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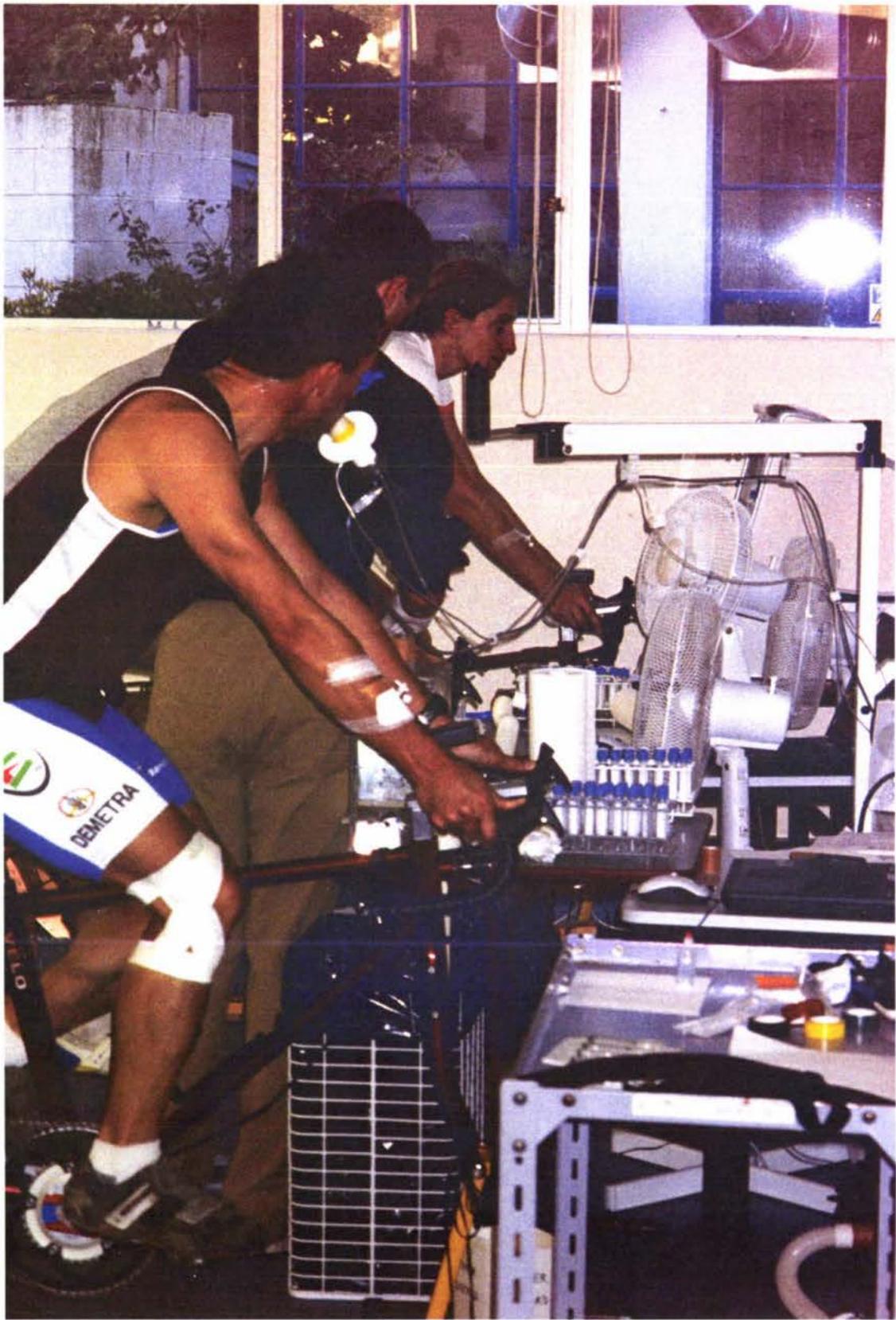
Adaptation to an MCFA-Rich Diet: Effect on Gastric Tolerance, the Capacity for MCFA Oxidation, and Performance while Ingesting Exogenous Carbohydrate and Structured Oils during Endurance Exercise.

A thesis presented in partial fulfilment of the requirements for the degree of
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Abstract

Introduction: Elevating the availability of fatty-acids to the muscle can potentially benefit endurance exercise performance by reducing intramuscular-glycogen utilisation. Digestion of triglycerides containing long-chain fatty acids (LCFAs) is slow, and fatty acids must pass through the carnitine palmityl transferase (CPT) transport system to enter the mitochondria, which potentially limits fat oxidation during prolonged-heavy exercise. Conversely, medium-chain triglycerides (MCTs) are rapidly digested and their constituent fatty acids (MCFAs) by-pass the CPT transport system. Ingestion of MCFAs may therefore supply mitochondrial acetyl-CoA, potentially reducing the requirement for glycolytic flux during exercise. However, studies comparing carbohydrate (CHO) with CHO-containing MCFA-rich exercise supplements have revealed inconsistent results, probably because of the variation in gastrointestinal (GI) distress suffered by participants associated with MCT ingestion.

Purpose: To investigate whether 2-weeks of dietary adaptation to MCFA-rich supplements reduces the severity of gastrointestinal (GI) distress, or increases the rate of MCFA oxidation during endurance exercise. A decrease in ratings of GI distress, or an increase in MCFA oxidation was anticipated to lead to performance benefits.

Method: Nine well-trained male endurance cyclists participated in a double-blind, pseudo-randomised, triple-crossover protocol. Participants were 37 ± 7.26 years, 81.36 ± 7.67 kg, training at least 8-10 h per week and riding competitively. Mean $\dot{V}O_{2\max}$ and peak power output (PPO) were 4.84 ± 0.46 L \cdot min⁻¹ and 357.33 ± 20.55 W respectively. The effects of a 2-week MCFA-rich diet + ¹³C-enriched MCFA+CHO exercise supplement (MC-MC) on GI distress, MCFA-oxidation rate and sprint performance variables were compared against a 2-week LCFA-rich diet with either: (a) a ¹³C-enriched MCFA+CHO exercise supplement (LC-MC), or (b) a CHO-only supplement (LC-CHO). Dietary and exercise MCFA-rich supplements were consumed in the form of randomised-structured triacylglycerols made with a 3:1 molar ratio of MC- and LCFAs randomly esterified to glycerol backbones. Participants followed a controlled training regime whilst on the diets.

The performance test consisted of a 3-h ride at 50% PPO followed by 10 maximal sprints. At rest and every 20-min throughout the ride, participant ratings of GI and exertion sensations were recorded, followed by external respiratory-gas analysis, collection of a breath sample for breath ¹³C-enrichment analysis, a venous blood sample and ingestion of a supplement.

Similarly, after sprints 1, 4, 7 and 10 participants recorded their GI ratings followed by a blood sample.

