent form. The experimental protocol of this study was approved by the Massey University Ethics Committee (Protocol 03/143).

General Design

Cyclists participated in a randomized double-blind triple-crossover protocol in which three dietary conditions modifying the normal fatty-acid composition were completed over three consecutive 2-week experimental blocks (Figure 1). The conditions were: *a*) *Placebo* – 2-week diet containing long-chain fatty-acid rich oil with the intervention CHO+MCFA-rich esterified oil emulsion ingested during exercise (LC-MC); *b*) *Intervention* – 2-week diet containing MCFA-rich esterified oil with the CHO+MCFA-rich oil emulsion ingested during exercise as above (MC-MC); *c*) *Control* – 2-week diet containing LCFA-rich oil with a CHO-only exercise supplement (LC-CHO). All subjects began an individualized standardized training regime two weeks before the baseline 3-h ride and performance test (Figure 1). The training was repeated for each subsequent 2-week dietary condition. The training protocol included three-to-five supervised and workload-standardized lab sessions

where they ingested 500 ml of the dietary supplement during exercise. On the morning of each test, subjects reported to the lab between 6-8 AM in a fasted state, and the parameters of the cycle ergometers were adjusted according to the subject's own racing cycle angles.

Menstrual cycle phase was not controlled. This decision was made because, although suggested, a substantial effect of menstrual cycle phase on carbohydrate and fat metabolism during exercise has not been clearly established (Kanaley, Boileau et al. 1992; Frankovich and Lebrun 2000). Additionally, non-control reduced time that subjects would have to commit to the study from 14 to 8 weeks, thereby increasing the chance of retention, reducing the drift in performance ability, and replicating the conditions employed with males in our lab (Thorburn, Vistisen et al. 2006).

[Insert Figure 1 near here]

Protocols

Maximal Oxygen Uptake (VO_2max) and Peak Power Output (PPO) were measured using a progressive exercise protocol on an electronically-braked cycle ergometer (VeloTron Racer Mate, Seattle, WA). After warm-up, the test started at a workload of 2 $W \cdot kg^{-1}$ body mass. The first stage duration was 150 s, after which the load was increased by 50 W, and then by 25 W for every subsequent 150-s stage. Exhaustion was defined as the time at which the cyclist could no longer maintain a pedal cadence of 50 RPM after 3 warnings. Maximal oxygen uptake was measured on-line with a calibrated SensorMedics Vmax Spectra Series gas analyzer (SensorMedics Corp., Yorba Linda, CA, USA) and taken as the highest attained 20-s average oxygen uptake. PPO was defined as the last completed

work rate plus the fraction of time spent in the final non-completed work rate multiplied by the 25 W work rate increase.

Familiarization. Approximately 1-week following the $\dot{V}O_2$max test, a shortened version of the performance test (see below) was performed as a procedural familiarization trial. Subjects cycled for 2-h at 50% PPO, and performed 6 maximal sprints interspersed with recovery periods at 40% PPO. An 8% CHO-based solution was ingested every 20-min and subjects practiced recording gastrointestinal distress and ratings of relative perceived exertion.

Baseline Test. This test provided information on gastric responses to naïve exposure of the CHO+MCFA-rich esterified-oil emulsion supplement (see below), doubling as a full practice trial of the testing procedure and a ride to deplete endogenous-glycogen stores to lower background ^{13}C enrichment. Subjects rode for 3-h at 50% PPO, primarily for assessment of physiological responses, but also as a long preload/depletion ride prior to the repeated-sprint performance test, which consisted of 10 maximal-effort sprints interspersed with recovery periods as described below.

Immediately before the start of exercise, subjects ingested a double quantity of the CHO+MCFA-rich esterified oil supplement. Every 20-min throughout the 3-h steady-state ride, gastrointestinal distress and exertion ratings were recorded before ingesting the next supplement, the quantity of which was based on individual PPO calculated from a base reference of 220 ml·20 min^{-1} for a PPO of 400 W. Subjects ingested between 127-141 ml of supplement/20 min (254-282 ml double bolus). During the sprints, subjects ingested each drink supplement throughout the 20 min as they liked, and reported gastrointestinal distress

and exertion immediately after sprints 1, 4, 7 and 10. Heart rate was continuously recorded by radiotelemetry (S610i Heart Rate Monitor, Polar Electro Oy, Finland).

^{13}C Background Trial. A 3-h ride at 50% PPO was performed on day 11 of each 2-week supplementation block (Figure 1) to determine background breath ^{13}C-enrichment required for later calculation of octanoic-acid oxidation. During the ride, subjects ingested the experimental drink supplement, containing all components except the esterified oil. Every 20-min during the 3-h ride external respiratory-gas was collected for indirect calorimetry (Sensormedics) and ^{13}C breath enrichment, the latter into 10-ml evacuated rubber-capped test tubes (Labco Ltd., High Wycombe, UK) from a 5-L mixing chamber. For all experiments, the Sensormedics mass flow sensor and gas analyzers were calibrated before testing and every hour during the 3-h exercise test. Following testing, the raw minute volume and gas fractions were adjusted if drift was greater than 3% following each hour of sampling. Drift between the initial and verification calibrations was assumed to be linear and the raw data were adjusted accordingly.

3-h Ride. On day 14 of each 2-week block subjects repeated the protocol undertaken during the baseline exercise test (Figure 1). Upon reporting to the lab, subjects had a 20GA cannula inserted into the antecubital vein of their right forearm (Becton Dickinson Medical Pte Ltd, Singapore). A 2-way stop-cock valve (Becton Dickinson Medical Pte Ltd, Singapore) was connected to the cannula and a 15-ml blood sample was taken immediately and transferred into vaccutainers (Becton Dickinson & Co., Franklin Lakes, USA) containing lithium heparin (5 ml) and EDTA (10 ml). Triplicate breath samples were collected from the mixing chamber for resting breath ^{13}C enrichment. Immediately before exercise began, subjects consumed a double bolus of drink supplement. Every 20-min during the 3-h ride data variables were collected in the order of exertion and gastrointestinal

distress scales, indirect calorimetry, ^{13}C breath enrichment, and a blood sample. Immediately following data collection another bolus of supplement was ingested. Heart rate was continuously recorded.

Performance Test. After completion of the 3-h ride, subjects dismounted their cycles and were allowed to toilet and stretch. Upon remounting their ergometers, subjects completed ten maximal sprints interspersed and beginning with a recovery interval at 40% PPO. The duration of the sprint (2-3 min) and recovery (5-6 min) periods were based on work done (kilocalories), determined by individual PPO (0.125 x PPO). Fixed linear workloads approximately equivalent to riding a 39 or 56 front chain ring and a 10-spocket 21 to 11 tooth rear cluster were programmed into the Velotron software. A gear switch was positioned on the end of the right handlebar break hood to provide convenient changing of linear resistance. Cyclists self-selected cadence and gearing but were instructed to sprint as fast as possible until the required kilocalories were achieved. No verbal encouragement was provided to the subjects, the only information provided during the sprints was elapsed work completed (kilocalories) shown on the computer screen. Subjects were given a verbal count down in preparation for the start of each sprint and at 20, 10, 5 and 2 kilocalories to go in preparation for the end of each sprint.