Results: Peak MCFA-oxidation rates were $0.38 \text{ g}\cdot\text{min}^{-1}$ (95% CI 0.31-0.47) and $0.43 \text{ g}\cdot\text{min}^{-1}$ (0.30-0.61, p -value = 0.21) in the MC-MC and LC-MC conditions respectively, but there was no evidence for CHO sparing following MCFA adaptation. Participant ratings of GI distress decreased slightly during exercise with 2-weeks of a diet high in MCFAs relative to LCFAs. Ratings of reflux, bloatedness, nausea, and urge to vomit were, respectively, 1.34 (0.88-3.14), 1.03 (0.74-2.27), 0.81 (0.62-1.69) and 0.93 (0.64-2.45) scale units lower in the MC-MC condition relative to LC-MC. The attenuation in GI distress corresponded with a tendency toward increased sprint mean power, which was 3.4% ($\pm 5.9\%$, 0.25) higher in the MC-MC condition relative to LC-MC. However, sprint mean power was still lower in both the MC-MC ($6.8\% \pm 2.8\%$, <0.0001) and LC-MC ($10.4\% \pm 5.5\%$, 0.0004) conditions relative to LC-CHO.

Mechanism covariate analysis illustrated a negative effect of the GI distress marker nausea on sprint performance. For every 1 unit increase in nausea for the MC-MC and LC-MC conditions, sprint power decreased by 6 W (± 3.8 , 0.004) relative to LC-CHO.

Conclusion: No clear metabolic adaptation was evident with high dietary MCFA relative to LCFA. In addition, MCFA-rich exercise supplements caused a decrement in performance relative to CHO ingestion in both MC-MC and LC-MC conditions, suggesting that light-moderate GI distress still causes substantial performance detriments. There was little evidence to support the ingestion of randomised structured triglycerides high in MCFA with the intention of enhancing endurance performance.

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Introduction

The finite availability of endogenous glycogen as an energy substrate is a limiting factor in athlete performance during prolonged exercise (Jeukendrup & Jentjens, 2000; Jeukendrup, 2002). Several dietary supplementation methods have been investigated to increase the supply of, or spare the utilisation of endogenous glycogen; for example, carbohydrate (CHO) loading and ingestion of exogenous-CHO during exercise (Coyle et al., 1986; Jeukendrup et al., 1997; Pitsiladis & Maughan, 1999; Walker et al., 2000). In most cases, these interventions increase the intensity or duration that an athlete can sustain an endurance exercise effort, and thereby increase performance. The mechanisms most consistently linked with enhanced performance under conditions of carbohydrate supplementation are maintenance of blood glucose, increased total-CHO availability and endogenous-glycogen sparing (Coyle et al., 1986; McConnell et al., 1994). An increase in plasma free fatty acid (FFA) and fat oxidation has also been shown to spare endogenous glycogen, most reliably via fat infusion studies (Jansson & Kaijser, 1984; Vukovich et al., 1993). Conversely, long chain fatty acid (LCFA) ingestion has not reliably been shown to enhance performance. The digestion and absorption of LCFAs is slow because they have to be packaged into chylomicrons and transported through the lymphatic system before reaching the blood stream. In addition, LCFAs rely on carnitine palmitoyl transferase (CPT) for transport in to the muscle mitochondria, and the human body already has an abundant endogenous supply stored both in muscle and adipose tissue (Hawley, 2002b; Vukovich et al., 1993; Whitley et al., 1998). Therefore, the body cannot efficiently use exogenous LCFAs for energy metabolism. More recently, research interest has focused on the ingestion of medium-chain fatty acids (MCFAs) as an energy source because they are digested and oxidised much more rapidly than LCFAs (Decombaz et al., 1983; Jeukendrup et al., 1995, 1996, 1998; Van Zyl et al., 1996; Vistisen et al., 2003). One study thus far has found glycogen sparing and a subsequent performance benefit with MCFA supplementation (Van Zyl et al., 1996).

Medium-chain triacylglycerols (MCTs) are lipids with a chain length between 6 and 12 carbons (6-12C). Most of these fats are rapidly hydrolysed in the intestinal lumen and converted into MCFAs that can directly diffuse into the blood stream. Medium-chain fatty acids also cross the mitochondrial membrane of liver and muscle independently of the acyl-carnitine transfer system (Bach & Babayan, 1982). However, some MCTs are incorporated into chylomicrons in the intestinal lumen and very low density lipids in the liver, and therefore take longer to be metabolised for energy (Bach & Babayan, 1982). In contrast, all

long-chain triacylglycerols (LCTs, >12C) are transported in chylomicrons through the lymphatic system and require transporters to enter the mitochondria. Consequently, ingested LCTs take longer to oxidise than MCTs during exercise (Cohen et al., 1971; Greenberger et al., 1966).

Fat and oil products with high MCFA concentrations are currently used in the treatment of fat malabsorption and metabolism difficulties related to AIDS, cystic fibrosis, cirrhosis, and anorexia, and are also used in parenteral formulations for surgery, respiration problems, and severely malnourished patients (Willis et al., 1998; Jeukendrup & Aldred, 2004). Investigation into the effect of MCT ingestion on exercise performance began in the 1980s. Theoretically, ingestion of MCTs may enhance exercise performance by elevating plasma free fatty acid (FFA) levels and sparing muscle glycogen, the latter of which is connected to improved performance (Van Zyl et al., 1996; Jeukendrup, 1997; Vistisen et al., 2003).

Early studies investigating MCT effects on endurance performance required participants to ingest ≤ 30 g of MCT added to a drink supplement. For example, Decombaz et al. (1983) studied the effect of a coffee drink containing 25 g of MCT against a carbohydrate (CHO) equivalent ingested 1-h before exercise. Decombaz and colleagues found no performance enhancement with MCT ingestion compared to CHO alone, with only 30% of exogenous MCT being oxidised. In a later study, Jeukendrup et al. (1995) found that gastric emptying of MCTs is enhanced when ingested with carbohydrate during 3-h of exercise. Approximately 70% of the 30 g MCT co-ingested with carbohydrate was oxidised, in contrast to only 30% in the MCT-only solution (Jeukendrup et al., 1995). The authors suggested that this latter phenomenon could be due to MCTs being more soluble in the presence of CHO, allowing accelerated entrance of MCT into the systemic circulation.

The lack of an observed performance enhancement with MCT feeding in early studies was originally thought to be due to the low doses (20-30 g) of MCT given to subjects, as little or no effects on carbohydrate and lipid oxidation were observed (Van Zyl et al., 1996; Jeukendrup et al., 1995, 1998; Decombaz et al., 1983). However, more recent studies investigating the effects of >30 g of MCT ingestion during endurance exercise have also failed to observe a performance benefit, and reported development of moderate-to-severe gastrointestinal (GI) distress either during or after cessation of exercise (Jeukendrup et al., 1998; Goedecke et al., 1999; Vistisen et al., 2003). To date, only one study has demonstrated a beneficial effect on performance, with supplements of ~86-g MCT co-ingested with CHO during a steady state 2-h cycle followed by a 40-km time trial (Van Zyl et al., 1996). This

level of MCT supplementation resulted in a significant elevation of serum FFA concentration, reduced reliance on muscle-glycogen utilisation, and a 2.5% decrease in time trial time relative to CHO-only ingestion. Participants were not reported to suffer GI distress. In contrast to the results of Van Zyl et al. (1996), Jeukendrup et al. (1998) found no performance benefit when feeding 85 g of MCT in a MCT+CHO emulsion. Unlike the Van Zyl et al. (1996) study, no differences were observed between fat and CHO oxidation with or without MCT ingestion, and participants suffered severe GI distress. Similarly, Goedecke et al. (1999) did not observe a performance benefit, and found a trend towards worse performance with MCT supplementation when investigating low (27.5 g) and high (55 g) MCT supplement doses during exercise. As with previous studies, fat and CHO oxidation in the MCT-supplement conditions were comparable to placebo, indicating no change in substrate use with MCT supplementation. GI distress was not significantly higher in the MCT-supplement conditions than CHO-only, and was reported by participants not to affect exercise performance.