Supplement ingestion continued during the sprint procedure with the allocated quantity ingested ad libitum every 20 min. Immediately after sprints 1, 4, 7 and 10, exertion and gastrointestinal distress data were collected. A final 10 ml blood sample was taken immediately upon completion of the final sprint.

Diet and Supplements

Diet and Dietary Supplementation. Immediately following the baseline test subjects started their first 2-week diet condition. The diets consisted of food and drink supplements containing either the MCFA-rich (⅓ canola and ⅔ trioctanoin esterified oils) or LCFA (canola only) oils in prescribed meal and snack replacements. Both MCFA and LCFA diets were administered in the same way so as to blind the subjects to each condition. Foods and supplements were prepared on site by food technologists and catering services and included muffins, sports fudge bars, curries, bolognese and milk-like drinks. Each item was produced to provide 15 g of experimental oil (either LCFA or MCFA-rich) per serving and a fixed macronutrient composition: 15, 55, and 30 percent energy from protein, carbohydrate, and oil, respectively, so that fortnightly consumption of the supplements and other foods could be replicated. The amount of food and supplements ingested by each subject was determined by body mass, relative to a model 65-kg female cyclist ingesting 78 g of the experimental oils per day. In practice, the quantity of randomized esterified oil ingested during the 2-week diet (intervention condition) was 81 $g \cdot d^{-1}$ (SD 6) (54 g $MCFA \cdot d^{-1}$ SD 4). Subjects recorded all food ingested over the first two-week experimental block and replicated that daily pattern over the proceeding two blocks. In addition to eating the foods and supplements during the day, subjects were instructed to ingest the sports bars and milk-like drinks during training sessions to facilitate exercise gastrointestinal adaptation. Subjects were also provided with extensive lists and instructed not to eat any foods naturally enriched in ^{13}C (cane sugar and maize products) beginning immediately after the baseline test, but to otherwise maintain their normal diet. Instructing subjects in this way has been shown to be effective in reducing background ^{13}C enrichment (Jeukendrup, Saris

et al. 1995; Jeukendrup, Saris et al. 1995; Jeukendrup, Saris et al. 1996). Subjects verbally reported adhering to the dietary interventions. Two subjects reported experiencing slight cases of diarrhea and belching while on the intervention (MC-MC) diet though none claimed to be aware of which condition they were on at the time. Both the LCFA- and MCFA-rich diets were otherwise well tolerated.

Exercise Supplements. Four different milk-like drinks and emulsions were formulated for use during exercise. The drinks were flavored identically for placebo purposes. The drink containing the CHO+MCFA-rich esterified oil was made in two forms: the supplement ingested during the baseline, LC-MC and MC-MC performance (sprints) tests contained 5.7% esterified oil, 2.6% wheat-derived dextrose and 5.6% maltodextrin, 1.5% sodium caseinate emulsifier, 0.08% salt, colors, chocolate and vanilla flavoring; an identical supplement was ingested during the 3-h ride in the MC-MC and LC-MC dietary conditions, but also contained 1-^{13}C octanoic-acid tracer incorporated into the esterified oil. The third, placebo-control exercise drink used in the LC-CHO condition contained the same ingredients as above minus the esterified oil, and with addition of 4.0% milk powder to emulate color and taste. The fourth drink used during the three ^{13}C background trials consisted of identical ingredients to the drink containing the MCFA-rich esterified oil minus the oil and ^{13}C tracer. The mean quantities of MCFA-rich esterified oil emulsion ingested were 77 g (SD 3) (51 g MCFA SD 2) and 31 g (SD 1.2) (21 g MCFA, SD 0.8) during the 3-h ride and performance test, respectively.

In producing the exercise supplements, the oil was first heated to 50°C. Dry ingredients were mixed with water, colors and flavors, and the two mixtures were then combined and homogenized to disperse the fat droplets (giving the drinks a milky appearance) and

refrigerated. The drinks were made 1-5 days before ingestion and had a minimum refrigerated shelf life of 10 days.

Randomized-Esterified Oil

A blended oil mixture consisting of ⅓ canola (LCT) and ⅔ trioctanoin oil was mixed in a batch reactor by an impeller at 230 rpm. After the vacuum reached 100 mbar, the oil was dried for 30 min at 90°C. Temperature was then decreased to 60°C and 0.1 wt% sodium methoxide was added while stirring. Any air in the reactor was removed using vacuum and N_2 systems. After 30 min, the reaction was stopped by addition of a 5 wt% citric-acid water solution. The oil was washed 3-4 times until pH was below 7, and dried again before deodorization in a conventional batch deodorizer. Vacuum was adjusted to less than 5 mbar, stripping steam consumption was adjusted to 4 wt%, and temperature was raised to 160°C for 2 h. After deodorization, the oil was cooled by tap water circulation with N_2 protection, and stored in a -25°C freezer.

1-^{13}C-Octanoic Acid-Enriched Esterified Oil

The 1-^{13}C-octanoic acid-enriched esterified oil was produced by esterification of ^{13}C-labelled octanoic acid (C8:0) (Cambridge Isotope Laboratories, Andover, MA), oleic acid (18:1n-9) and glycerol in the proportion of 3:1:1.6. Components were mixed 10:1 with lipase (Novozym, Novozymes, Bagsvaerd, Denmark) at 60°C with constant stirring. Vacuum removed excess water from the process by passing the air through anhydrous Na_2SO_4 (JT Baker, Deventer, Holland). After a 24 h incubation period, the reaction was stopped by separating the oil and enzymes with filtering. The oil was then kept at -20°C

until use. The ^{13}C-enriched oil was mixed into the randomized esterified oil that yielded a mean oil ^{13}C-enrichment of 84 $\delta \cdot mil^{-1}$ vs. PDB (SD 30) (0.006854 $^{13}C/^{12}C$ ratio).

Psychometric Scales

Perceived exertion (soreness of legs and effort of cycling) and gastrointestinal distress (nausea, fullness/bloatedness, stomach cramp, reflux/burping) markers were measured using scales modeled from Borg's CR10 scale (Borg et al. 1998). Verbal descriptors were associated with the scale: nothing 0, very weak 1, weak or mild 2, moderate 3, strong 5, very strong 7, extremely strong 10, and absolute maximum 13.5. Subjects were instructed to make a pen mark on a continuous scale, rating the strength of their exertion or distress.

Analyses

^{13}C Enrichment. Breath samples were analyzed for $^{13}C/^{12}C$ by gas chromatography continuous flow isotope ratio mass spectrometry (Finnigan Delta XP, Bremen, Germany). The enrichment of the esterified oil was measured using Elemental Analyzer isotope ratio mass spectrometry.

Calculations. Oxidation rates ($g \cdot min^{-1}$) of exogenous octanoic acid and total fat and CHO were calculated from ^{13}C-enrichment and indirect calorimetry measurements. Isotopic enrichment of expired air was expressed as the delta per million difference (δ‰) between $^{13}C/^{12}C$ ratio of the sample and a known laboratory reference standard (Pee Dee Belemnite; PDB) according to the formula: $\delta^{13}C = [(^{13}C/^{12}C \text{ ratio sample}/^{13}C/^{12}C \text{ ratio standard}) - 1] \cdot 10^3$ ‰, where, $^{13}C/^{12}C$ standard = 0.0112372 (Craig 1957). The amount of octanoic-acid oxidized is then calculated according to the formula: Exogenous octanoic-acid oxidation ($g \cdot min^{-1}$) = $VCO_2 \cdot (\delta_{Exp} - \delta_{bkg}/\delta_{Ing} - \delta_{bkg}) \cdot (1/k)$, in which δ_{bkg} is the ^{13}C enrichment of expired

air in the 3-h background trial, δ_{Exp} is the ^{13}C enrichment of expired air during the 3-h ride with ^{13}C-enriched esterified oil ingestion, δ_{Ing} is the ^{13}C enrichment of the oil in the ingested exercise supplement, and k is the amount of CO_2 (liters) produced via oxidation of 1 g octanoic acid on a glycerol backbone (k = 1.2369 L CO_2·g trioctanoin^{-1}) (Jeukendrup, Saris et al. 1995). A conversion factor of 34.19 kJ·g^{-1} was used to estimate MCFA contribution to energy expenditure (Livesey and Elia 1988).

Total carbohydrate and fat-oxidation rates were calculated using the non-protein respiratory quotient (Jeukendrup and Wallis 2005): Carbohydrate oxidation (g·min^{-1}) = $4.210 \cdot VCO_2 - 2.962 \cdot VO_2$, Fat oxidation (g·min^{-1}) = $1.695 \cdot VO_2 - 1.701 \cdot VCO_2$. Conversion factors of 15.64 kJ·g^{-1} (Ferrannini 1988) for CHO, and 40.81 kJ·g^{-1} (Péronnet and Massicotte 1991) for fat oxidation were used to estimate contribution to energy expenditure. The oxidation rate of other fats was the total fat-oxidation rate minus the octanoic acid oxidation rate.

Calculation of exogenous substrate oxidation is affected by the delayed equilibration of $^{13}CO_2$ originating from the tissues with the large endogenous HCO_3^- pool. However, a physiological steady-state condition occurs relatively rapidly during exercise, and $^{13}CO_2$ in the expired air will be equilibrated with the $^{13}CO_2/H^{13}CO_2$ pool from around 60 min of exercise (Robert 1987). As a consequence, calculations on substrate oxidation were only reported from 60 to 180 min of exercise.

Plasma. Blood was collected into lithium heparin and EDTA-containing tubes, immediately centrifuged at 2000 G for 12 min, aspirated into eppendorf tubes and snap-frozen in liquid nitrogen before being stored in a -80°C freezer until analysis. Plasma lactate, glucose, potassium, and acid-base variables were analyzed using an automated

blood gas analyzer (Bayer Rapidlab 800 system, Bayer HealthCare LLC, Tarrytown, NY, U.S.A). Before analysis, a quality control test was run to evaluate the system for imprecision and inaccuracy. Two-point calibrations for all parameters were performed every 45-60 samples. One-point calibrations for all parameters were performed every 15-20 samples. One-point calibrations for PCO_2, glucose and lactate were performed every 3 samples.

Triacylglycerol and glycerol concentrations were determined by thin layer and gas chromatography as described previously (Vistisen, Nybo et al. 2003). Extraction of plasma fatty acid for the determination of fatty acid concentration and isotopic enrichment was performed according to Patterson and colleagues (Patterson, Zhao et al. 1999). Fatty acid concentration was measured by gas chromatography with FID (Autosystem XL; Perkin Elmer, Northwalk, CT, USA), using 9:0 and 17:0 as internal standards. Plasma fatty acid ^{13}C enrichment was measured by gas chromatography-combustion isotope ratio mass spectrometry (GC-C-IRMS, Hewlett Packard 5890, Finnigan GC combustion III, Finnigan Deltaplus; Finnigan MAT, Bremen, Germany) as described previously (Van Hall, Bulow et al. 2002). 8:0 enrichment was corrected by a factor of 9/8 to account for the extra methyl group of the methyl octanoate derivative. A standard sample (Nu-Chek Prep, Elysian, MN) with known composition and quantities of fatty acid methyl esters was run daily. This standard contained fatty acid methyl esters with chain length of C6 to C22, which ensured detection of all plasma fatty acid methyl esters within this range. Fatty acids were identified by retention times of the standard fatty acid methyl esters.

Sprint Data. Mean power ($J \cdot s^{-1}$) for each interval was calculated from the inbuilt efficiency factor and J conversion factors in the Velotron software code: kcal·4.186 ·1000·0.25/t, where kcal is the energy (kilocalories) expended by the subject during the

sprint, 4.186 is the conversion factor from kilocalories to kilojoules, 0.25 is the efficiency factor of an exercising person, and t is the time it took the subject to complete the sprint in seconds.

Statistics

General Method. The effect of diet condition on the outcome variables was estimated with mixed modeling in the Statistical Analysis System (SAS9.1, SAS Institute, Cary, NC). Most dependent variables were analyzed after log transformation to reduce the effects of non-uniformity of error. For measures of performance, metabolism, and other dependent physiological variables, the effects of the diets were compared in a three-way model, whereas the analysis of psychometric variables also included the Baseline test responses. Quantitative mixed linear models were applied to the time-series data sets for the 3-h exercise and during the sprint procedure where sample time or sprint number were numeric effects. For all datasets treatment was a fixed effect and for the performance and psychometric analyses an order term was included. In all data sets, the random effects were subject and the interaction between subject and condition. The random variability associated with moving between sprints (fatigue) or progression along a time series was also included in the analysis for the relevant data sets. The within-cyclist standard deviation was estimated from the residual variance. A mechanisms analysis of the relationship between power output and nausea was conducted as described previously (Thorburn, Vistisen et al. 2006).

Presentation of Data. Measures of centrality and spread for subject descriptive, raw stable isotope, and dietary variables are raw means and standard deviations (SD). Means derived from the analysis of log-transformed variables are back log-transformed least-

squares, or *adjusted*, means. The associated spread around these least-squares mean is represented by percentage (geometric) standard deviations or factor standard deviations (SD_f), implying ×/÷. For example, for a plasma-glucose concentration of 5 mmol·L^{-1} with a between-subject standard deviation of 20% (SD_f 20), the typical variation is 5 × 1.20 to 5 ÷ 1.20, or 6 to 4.16 mmol·L^{-1}. Data in graphs and text are shown as least-squares means. Data are rounded to two significant digits or in some cases three where the first digit is "1".

Precision of the Estimate and Practical Inference. In keeping with recent trends in inferential statistics, e.g., (Curran-Everett and Benos 2004; Sterne and Smith 2001), we made quantitative inferences about population (true) effect by expressing uncertainty as 90% confidence limits (CL) or interval (CI) and as probabilities that the true value represents a practically-important *substantial* change (beneficial or detrimental, increase or decrease) (Batterham and Hopkins 2006). The smallest substantive change in sprint power was 1.1% as previously (Thorburn, Vistisen et al. 2006), with females assumed to be similar (Paton and Hopkins 2005). The smallest standardized (Cohen) change (i.e. effect-size statistic) in the mean for biochemical and psychometric variables was 0.20 times the between-subject SD for the value in the control group (LC-CHO condition) (Cohen 1988). Probabilities of a substantial benefit or detriment, increase or decrease were calculated for 90% CL from the two-tailed Student t-distribution (Hopkins 2001) and inferred as follows: <1%, almost certainly not; 1–5%, very unlikely; 5–25%, unlikely; 25–75%, possible; 75–95%, likely; 95–99%, very likely; >99%, almost certain (Batterham and Hopkins 2006). An effect was qualified *unclear* (not clear, inconclusive) if its confidence interval overlapped both thresholds for substantiveness by more than 5%. In the case where the chance that the

benefit or detriment is <5% and the chance of the effect being trivial is greater than a substantial benefit or detriment, the *trivial* likelihood is qualified.