There is evidence that a period of ≥ 7 -d dietary supplementation with long chain fat can cause metabolic adaptations that substantially enhance fat absorption and oxidation during exercise and probably prolong exercise performance in well-trained cyclists (Rowlands & Hopkins, 2002). Similarly, a chronic dietary adaptation period to MCT ingestion may be one way to decrease gastric distress suffered by athletes when ingesting MCTs during exercise. Fushiki et al. (1995) reported metabolic adaptation in mice with 6 weeks of dietary MCT supplementation, which also resulted in increased endurance swimming capacity. With respect to GI distress, human studies have shown a decrease in severity of symptoms at rest with dietary MCT supplementation. Misell et al (2001) studied the effects of a 2-week MCT supplementation period on metabolism during exercise. It was hypothesised that the chronic ingestion of MCT would cause gastric-adaptation, and would therefore cause performance enhancement due to a decrease in GI distress. Though GI distress was not formally assessed, participants reported a decrease in severity of discomfort over the 2-week supplement period. This suggests that adaptation to MCT ingestion did occur, and could increase the likelihood of inducing performance enhancement. However, no increase in performance was observed by Misell et al. (2001) during a run to exhaustion in the MCT condition in comparison to a placebo (LCT) supplement. Unfortunately, the participants were not fed any MCT supplement during exercise, which could explain the lack of performance benefit, as the last feeding was 11 hours prior to exercise, and MCTs are rapidly absorbed and oxidised. In a

similar study, Oöpik et al (2001) supplemented their participants' diets with medium-chain fats for 7 days and found no performance benefit. Again, no MCTs were supplemented during exercise. In addition, one week did not seem to be sufficient to decrease all participants' GI symptoms.

For the most part, the disadvantages of MCT consumption (their toxicity at high concentrations, and potential for inducing systemic acidosis) have been shown to be overcome when ingested at rest by incorporating them into fats and oils in the form of structured triacylglycerols (TAGs) (Sandström et al., 1993, 1995; Willis et al., 1998; Vistisen et al., 2003). Structured TAGs are mixtures of long and medium-chain fatty acids (L- and MCFAs) incorporated on the same glycerol backbone by hydrolysis and re-esterification of the constituent oils. Structured triacylglycerols may be better tolerated because of an altered gastric response to that of identical physical blends of oils that have not been re-esterified (Tso & Crissinger, 2000). Specifically, structured TAGs containing MCFAs in the sn-1 and sn-3 positions and a LCFA in the sn-2 position (creating a MLM triacylglycerol – medium-long-medium) have been shown to be well tolerated and rapidly metabolised when ingested at rest (Sandström et al., 1993, 1995; Willis et al., 1998; Vistisen et al., 2003). Supplements ingested in the form of MLMs during endurance exercise have been investigated by Vistisen et al. (2003). Though there was no clear difference in GI distress ratings between MLM and CHO-only supplements during exercise, some participants experienced diarrhoea and vomiting in the hours following exercise after ingesting the MLM supplements. Despite similar GI distress between MLM and CHO-only supplement conditions during exercise, MLM ingestion did not improve performance. Vistisen et al. (2003) suggested that the lack of performance benefit may have been due to a lack of MCFA appearing in the systemic circulation, which might be altered by dietary adaptation.

Rationale and Hypotheses

Medium-chain triacylglycerols have the potential to spare glycogen during exercise, and thereby increase endurance performance due to their rapid absorption and oxidation. However, previous studies do not show a consistent performance enhancement with MCT ingestion, possibly due to the GI distress experienced by exercising participants with supplementation of greater than 30-g. Although chronic adaptation to an MCT-rich diet and co-ingestion of MCFAs with LCFAs in the form of structured TAGs (relative to MCT alone) have been shown to improve gastric tolerance, these studies still show no performance benefit.

The main purpose of this study was, therefore, to investigate whether 2-weeks of dietary adaptation to MCFA-rich supplements reduces the severity of gastrointestinal (GI) distress markers, or increases the rate of MCFA oxidation during endurance exercise. A decrease in GI distress markers or increase in MCFA oxidation was anticipated to lead to performance benefits.

Literature Review

Fatty acids and glucose are the principal substrates for aerobic energy, in the form of adenosine triphosphate (ATP) re-synthesis in muscle mitochondria (Figure 1) (Spriet, 2002; Van Loon et al., 2001; Brouns & Van der Vusse, 1998). During exercise the oxidation of endogenous substrates increases greatly in order to meet energy expenditure needs. Endurance exercise can cause glycogen stores to deplete, which leads to fatigue and impairs athlete performance. High dietary intake and exogenous supplementation during exercise have been shown to be beneficial to athlete performance by maintaining sufficient muscle-glycogen concentrations (Bergstrom et al., 1967; Jeukendrup et al., 1997; Walker et al., 2000). However, fat supplementation has been less successful, possibly due to limitations on the length of dietary interventions and tolerability of fat intake during exercise.

Fatty Acid Metabolism

Fat is often depicted as the less important fuel, because CHO becomes the dominant substrate during intense aerobic exercise, and fat cannot be used to generate ATP via anaerobic metabolism (Jeukendrup, 1997; Spriet, 2002). In addition, CHO feedings during exercise have repeatedly been shown to delay fatigue, and depletion of the body's glycogen stores has been linked to fatigue onset (Jeukendrup, 2002; Jeukendrup & Jentjens, 2000). The endogenous-glycogen pool decreases in relation to exercise intensity and duration because the rate of glycogen resynthesis becomes insufficient to meet the rate of use. As glycogen levels decrease, so does phosphocreatine and ATP re-synthesis, and so muscle force (number of fibres recruited) must reduce to a rate that equals glycogen synthesis. It is therefore important to maintain sufficient carbohydrate availability during exercise in order to maintain force production (Jeukendrup, 2002; Jeukendrup & Jentjens, 2000). However, the importance of lipids must not be dismissed, as they provide the largest nutrient store of chemical energy used to power biological processes, with the total amount of energy stored greater than 60 times that of glycogen (Horowitz & Klein, 2000). For example, glycogen stores contain ~8-16 MJ of energy, in comparison to fat stores which contain ~192 MJ in an 80 kg person with 15% fat (Jeukendrup & Aldred, 2004), and therefore fat supplementation warrants investigation as a means to spare endogenous glycogen use.