Results

Performance

No clear effect of diet on mean power or fatigue (slope) was observed between the three supplemental conditions (Figure 2, Table 1).

Mechanisms Analysis

For every 1 unit increase in the position of the nausea curve, the position of the mean power curve decreased by 0.4 W (90% CI: -3.6–4.4 W; effect unclear). Nausea had no detectable influence on sprint mean power when added as a covariate in the polynomial analysis (Figure 3).

Substrate Metabolism

Breath ^{13}C-enrichments during the 3-h background trials are shown in Figure 4. Mean peak octanoic-acid oxidation rates at the 180-min sample during the LC-MC and MC-MC conditions, respectively, were 0.31 $g \cdot min^{-1}$ (SD_f 32%) and 0.32 $g \cdot min^{-1}$ (32%). There was no clear effect of MCFA adaptation (MC-MC condition) on exogenous octanoic-acid oxidation (vs. LC-MC), but interestingly, the oxidation efficiency (ingestion rate/oxidation rate) over the period 60-180 min was 98% in the LC-MC condition and 99% in the MC-MC condition.

Overall relative energy contributions are shown in Figure 5. Energy derived from the oxidation of endogenous fat was substantially reduced by the oil emulsion (MC-MC: -88; 59–117%, decrease almost certain; LC-MC -74; -108– -40%, decrease almost certain) conditions relative to LC-CHO, but there was no effect of MCFA adaptation evident. There

was also a likely reduction (-14%; -29–1%) in energy derived from endogenous-CHO oxidation in the LC-MC condition relative to LC-CHO, but the differences between the other comparisons were unclear.

The pattern of substrate oxidation over the 3-h ride is illustrated in Figure 6. The increase in the octanoic-acid oxidation rate from the 60th to the 180th min sample points was 0.09 g·min^{-1} in the LC-MC condition and 0.10 g·min^{-1} in the MC-MC condition, but consistent with the overall average response, there was no clear difference between conditions. Total CHO oxidation rates changed little (<10%) from 60 to 180 min; in contrast endogenous fat-oxidation rates declined by 0.04 and 0.11 g·min^{-1} in the LC-MC and MC-MC, respectively, with only a 0.01 g·min^{-1} decline in the LC-CHO condition. The difference in the decline in MC-MC relative to LC-CHO was 3.2-fold (0.7–14). The remaining observed between-treatment slope differences for endogenous-fat oxidation were inconclusive.

Psychometric Scales

A statistical summary of gastrointestinal distress (nausea, fullness/bloating, stomach cramp, reflux) and exertional parameters (leg soreness, perceived effort) is shown in Table 2. The responses for nausea, fullness/bloatedness, and perceived effort were selected to illustrate the magnitude and temporal effects of treatment and exercise duration (Figure 7).

3-h ride. The treatment effects on gastrointestinal distress and exertion were similar, but the effect of the MC-MC condition relative to LC-CHO on fullness/bloating, stomach cramp, and leg soreness were unclear (Table 2). There were no clear consistent patterns in slope (increase in scale unit rating) from the 20th min to the 180th min sample points.

Performance test. The performance test accentuated the effect of the LC-MC treatment on nausea and fullness/bloating, relative to the LC-CHO condition. The exertional parameters were also substantially increased in the LC-MC condition but not clearly in the MC-MC condition, relative to the LC-CHO control. The effect of the MC-MC condition on gastrointestinal distress markers relative to LC-MC was unclear. There were no clear consistent slope patterns from sprints 1 to 10 (not shown).

Plasma, Electrolytes, Acid-base Status, Glucose and Lactate

No clear differences were observed between conditions for hydrogen ions or standard bicarbonate concentrations. Glucose was 5.2% (1.5–8.9%) lower in the MC-MC condition, relative to LC-CHO; no clear effect was observed between LC-MC and LC-CHO or LC-MC and MC-MC conditions. Lactate was 7.9% (1.6–14.2%) lower in the LC-MC condition, relative to LC-CHO; no clear effect was observed between MC-MC and LC-CHO or MC-MC and LC-MC conditions. The outcomes for the remaining electrolyte comparisons were qualified as possible increases or decreases (25–75% likelihood) or unclear.

Plasma Lipids

The effects of treatment condition on plasma lipids and octanoic-acid ^{13}C enrichment are shown in Figure 8. At rest and during the 3-h steady-state ride plasma triacylglycerol concentrations were overall lower by 17% (23– -4%; reduction likely) and 15% (20– -3%; reduction likely) in the LC-MC and MC-MC conditions, respectively, relative to LC-CHO; there was no clear effect of exercise, however. Plasma glycerol concentrations trended

overall lower by 25% (54– -18%) and 45% (84– -17%) in the LC-MC and MC-MC conditions, respectively relative to LC-CHO, but the uncertainty meant these effects were qualified unclear. At the end of the sprints, there was a 50% lower (137– -6%; likely reduction) plasma glycerol concentration. Total plasma free fatty acids were also 27% (73– -7%; unclear) and 20% (39–3%; reduction likely) lower in the LC-MC and MC-MC conditions, respectively, relative to LC-CHO. There was no effect of MCFA adaptation on total and ^{13}C-enriched plasma octanoic-acid concentrations. There were no detectable elongation products (^{13}C-enriched fatty acids >8:0) seen in the plasma in either the MC-MC or LC-MC condition (data not shown). All remaining comparisons for the respective variables were unclear.

Discussion

The purpose of this study was to determine if a 2-week high-MCFA dietary adaptation period would attenuate gastrointestinal distress, increase octanoic-acid oxidation rate, and enhance performance while ingesting a CHO+MCFA-rich oil emulsion during endurance cycling in a sample of well-trained female cyclists. The CHO+MCFA-rich oil emulsion resulted in mild-to-moderate gastrointestinal distress ratings, relative to the LC-CHO condition, and there was no evidence for any attenuation with dietary-MCFA adaptation. High amounts of ingested MCFA were oxidized during the 3-h pre-load exercise, and a substantial reduction in endogenous fat availability and metabolism was observed with MCFA-rich oil emulsion ingestion. However, there was no clear effect of MCFA adaptation on octanoic-acid oxidation, ingested ^{13}C-octanoic acid and endogenous carbohydrate metabolism, or endurance cycling performance.