Humans normally (absent of absorption disorders) excrete less than 5% of their fat intake (Tso & Crissinger, 2000). Instead, excess lipid is principally stored as triacylglycerol (TAG)

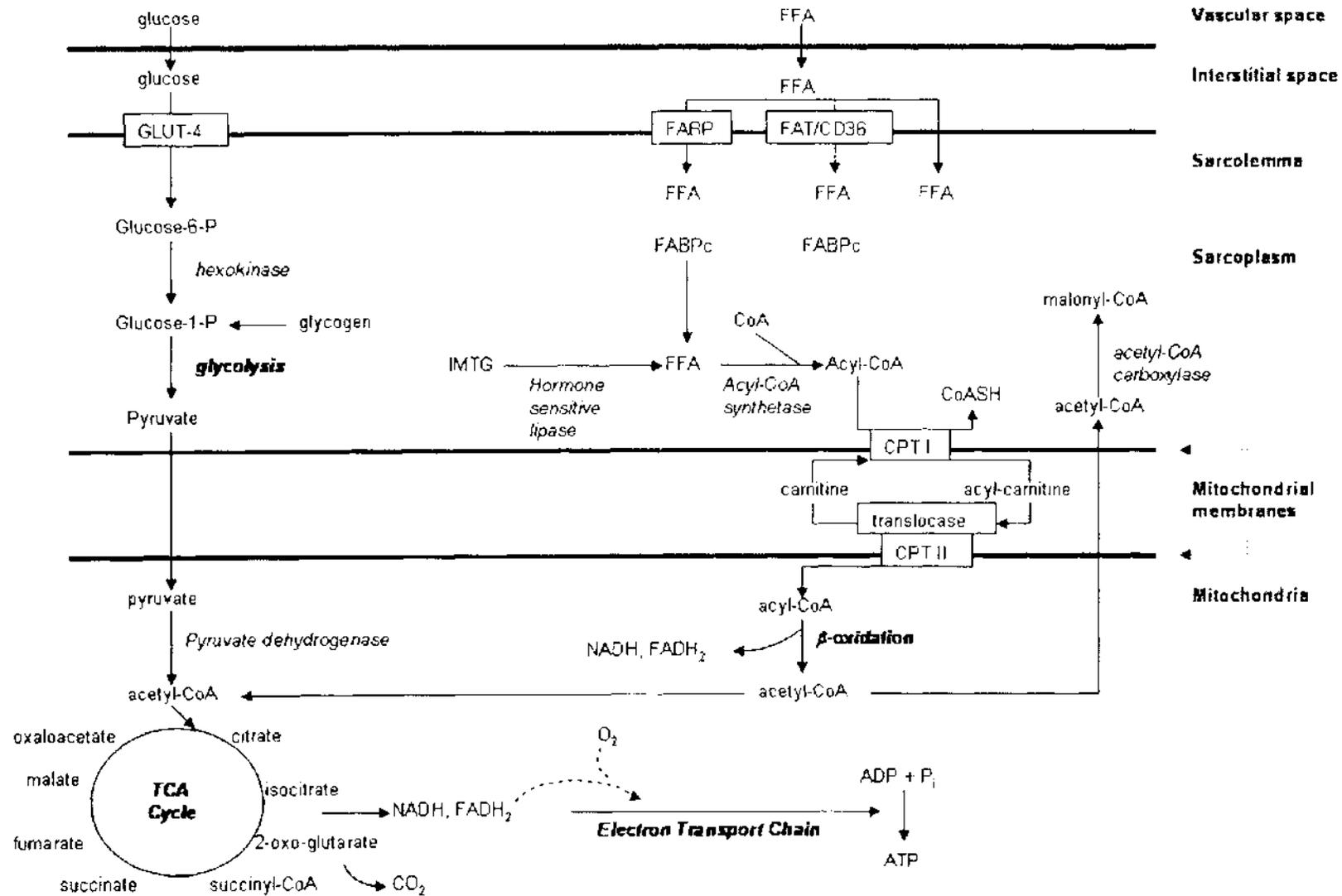


Figure 1. Pathways for transportation of glucose and fat from the vascular space to the mitochondria, and their subsequent oxidation for production of ATP. FABP = fatty acid binding protein; IMTG = intramuscular triglyceride; CPT = carnitine palmitoyl transferase; TCA cycle = tricarboxylic acid cycle (adapted from Jeukendrup, 2002 pp. 222, 225).

in adipose tissue, with small deposits also found within skeletal muscle (intramuscular triglycerides) adjacent to the mitochondria (Figure 1) (Hawley, 2002a; Spriet, 2002). In addition to the large store of TAG, fat is energy dense, with a high-energy yield per unit mass ($\sim 38 \text{ kJ}\cdot\text{g}^{-1}$) in comparison to carbohydrate ($\sim 16 \text{ kJ}\cdot\text{g}^{-1}$). Therefore, an increase in fat and decrease in glycogen utilisation during aerobic exercise potentially increases endurance capacity (duration and/or power output at which exercise can be maintained) via glycogen sparing (Spriet, 2002).

Lipid classification

Lipids are classified according to their FA composition (chain length, degree of saturation) or source. In general, vegetable oils are an important source of unsaturated LCFAs, e.g. linoleic and linolenic acids. Milk fat contains more short chain fatty acids (SCFAs; butyric and caproic acid), MCFAs (caprylic, capric and lauric acid), and more saturated FAs. Marine and algal oils are rich sources of polyunsaturated fatty acids. Canola, soybean and safflower oils are rich in linoleic and linolenic acids, while coconut oil contains high concentrations of caprylic, capric and lauric acids, as well as the saturated LCFAs myristic and palmitic acid. Animal-derived fats e.g. butter and lard, have a much more diverse distribution of FAs, with more emphasis on MCFAs and saturated FAs (Willis et al., 1998).

Digestion and oxidation of fatty acids

The composition and position of FAs in exogenous TAGs can affect the digestibility of fats and oils. In general, hydrolysis of lipids begins with *lingual lipase* in the mouth and stomach, where it hydrolyses TAGs to give monoacylglycerols, diacylglycerols and free fatty acids (Willis et al., 1998). Once fat reaches the stomach, it comes in contact with *gastric lipase*, which is secreted by gastric mucosa and continues to produce monoacylglycerols, diacylglycerols and free fatty acids. Triacylglycerols containing both MCFAs and LCFAs are hydrolysed in the stomach, but SCFAs are soluble in aqueous media and can be absorbed through the stomach directly into the systemic circulation. Some MCFAs are also absorbed directly in the stomach. These FAs travel via the portal vein to the liver and skeletal muscle, where they are oxidised and used as a rapid source of energy, as they do not require carnitine transport into the mitochondria (Willis et al., 1998; Jeukendrup et al., 1998; Tso & Crissinger, 2000; Jeukendrup & Aldred, 2004).