The purpose of the 2-week MCFA-dietary adaptation intervention was to attempt to alleviate the gastrointestinal distress normally associated with high MCFA ingestion rates during exercise and, therefore, to observe any potential benefit to substrate supply and high-intensity endurance performance. In male subjects, we (Thorburn, Vistisen et al. 2006) previously observed a clear substantial reduction in gastrointestinal distress parameters with MCFA adaptation; and in the covariate analysis, nausea had a moderate-strong reducing effect on sprint mean power. Conversely, in the current cohort of female subjects MCFA adaptation did not clearly attenuate gastrointestinal distress symptoms, and performance was not adversely affected by the intervention relative to the smallest worthwhile detriment in mean power (Table 1), or in relation to the distress marker nausea (Figure 3). However,

only five female subjects completed the present study leading to modest power and contributing to the uncertainty. The confidence limits for mean sprint power suggest that, relative to the LC-CHO condition, the true mean population effect of MCFA adaptation (MC-MC condition) on performance could be a substantial benefit of up to 2.8%, or an impairment in the order of 5.0%; for the control LC-MC condition the true effect could be an enhancement of up to 6.6%, or impairment of 10%. This uncertainty in the direction of the true population response implicates individual differences in response to the treatment, or random error. The sample size required to make a confident statement about whether the true population mean effect for the MC-MC vs. LC-CHO comparison is trivial (≤1.1% impairment or enhancement) or substantially negative is *infinite*, because the observed effect (1.1% impairment, Table 1) is the same as the smallest worthwhile effect (1.1%), so no matter how many subjects are tested, there will be 50% probability that the true population mean will be both substantially negative or trivial. For the LC-MC vs LC-CHO comparison, however, the sample size to conclude a *very likely* substantial detriment is 17 (n required for clear-cut substantial positive or negative effect = (SQRT(width or observed CI/width of acceptable CI)) × existing n; source: W. Hopkins, 2006 unpublished). Therefore, were we able to have completed testing on all 8 subjects, or extended the cohort to 10-12 – a large sample for these types of studies – our conclusions may have been little different.

Meanwhile, the finding of an attenuated response to the intervention relative to the male cohort studied previously (Thorburn, Vistisen et al. 2006) is consistent with the generalization that trained females respond with lower amplitude to nutritional-exercise

interventions, relative to their male counterparts. Compared with males, the observed average reductions in sprint performance and endogenous-fat oxidation with naïve exposure to the MCFA-CHO exercise supplement (LC-CHO condition) were less in magnitude, and following MCFA adaptation (MC-MC condition) the attenuating effects of octanoic acid and gastrointestinal distress markers were unclear in the females. Other's have found that trained females respond in a lesser magnitude to a number of nutritional interventions including recovery in response to high-protein recovery supplements (D.S. Rowlands, 2005 unpublished), carbohydrate loading, and creatine supplementation (Tarnopolsky 2000).

All previous published work examining the ergogenic potential and metabolic effects of MCT-CHO ingestion during exercise has been conducted in well-trained males with the outcomes equivocal. In response to naïve exposure, Goedecke et al. (2005) found CHO+MCT ingestion to clearly impair performance relative to CHO-only during ~5-h of exercise. In their study, the performance of intermediary sprints became increasingly worse, and time to complete the final 200-kJ time trial was longer by 13% (90%CL: 8 to 18%) with CHO+MCT ingestion. In an earlier study, Goedecke et al. (1999) observed mean impairments of 3% and 2.5% in 40-km time-trial performance following a 2-h ride reported as statistically insignificant (P-values not provided) when subjects ingested high MCT+CHO and low MCT+CHO supplements, respectively, in comparison to CHO-only. On the other hand, other authors have reported non-statistically clear (P-value >0.05) trends toward performance improvements with CHO+MCT ingestion. Jeukendrup et al. (1998) and Vistisen et al. (2003) observed on average 1% and 1.5% improvements in constant work or constant time tests, respectively, when subjects ingested a CHO+MCT supplement in comparison to CHO-only. The only group reporting a clear performance benefit as a

2.5% (90%L: 0.5–4.5) enhancement in 40-km time following a 2-h pre-load was also the only group reporting no gastrointestinal distress (Van Zyl, Lambert et al. 1996). This finding is in contrast to all of the other studies feeding >30 g of MCT, including our earlier adaptation study (Thorburn, Vistisen et al. 2006). Van Zyl et al. (1996) suggested that the increase in performance in their study was due to the observed increased availability of plasma fatty acids and the subsequent sparing of endogenous carbohydrate (implying muscle glycogen). This result contrasts the present findings, as well as that of others (Goedecke, Elmer-English et al. 1999; Jeukendrup, Saris et al. 1995; Jeukendrup, Thielen et al. 1998; Thorburn, Vistisen et al. 2006) which show that MCT ingestion during exercise spares only endogenous fat rather than endogenous carbohydrate utilization. In addition, the present study showed a possible reduction in plasma lipid (triacylglycerol, glycerol and total fatty acid) availability with MCFA ingestion in comparison to the LC-CHO condition, suggesting reduced rather than increased plasma fatty-acid availability (Figure 8).

That there was no clear evidence supporting a performance benefit in this and several previous studies might be attributed to the substantial gastrointestinal distress (Jeukendrup, Thielen et al. 1998; Goedecke, Elmer-English et al. 1999; Goedecke, Clark et al. 2005; Thorburn, Vistisen et al. 2006); indeed we previously provided evidence through quantifying the relationship with nausea (Thorburn, Vistisen et al. 2006), though in the present study we found no evidence for a relationship between gastrointestinal distress and impaired performance. We previously suggested that MCFAs may not even reach the systemic circulation as MCFAs, which would exclude utilization of their potentially beneficial properties in the muscle cells (Vistisen, Nybo et al. 2003; Thorburn, Vistisen et al. 2006). Fatty-acid elongation or storage in the liver prior to release into the circulation,

conversion to ketone bodies which were then later oxidized or excreted through the urine or breath, or malabsorption by the gut and excretion in the feces we proposed as possible fates. However, several observations in the present study suggest that the muscle was the more likely site for MCFA disposal during exercise: we observed ^{13}C-octanoic acid in the plasma and the oxidation rates were rapid and exclusive; there were no measured elongation products; and the average energy provided by octanoic-acid oxidation was approximately matched by the reduction in energy provided from endogenous-fat oxidation (Figure 5).

Although we can not be certain that all of the ingested MCFAs were metabolised by the exercising muscle, we did make some further interesting and novel observations pertaining to the CHO+MCFA-rich esterified oil emulsion. In males, we found a high efficiency of octanoic-acid oxidation of up to ~100% ingestion rate (0.43 g·min^{-1}, 5.3 mg·kg^{-1}·min^{-1}) when co-ingested with CHO (Thorburn, Vistisen et al. 2006). In the present study the maximum octanoic-acid oxidation rate reached in the 180th minute of the 3-h ride was similar (4.9 mg·kg^{-1}·min^{-1}), which also approximated the MCFA ingestion rate (Figure 6). These oxidation rates are 2-3 fold higher than previously reported (0.15 g·min^{-1}, (Jeukendrup, Saris et al. 1996) and suggest a unique quality of the exercise supplements promoting high absorption and oxidation rates. Fat digestion is normally slow because triacylglycerols aggregate into large droplets in the small intestine, slowing the action of pancreatic lipase. However, our supplements were suspended in an emulsion, which distributes lipids into smaller droplets, thereby increasing their accessibility to pancreatic lipase. Also, cyclists were fed MCFAs and long chain fatty acids in the form of structured triacylglycerols, which may have enhanced MCFA absorption due to the increase in

pancreatic secretion which is stimulated by long-chain triglyceride, but not MCT ingestion (Mott, Sarles et al. 1972). Therefore, more extensive hydrolysis of the structured triacylglycerols may have occurred in comparison to MCT solution/suspensions used in previous MCFA-oxidation rate studies (Jeukendrup, Saris et al. 1996), leading to greater absorption, blood concentrations, and mitochondrial oxidation. Despite the higher oxidation rates, there was little evidence for an effect of MCFA adaptation on absorption or metabolic clearance rates based on the observation of similar plasma ^{13}C-enriched octanoic-acid concentrations, relative to the LC-MC control.

In summary, the ingestion of a CHO+MCFA-rich esterified oil emulsion resulted in high efficiency of exogenous MCFA oxidation, but induced mild-moderate gastric distress symptoms during prolonged exercise in female cyclists. Two weeks' adaptation to a MCFA-rich diet did not attenuate the gastrointestinal distress symptoms, and no clear performance benefit was observed with ingestion of the CHO+MCFA-rich exercise supplement or following adaptation. Similar to the consensus from previous adaptation and naïve exposure studies in males, there was little evidence to suggest that increasing exogenous energy supply through the addition of MCFAs to the training diet or exercise supplements will result in ergogenic outcomes in trained female cyclists. While some uncertainty remains, the results of our investigations lead to the conclusion that further investigation of MCFA ingestion as a potential ergogenic aid for exercise performance is not warranted.

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Tables

Table 1. Comparison of the three dietary conditions on performance.

	Effect Comparisons (%) ±90% CL[a] and Qualitative Inference		
PERFORMANCE MEASURE	MC-MC minus LC-CHO	LC-MC minus LC-CHO	MC-MC minus LC-MC
Sprints 1-9			
Mean Power[b]	-1.1 ±3.9	-1.9 ±8.5	0.9 ±9.5
	Unclear	Unclear	Unclear
Fatigue[c]	-0.9 ±6.4	-0.4 ±5.3	0.4 ±6.1
	Unclear	Unclear	Unclear
Sprint 10			
Mean Power	-2.2 ±6.7	-2.8 ±9.2	0.6 ±9.6
	Unclear	Unclear	Unclear

[a]±90%CL: add and subtract this number to the mean effect to obtain the 90% confidence limits for the true difference.

[b]Mean power is the overall mean power effect for sprints 1-9. The sprint 10 effect was excluded in the linear model because is approached differently by the subjects; it was extracted separately from the repeated-measures model.

[c]Fatigue is the percentage difference between the conditions in the linear decline in

power evaluated between sprints 1 and 9. Analysis is based on the reduction in mean power per sprint. More negative means greater relative fatigue.

Abbreviations: MCFA-rich esterified oil diet with the CHO+MCFA-rich oil emulsion ingested during exercise (MC-MC); long-chain fatty-acid rich diet with the CHO+MCFA-rich esterified oil emulsion ingested during exercise (LC-MC); long-chain fatty-acid rich diet with a CHO-only during exercise (LC-CHO).

Table 2. Summary of the overall effect of the three dietary conditions on gastrointestinal distress and exertional parameters during the 3-h ride and performance test.

	Mean Effect Comparisons[a] ±90% CL[b] and Qualitative Inference					
	3-h Ride			Performance Test		
PARAMETER	MC-MC minus LC-CHO	LC-MC minus LC-CHO	MC-MC minus LC-MC	MC-MC minus LC-CHO	LC-MC minus LC-CHO	MC-MC minus LC-MC
Nausea	0.8 ±0.5 Increase very likely	0.4 ±0.5 Increase possible	0.4 ±0.8 Unclear	2.5 ±0.7 Increase almost certain	2.1 ±0.5 Increase almost certain	0.3 ±0.6 Increase unlikely
Fullness / Bloating	1.9 ±2.6 Unclear	1.6 ±0.2 Increase	0.4 ±3.1 Unclear	3.5 ±1.9 Increase very likely	2.7 ±0.4 Increase almost	0.8 ±2.7 Unclear

		almost certain			certain	
Stomach	0.6 ±1.4	0.3 ±0.2	0.4 ±1.6	0.6 ±1.8	0.9 ±1.5	-0.2 ±2.4
Cramp	Unclear	Increase likely	Unclear	Unclear	Unclear	Unclear
Reflux	1.8 ±1.3	1.7 ±0.2	0.0 ±1.6	2.6 ±0.4	3.3 ±0.5	-0.6 ±0.9
	Increase very likely	Increase almost certain	Unclear	Increase almost certain	Increase almost certain	Decrease possible
Leg Soreness	0.1 ±0.4	0.2 ±0.3	-0.1 ±0.3	0.4 ±1.7	1.9 ±0.5	-1.5 ±1.6
	Unclear	Increase possible	Decrease unlikely	Unclear	Increase almost certain	Decrease likely
Perceived Effort	0.3 ±0.2	0.1 ±0.2	0.2 ±0.2	0.8 ±1.3	1.3 ±0.6	-0.5 ±1.3
	Increase likely	Increase possible	Increase possible	Increase possible	Increase very likely	Unclear

[a]Mean effect comparisons are presented in Likert Scale Units

[b]±90%CL: add and subtract this number to the mean effect to obtain the 90% confidence limits for the true difference.

Abbreviations: MCFA-rich esterified oil diet with the CHO+MCFA-rich oil emulsion ingested during exercise (MC-MC); long-chain

fatty-acid rich diet with the CHO+MCFA-rich esterified oil emulsion ingested during exercise (LC-MC); long-chain fatty-acid rich diet with a CHO-only during exercise (LC-CHO).

Figure Captions

Figure 1. Study Design. Three 2-week dietary adaptation periods including a ^{13}C background breath-enrichment trial on day 11 and performance test on day 14 with ingestion of either the CHO+MCFA-rich esterified oil emulsion or placebo CHO-only exercise supplement.

Figure 2. Sprint mean power in the performance tests. Data are back log-transformed least squares means. Bars are between-subject SD_f, those marked with an × represent the within-subject deviation. Y-axes are presented on logarithmic scales.

Figure 3. Effect of covariate nausea on mean sprint power (y-axis position coefficient) derived from the within-subject polynomial analysis. Data points are the observed mean effect of treatment relative to the reference condition and bars are 90% confidence intervals. The proportion of the confidence interval with beneficial, trivial or detrimental population outcomes is defined and the practical inference of the observed outcome qualified on the right.

Figure 4. Breath ^{13}C-enrichment. Data are raw means ± between-subject SD.

Figure 5. Mean substrate oxidation as percentage contribution to total energy expenditure during the 3-h rides.

Figure 6. Pattern of substrate oxidation during the 3-h rides. Data are back-log transformed least-squares means. Bars are between-subject SD_f, those marked with an × represent the within-subject deviation. Y-axes are presented on logarithmic scales.

Figure 7. Exertional parameters and gastrointestinal distress ratings during the 3-h and performance tests. Ratings are least-squares means ± between-subject SD. SD bars marked with an × represent the within-subject deviation.

Figure 8. Effect of treatment on plasma lipid concentration during exercise. Octanoic acid closed symbols = total octanoic acid; open symbols = ^{13}C-enriched octanoic acid. Bars are between-subject SD_f. SD bars marked with an × represent the within-subject deviation. Y-axes are presented on logarithmic scales.