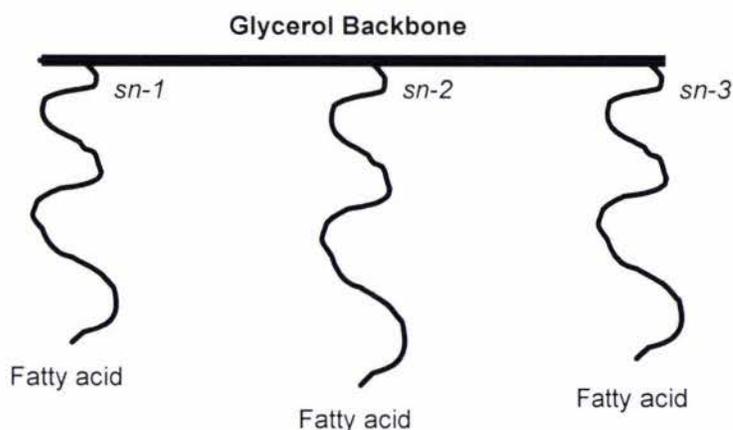


Figure 2. Structure of triacylglycerols. A glycerol backbone binds three fatty acids of any length.

Acid lipases in the stomach hydrolyse TAGs containing both MCFAs and LCFAs, preferentially cleaving FAs in the sn-3 position (Figure 2) of the TAG molecule, regardless of the FA esterified to this position. Grinding and mixing of the gastric contents helps disperse the lipid droplets before the lipid emulsion enters the small intestine (Tso & Crissinger, 2000). Most of the digestion of TAGs is done by *pancreatic lipase* in the lumen of the upper intestinal tract (Figure 3). Pancreatic lipase works at the interface between the oil and aqueous phases, acting mainly on the sn-1 and sn-3 positions of the TAG molecule. The smaller molecular weights of MCTs facilitate the action of *pancreatic lipase*, and are consequently hydrolysed both faster and more completely than LCTs. In the case of mixed TAGs, MCFAs are liberated preferentially. At this stage of digestion, few individual molecules are absorbed by the enterocytes in the intestine due to limited solubility of the lipid digestion products (Bach & Babayan, 1982; Tso & Crissinger, 2000).

To increase the solubility of the lipid digestion products, they are mixed with bile salt micelles, which readily cross the water layer (Tso & Crissinger, 2000). The digested lipids are then presented to the brush border membrane of the enterocytes. Passive uptake of some lipids occur due to the concentration gradient between lipids in the brush border and those in the intracellular compartment of the enterocyte, but specific binding proteins have also been identified that may participate in the uptake process of some lipids. These binding proteins are located at the apical membrane of the small intestinal epithelial cells (Bach & Babayan, 1982; Tso & Crissinger, 2000). Once inside the enterocyte, MCTs are hydrolysed by intestinal lipase and rapidly leave the intestine. In the mucosa, LCFAs are converted into acyl-CoAs by *acyl-CoA synthetase*, which are then re-esterified into TAGs and incorporated into chylomicrons (Figure 3) (Willis et al., 1998; Jeukendrup et al., 1998; Bach & Babayan,

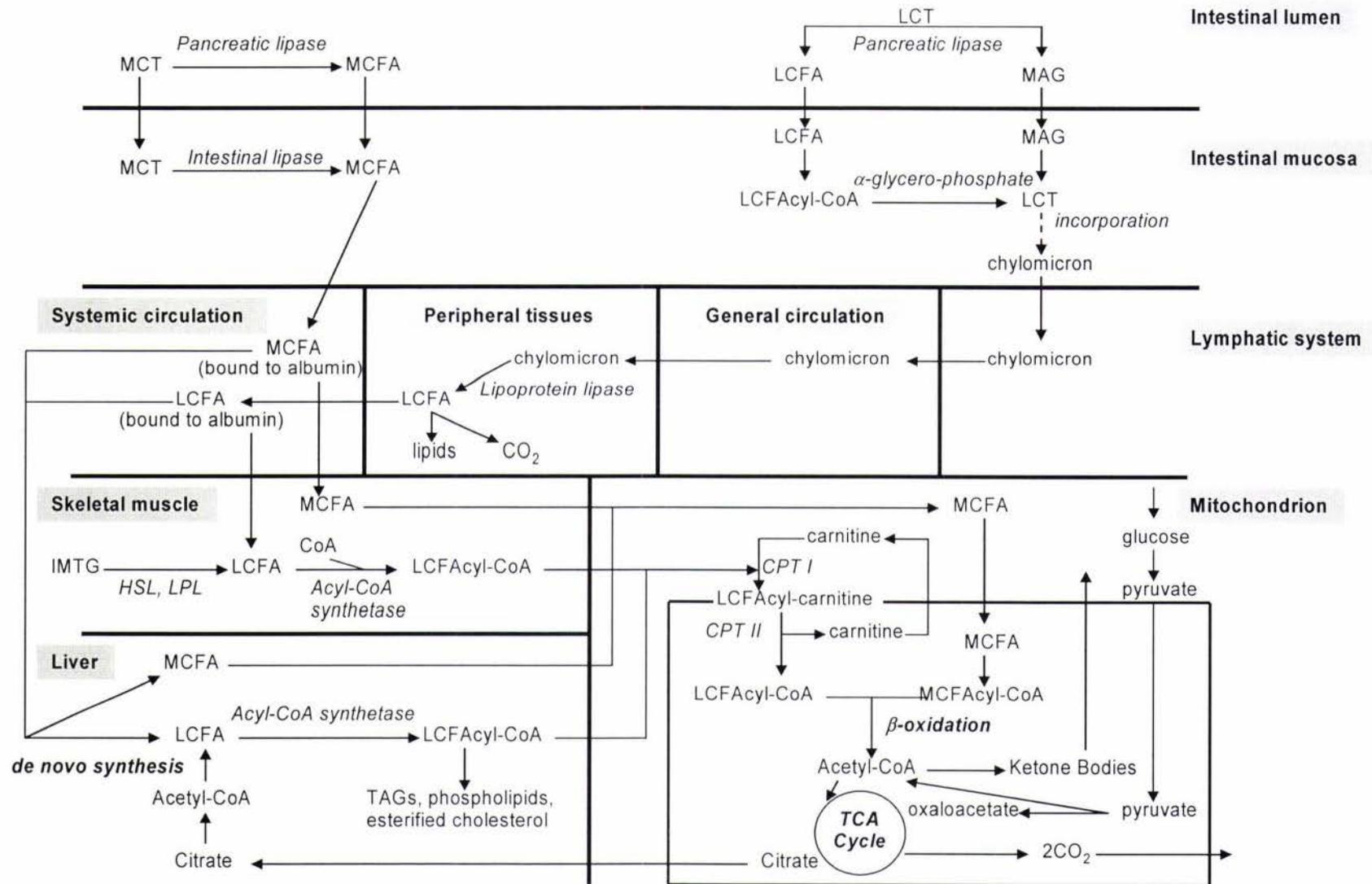


Figure 3. Digestion, absorption, transport and oxidation of long- and medium-chain fats. MCT = medium-chain triacylglycerol; LCT = long-chain triacylglycerol; M/LCFA = medium/long-chain fatty acid; IMTG = intramuscular triacylglycerol; CPT = carnitine palmityl transferase; TCA cycle = tricarboxylic acid cycle; HSL = hormone sensitive lipase; LPL = lipoprotein lipase (adapted from Bach & Babayan, 1986 pp. 951-952).

1982; Tso & Crissinger, 2000). Lipoproteins – chylomicrons, low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs) – are lipid-protein complexes formed by the small intestine and liver for the export of lipids from these organs. Only the intestine secretes chylomicrons (Tso & Crissinger, 2000).

Under conditions where dietary fat is comprised primarily of MCTs, the small intestine can incorporate MCFAs into TAGs that are then transported into the circulation via chylomicrons (Swift et al., 1990). However, there is a preference in the human body for LCFAs to be taken up into chylomicrons, which are then transported through the lymphatic system, which ultimately drains into the systemic circulation for transport to the liver and skeletal muscle. In contrast, MCFAs follow the portal venous system in their soluble form, bound to albumin (Figure 3). Because MCFAs leave the intestinal mucosa by the portal venous system, they reach the liver more rapidly than LCFAs. At rest, the majority of MCFAs are retained in the liver, and only a small amount appears in the peripheral blood for a short time (Bach & Babayan, 1982).

MCFAs, in both liver and skeletal muscle, cross the double mitochondrial membrane very rapidly, where they are acylated by *octanoyl-CoA synthetase*. In contrast, LCFAs, or their acyl-CoA derivatives, can't cross the mitochondrial wall, and require the presence of a carnitine-palmityl transferase (CPT) carrier. In the presence of CPT I, LCFAs are transformed into acyl-carnitines that cross the outer membrane and regenerate LCFacyl-CoAs in the matrix by the action of CPT II (Figures 1 & 3). Both the long- and medium-chain mitochondrial acyl-CoAs then undergo β -oxidation, with production of acetyl-CoA and the reducing equivalents NADH and FADH₂ (Figure 1) (Bach & Babayan, 1982; Brouns & Van der Vusse, 1998; Spriet, 2002; Jeukendrup & Aldred, 2004). In a healthy person, relatively few LCFAs reach the mitochondrial matrix at the same time, but MCTs are rapidly available and oxidised. The result of the abundance of MCT is an excess production of acetyl-CoA, which then follows various metabolic pathways both in the mitochondria (TCA cycle, ketogenesis, elongation of FAs) and in the cytosol of the liver (de novo synthesis of FAs and cholesterol; Figure 3). A fraction of the acetyl-CoA supplied by the excess MCFAs enters the tricarboxylic acid (TCA) cycle and is oxidised into CO₂ and further reducing equivalents (Figures 1 & 3). The liver produces approximately ten times more CO₂ from C8:0 than C16:0, but the capacity of the TCA cycle is limited, and therefore a large part of the acetyl-CoA is directed toward the synthesis of ketone bodies. Medium chain triacylglycerols are much more ketogenic than LCTs (Bach & Babayan, 1982).

The electron transport chain accepts the reducing equivalents produced by the TCA cycle in order to generate proton motive force, which provides the chemical energy used to synthesise ATP from inorganic phosphate (P_i) and adenosine-diphosphate (ADP) in the process of oxidative phosphorylation (Figure 1) (Spriet, 2002). At rest, the role of extra-hepatic tissues in the metabolism of MCTs is small (except for the utilisation of ketone bodies), given the magnitude of hepatic uptake of MCFAs (Bach & Babayan, 1982). However, this is probably not representative of what occurs in exercise due to the increased energy requirements of skeletal muscle.

Regulation of fat and carbohydrate oxidation

Currently, the main sites for regulation of fat metabolism and oxidation during exercise are thought to be: 1) FFA movement across the muscle membrane. Until recently, it was believed that FFAs simply diffused through the lipid bilayer of the muscle membrane into the muscle cell. But there is now strong evidence that a major proportion of FFAs enter the muscle cells via protein transport or facilitation via initial binding to transport proteins (Fatty acid binding protein – FABP, and fatty acid translocase protein – FAT/CD36). This could therefore be a major site of regulation for fat metabolism (Jeukendrup, 2002); 2) Regulation of muscle TAG lipase activity, which determines the rate of TAG degradation to FFA during exercise (Spriet, 2002); 3) Regulation of FFA movement across the mitochondrial membranes via CPT I activity. The carnitine palmitoyltransferase complex consists of CPT I, acylcarnitine transferase, and CPT II and plays a regulatory role in the transport of LCFAs into the mitochondria for subsequent β -oxidation in skeletal muscle (described above). Specifically, CPT I is located on the outer surface of the outer mitochondrial membrane and catalyses the transfer of a variety of long chain fatty acyl groups from CoA to carnitine. CPT I is considered the rate limiting step in the oxidation of LCFAs, and is thought to be influenced by numerous regulators such as malonyl CoA, H^+ and carnitine (Spriet, 2002; Jeukendrup, 2002); 4) Adipose tissue lipolysis and FFA delivery to muscle. In certain situations, FA oxidation is impaired due to the rate of lipolysis failing to meet energy demands of the muscle (i.e. at intensities approximately $\geq 85\% \dot{V}O_{2max}$). However, even when lipid is infused during intense exercise, with concentrations far in excess of muscle requirements, less than 50% of total energy is derived from fat (Hawley, 2002a; Spriet, 2002).

Originally, it was proposed that carbohydrate metabolism was regulated by fat metabolism, thereby explaining the reciprocal relationship between fat and glucose oxidation, and the increase in carbohydrate metabolism seen with a decrease in fat metabolism at high

exercise intensities (Randle et al., 1963). This theory states that an increase in fatty-acid oxidation increases the mitochondrial ratio of acetyl-CoA:CoA. An increase in acetyl-CoA concentration will suppress *pyruvate dehydrogenase* activity directly (responsible for the breakdown of pyruvate to acetyl-CoA in the glucose oxidation pathway), and increase citrate levels, which inhibits *phosphofructokinase* (the rate-limiting enzyme in glycolysis). Inhibition of *phosphofructokinase* would in turn cause accumulation of glucose-6-phosphate in the muscle, which inhibits *hexokinase* activity and thus reduces muscle glucose uptake. According to this theory, the availability of plasma FFAs therefore determines the mix of carbohydrate and fat oxidised in a certain situation (Jeukendrup, 1997, 2002; Van Loon et al., 2001). However, previous studies have not supported this hypothesis during exercise (Dyck et al., 1993; Romjin et al., 1995; van Loon et al., 2001).