Illustrations

Figure 1.

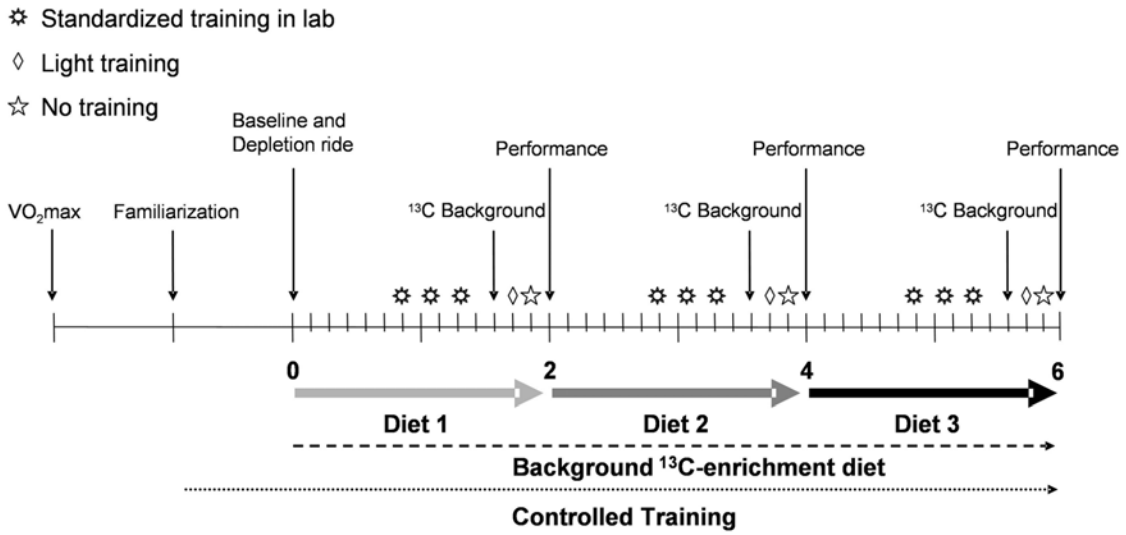


Figure 2.

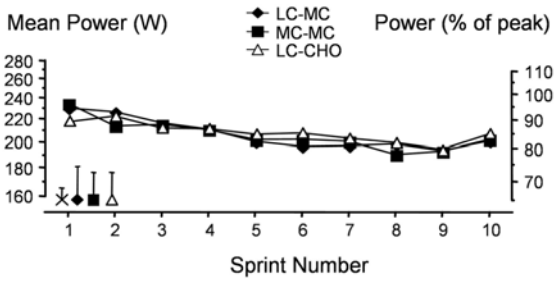

Mean Power (W)
LC-MC
MC-MC
LC-CHO
Power (% of peak)
280
260
240
220
200
180
160
110
100
90
80
70
1
2
3
4
5
6
7
8
9
10
Sprint Number

Figure 3.

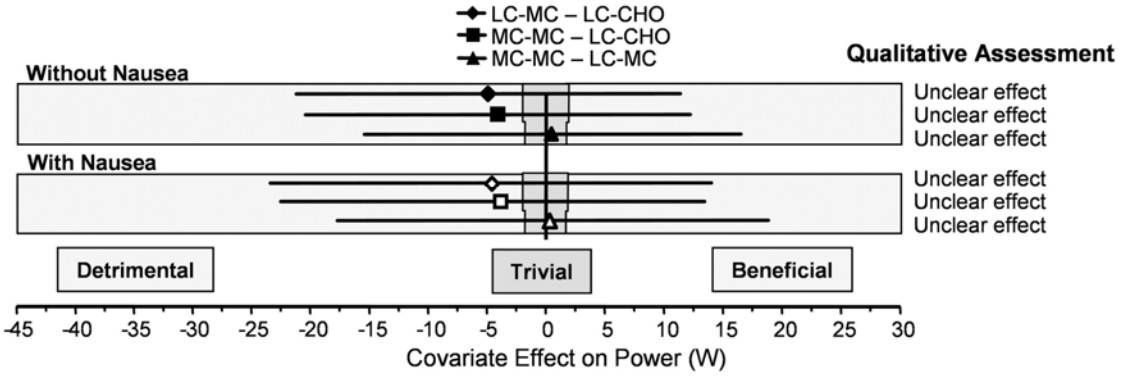

LC-MC – LC-CHO
MC-MC – LC-CHO
MC-MC – LC-MC
Qualitative Assessment
Without Nausea
Unclear effect
Unclear effect
Unclear effect
With Nausea
Unclear effect
Unclear effect
Unclear effect
Detrimental
Trivial
Beneficial
-45 -40 -35 -30 -25 -20 -15 -10 -5 0 5 10 15 20 25 30
Covariate Effect on Power (W)

Figure 4.

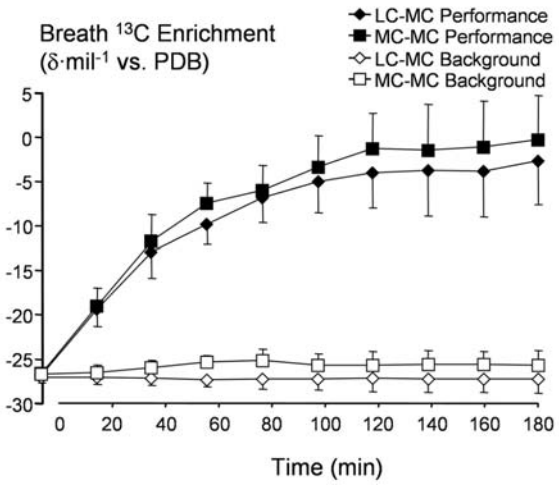

Breath 13C Enrichment
(δ·mil-1 vs. PDB)
LC-MC Performance
MC-MC Performance
LC-MC Background
MC-MC Background
5
0
-5
-10
-15
-20
-25
-30
0
20
40
60
80
100
120
140
160
180
Time (min)

Figure 5.

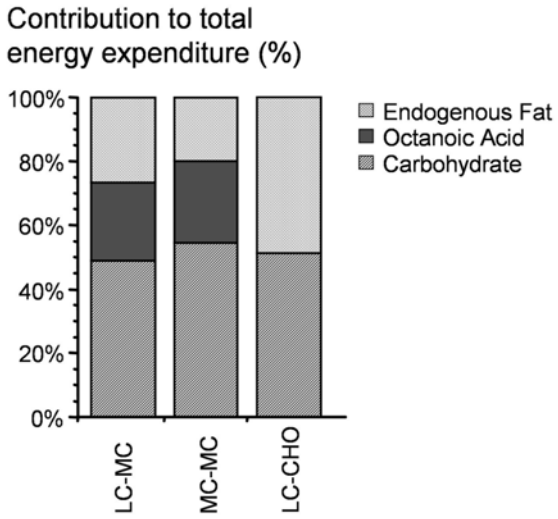

Contribution to total
energy expenditure (%)
100%
80%
60%
40%
20%
0%
LC-MC
MC-MC
LC-CHO
Endogenous Fat
Octanoic Acid
Carbohydrate

Figure 6.