A more recent proposal is that carbohydrate metabolism may largely regulate fat oxidation during exercise (Dyck et al., 1993; Sidossis et al., 1997; Van Loon et al., 2001). Three potential sites for carbohydrate inhibition of fatty acid oxidation have been identified. Firstly, malonyl-CoA is formed from acetyl-CoA in the TCA cycle, and is a potent inhibitor of CPT I. It has been proposed that the resting concentration of malonyl-CoA is sufficient to inhibit CPT I, whereas the onset of exercise causes a decrease in malonyl-CoA concentration, allowing CPT I activity to increase, and therefore allowing greater LCFA transport into mitochondria. Alternatively, at high intensities acetyl-CoA concentration in the muscle increases rapidly, which stimulates formation of malonyl-CoA, resulting in reduced FA uptake. However, human studies show little support for malonyl-CoA involvement in suppression of FA metabolism (Sidossis et al., 1996; Odland et al., 1996, 1998; Jeukendrup, 2002). A second potential regulator of FA oxidation may be hydrogen accumulation in the muscle, which has also been proposed to regulate CPT I activity. Sarritt et al. (2000) found that small changes in pH from 7.0 to 6.8, which can be observed during intense exercise ($\sim 80\% \dot{V}O_{2\max}$) in humans, inhibited CPT I activity by 50%. In addition, hydrogen ion accumulation in muscle may be responsible for reduction in intramuscular triacylglycerol hydrolysis due to the influence of the acidic environment on *lipase* enzymes. Therefore the reduction in fat oxidation at very high intensities ($>80\% \dot{V}O_{2\max}$) as well as the small reduction from moderate-high exercise intensities could be explained by the relative decrease in pH. A third hypothesis is that free carnitine availability could become rate limiting for FA transport into mitochondria. This theory proposes that during low intensity exercise the flux through *pyruvate dehydrogenase* (breaks down pyruvate to acetyl-CoA) is lower than the flux

through the TCA cycle, resulting in minimal acetylation of the carnitine pool. However, with increasing exercise intensity, the flux through *pyruvate dehydrogenase* may increase more rapidly than the TCA cycle, resulting in accumulation of acetyl-CoA. In order to free up the CoA, acetyl units are bound to free carnitine, reducing the free carnitine pool. It is thus possible that the reduced rates of fat oxidation are caused by a reduction in transport of FA into the mitochondria because the availability of free carnitine becomes rate limiting (Sidossis et al., 1996; Van Loon et al., 2001; Jeukendrup, 2002). However, there is currently no evidence that this mechanism is important, as the levels of free carnitine observed at high exercise intensities could still support significant rates of FA transport, especially since carnitine is recycled, not consumed (Jeukendrup, 1997, 2002).

Performance Enhancement

Muscle glycogen is one of the main sources of fuel used by skeletal muscle during prolonged aerobic exercise, and depletion of muscle glycogen is thought to be an important determinant of fatigue. Theoretically, if the concentration of glycogen in the exercising muscles falls below a critical concentration when the oxidation of the alternative fuel, fat, is also insufficient to sustain the required rate of ATP production, then work rate will fall to a pace at which the rates of ATP production and use are again in balance (Ivy, 2004; Noakes & Gibson, 2004). In light of this, several attempts to enhance performance have been based on the theory that glycogen sparing will increase the intensity and/or duration that an athlete can sustain exercise. The majority of these methods have aimed to increase the proportion of fat-to-carbohydrate substrate utilisation at high exercise intensities (Burke, Kiens & Ivy, 2004).

Carbohydrate-loading and supplementation

Carbohydrate-loading is based on increasing normal background stores through a high-carbohydrate diet. By contrast CHO supplementation replenishes the body throughout exercise with drinks and/or gels, thereby providing an additional source of carbohydrate for muscle (Burke & Hawley, 2002). Most studies using one or both of the CHO-loading and supplementation methods show a positive effect on performance when exercise duration is 45 min or longer. The mechanism by which these methods exert their effect is believed to be through maintenance of blood glucose, and increased rates of CHO oxidation during exercise rather than endogenous glycogen sparing (Jeukendrup, 2002; Burke & Hawley, 2002; Hargreaves et al., 2003). However, oxidation of ingested glucose or glucose and CHOs is limited to a rate of $\sim 1 \text{ g}\cdot\text{min}^{-1}$, or $1.5 \text{ g}\cdot\text{min}^{-1}$ when multiple-transportable glucose-fructose

mixtures are ingested (Wallis et al., 2005), possibly because intestinal transport mechanisms become saturated at these rates. Therefore, other mechanisms of increasing energy supply have been investigated in an attempt to further improve performance (Jeukendrup & Aldred, 2004).

Fat-loading and supplementation (long-chain triglycerides)

Studies have found little effect of acute LCT ingestion on exercise capacity. Fat supplementation during exercise is believed to be undesirable for several reasons. Firstly, endogenous fat stores are very large, and therefore fat ingested during exercise would not add appreciable TAG to the total body content. Digestion and absorption of LCTs is also slow, and LCTs are known to be potent inhibitors of gastric emptying, thereby inhibiting absorption of any carbohydrates and fluids that may also have been ingested (limiting rehydration and exogenous carbohydrate supply). In addition, glycogen is the essential fuel for the contracting muscle fibre, and oxidation cannot proceed without sufficient supply (Jeukendrup et al., 1997, 1998).

Chronic supplementation of LCTs has been shown to cause metabolic adaptations that enhance fat oxidation. Some of these adaptations occur within 5-6 days and include an increase in intramuscular triglyceride stores, increased fatty acid extraction by muscle, and an increase in the enzymes involved in fat transport and oxidation (Goedecke et al., 1999; Burke & Hawley, 2002; Hargreaves et al., 2003). However, studies investigating adaptation to high-fat diets have been restricted to a maximum of several weeks due to practical limitations and study-design constraints.

Training

Endurance training has been observed to result in a number of structural and metabolic adaptations that increase the relative contribution of lipid to exercise metabolism and attenuate the rate of glycogen utilisation. Training increases the sensitivity of β -adrenoreceptors for catecholamines in the adipocyte, which theoretically promotes delivery of FA from fat cells to blood. Capillary density in the muscles increases, which enlarges the surface area for exchange, promoting blood flow and thereby delivery of oxygen and FA. Training also induces an increase in sarcolemmal fatty acid binding protein, which enhances translocation of FA into muscle. Within the muscle, there is an increase in mitochondrial volume as well as enzyme activity. Trained muscles express higher activities of *lipoprotein lipase*, *muscle lipase*, *fatty acyl-CoA synthase* and *reductase*, *carnitine acyl-transferase*, and

3-hydroxyacyl-CoA dehydrogenase, which enhance FA supply to mitochondria, and subsequent oxidation. As a result, trained muscles are able to oxidise more substrate, which is also expressed as an increase in maximal oxygen consumption ($\dot{V}O_{2\max}$). Finally, trained muscles store more intracellular fat in lipid droplets located close to mitochondria, which theoretically enhances the capacity to supply and oxidise intramuscular triglyceride stores (Johnson et al., 2003; Horowitz & Klein, 2000; Brouns & Van der Vusse, 1998). Training therefore enhances total FA oxidation, especially by increasing intramuscular fat storage, and increasing maximal FA flux. In addition, endogenous carbohydrate stores will be conserved during exercise in endurance-trained athletes, which will increase the duration over which intense exercise can be performed (Johnson et al., 2003; Horowitz & Klein, 2000; Brouns & Van der Vusse, 1998).

MCT Supplementation

Early Studies

Extensive investigation into the effect of medium-chain triacylglycerols (MCTs) on exercise performance began in the 1980s. The first studies tested the effects of MCT ingestion before and/or during exercise in comparison to a placebo supplement. For example, Decombaz et al. (1983) studied the effects of a coffee drink containing 25 g of MCT (in a powdered emulsion with caseinate) against a carbohydrate equivalent. Participants ingested the drink 1-h before cycling for 60 min at 60% $\dot{V}O_{2\max}$. There was no difference in CHO oxidation between the MCT and CHO-feeding trials, indicating that the ingestion of 25 g of MCT before exercise offered no advantage over ingestion of CHO alone. In fact, only around 30% of the MCTs ingested were oxidised during exercise, contributing ~10% to total energy expenditure.