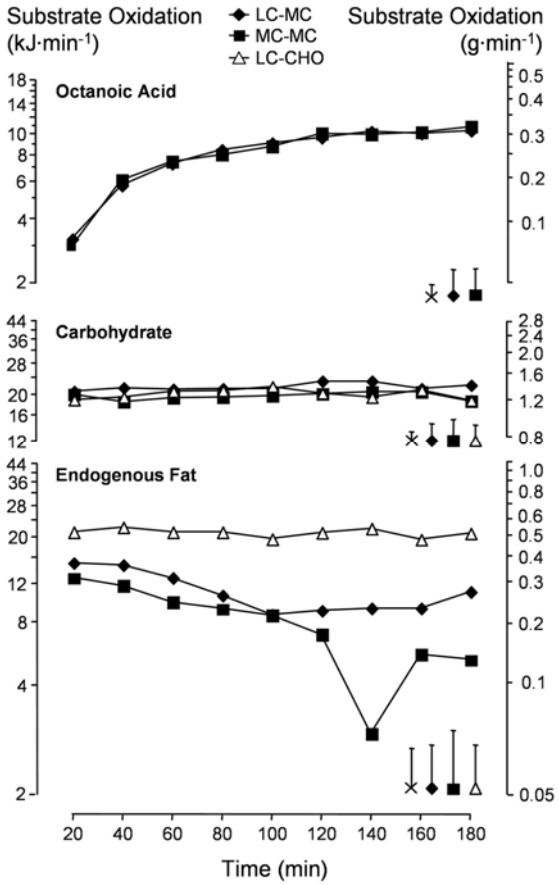

Substrate Oxidation (kJ·min-1)
LC-MC
MC-MC
LC-CHO
Substrate Oxidation (g·min-1)
Octanoic Acid
Carbohydrate
Endogenous Fat
Time (min)

Figure 7.

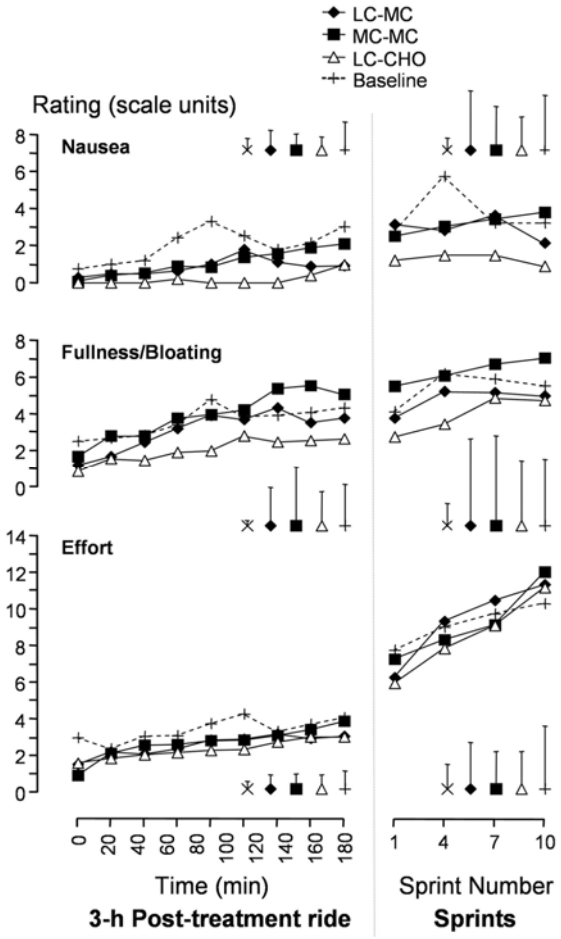
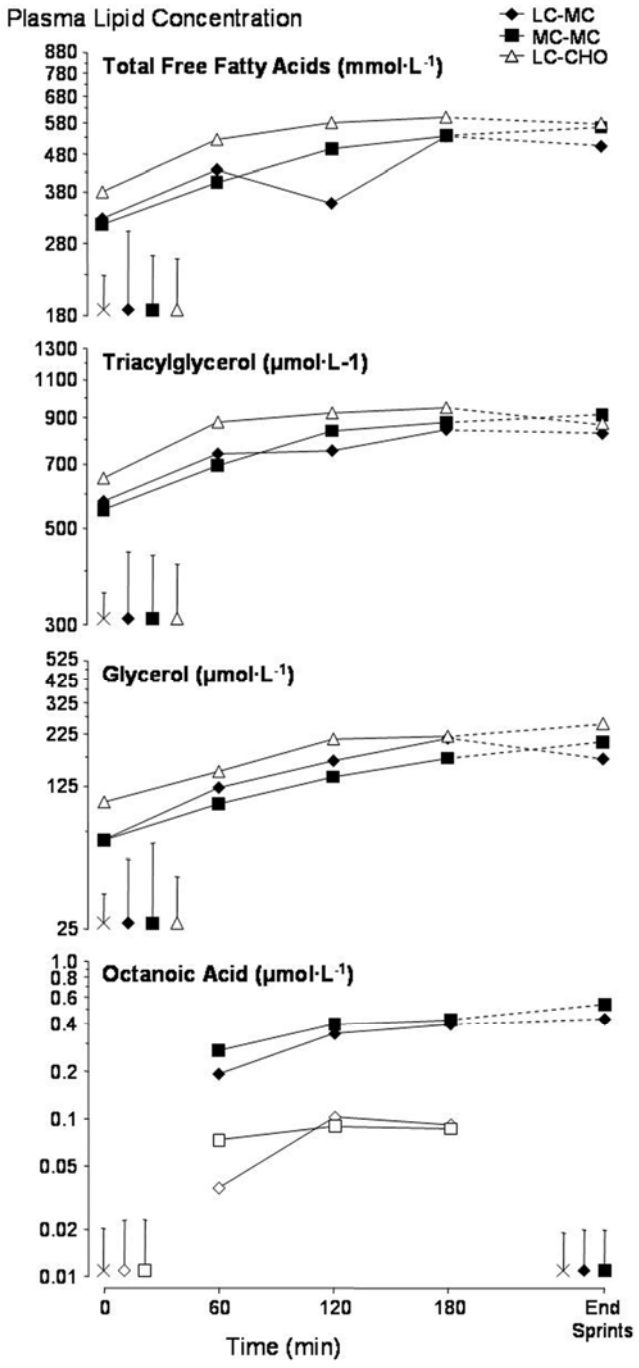

LC-MC
MC-MC
LC-CHO
Baseline
Rating (scale units)
Nausea
Fullness/Bloating
Effort
0
2
4
6
8
10
12
14
0 20 40 60 80 100 120 140 160 180
Time (min)
3-h Post-treatment ride
1 4 7 10
Sprint Number
Sprints

Figure 8.


Plasma Lipid Concentration
LC-MC
MC-MC
LC-CHO
Total Free Fatty Acids (mmol·L-1)
880
780
680
580
480
380
280
180
Triacylglycerol (µmol·L-1)
1300
1100
900
700
500
300
Glycerol (µmol·L-1)
525
425
325
225
125
25
Octanoic Acid (µmol·L-1)
1.0
0.8
0.6
0.4
0.2
0.1
0.05
0.02
0.01
0
60
120
180
End Sprints
Time (min)