In a study by Jeukendrup et al. (1995), participants performed four trials separated by at least 7 days. Each trial consisted of 3 h cycling at 50% maximal work rate (~57% $\dot{V}O_{2\max}$), during which participants ingested a supplement consisting of either CHO; LCHO+MCT (low CHO); HCHO+MCT (high CHO); or MCT only. Each MCT trial fed a total of 29 g MCT. Oxidation rates of MCT were assayed using gas combustion continuous flow isotope radio mass spectrometry (GC-IRMS) and reached a peak of ~0.12 g·min⁻¹ after 90-120 min of exercise in the CHO+MCT trials, in comparison to the MCT only trial, which showed a linear relationship and reached its highest oxidation rate at the end of exercise (~0.12 g·min⁻¹). In agreement with the results of Decombaz et al. (1983), Jeukendrup et al. (1995) found that

only ~33% of MCTs ingested during exercise were oxidised during the MCT-only trial. However, when co-ingested with CHO, up to 76% of the MCTs ingested were oxidised. Again, there was no indication that exogenous MCT feeding decreased CHO oxidation during exercise, which is evidence against the hypothesis that MCT ingestion may lead to glycogen sparing.

In a follow-up study, Jeukendrup et al. (1996b) investigated the effects of glycogen-depletion versus glycogen-loading on the metabolic response to a CHO+MCT suspension supplement during exercise. Eight highly-trained triathletes and cyclists performed two glycogen depleted (LG) and two glycogen-loaded (HG) trials with supplements of either CHO-only or CHO+MCT (26.6 g MCT total). Participants cycled for 90 min at 50% maximum workload. Peak oxidation rates were reached at the end of the 90-min exercise bout, and attained values of $0.15 \text{ g}\cdot\text{min}^{-1}$ in the LG condition, and $0.13 \text{ g}\cdot\text{min}^{-1}$ in the HG condition. Exogenous MCT oxidation contributed up to 7.6% and 6.5% of total energy expenditure, representing 85% and 69% of the total amount ingested in the LG and HG conditions respectively. Substrate utilisation between the LG and HG conditions was significantly different, but not between the CHO and CHO+MCT supplementation protocols within the LG and HG conditions. That is, CHO utilisation over the 90 min exercise period was significantly higher in the HG condition at 63% (CHO) and 53% (CHO+MCT) of total energy expenditure, in comparison with 37% (CHO) and 33% (CHO+MCT) in the LG condition. Therefore, the results did not indicate any influence of MCT ingestion on total CHO utilisation, which was in agreement with previous studies.

The main criticism of these early studies on MCT supplementation has been that a 20-30 g dose of MCT was not enough to influence fat metabolism sufficiently to cause glycogen sparing (Van Zyl et al, 1996; Jeukendrup et al, 1998; Goedecke et al, 1999). Although MCTs were rapidly oxidised, they contributed only marginally to total energy expenditure, and no rise in plasma fatty acids was observed after ingestion.

Increased Rates of MCT Ingestion

The only study to date that has shown a performance enhancement with MCT supplementation was by Van Zyl et al. (1996). In this study, six endurance-trained cyclists participated in a three-way crossover study consisting of 2 h cycling at 60% peak $\dot{V}O_2$, followed immediately by a 40-km time trial. Drink supplements consisted of either a 10% short-chain glucose polymer, an isocaloric 4.3% MCT emulsion, or a 10% CHO plus 4.3% MCT mixture. A total of ~86 g of MCT was ingested with the MCT supplements. The main

finding of this study was that time-trial performance was $\sim 2.5\%$ faster in the MCT+CHO condition relative to CHO-only ($p < 0.05$), and performance times in the MCT-only condition were $\sim 7.9\%$ slower than CHO-only ($p < 0.001$). Total rates of CHO oxidation were lower in the MCT and MCT+CHO trials in comparison to the CHO-only trial after 90 min of exercise. In the CHO+MCT trial, this was not due to a reduced rate of plasma glucose oxidation, and was therefore attributed to a decrease in the direct and/or indirect oxidation of muscle glycogen. This is in contrast to the findings of Decombaz et al (1983) and Jeukendrup et al (1995), who reported no measurable effect on CHO oxidation rate. The authors theorised that this was due to the large differences in total MCT ingested over the course of exercise (≤ 30 g in comparison to 86 g). It was concluded that the increase in performance in the CHO+MCT trial could be attributed to decreased reliance on CHO oxidation at comparable work rates, and less accumulation of hydrogen ions and lactate. It should, however, be noted that the contribution of MCT oxidation to total energy was $\sim 10\%$, which is akin to what was observed in studies feeding 20-30 g of MCT. Also, the rate of MCT oxidation was not directly measured, but was estimated to be less than $0.2 \text{ g}\cdot\text{min}^{-1}$, which is well below the feeding rate of $0.5 \text{ g}\cdot\text{min}^{-1}$, and approximately the same as that observed in previous studies feeding only ~ 30 g of MCT. These estimates suggest that there was no difference in the oxidation rate of MCTs in the Van Zyl (1996) study in comparison to that of previous studies feeding less than half the amount and, therefore, it may not have been the MCT ingestion that led to glycogen sparing, and performance enhancement. In fact, Jeukendrup et al. (1998) suggest that there may not have been any glycogen sparing at all, as the results may have been influenced by high plasma ketone levels. Elevated plasma ketone concentrations can fixate O_2 in the production of β -hydroxybutyrate, which affects $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ readings, and can therefore decrease estimation of CHO oxidation rates, which could be interpreted as glycogen sparing.

In an attempt to replicate the findings of Van Zyl (1996), Jeukendrup et al. (1998), investigated the effects of supplementing solutions of either CHO-only, CHO+MCT, MCT-only, or water (placebo) on performance in a crossover protocol. Both MCT supplements fed a total of 85 g over the course of exercise. Seven participants cycled at $60\% \dot{V}\text{O}_{2\text{max}}$ for 2 h consuming one of the four supplements, which was followed by a time trial of ~ 15 -min duration at $75\% \dot{V}\text{O}_{2\text{max}}$. No clear differences were observed between CHO and CHO+MCT conditions – on average, participants completed the time trial $\sim 1\%$ faster in the CHO+MCT condition. However, time trial performance was $\sim 18\%$ slower in the MCT-only condition. In addition, no significant differences in total fat oxidation or CHO oxidation were

found between conditions. However, there was a trend toward higher fat oxidation in the CHO+MCT condition in comparison to CHO-only. Unfortunately, exogenous MCT oxidation was not quantified (only total fat oxidation), therefore the authors were not able to determine whether the trend was due to greater exogenous MCT oxidation, or an increase in endogenous fat oxidation due to increased capacity of the TCA cycle with CHO co-ingestion. The results of Jeukendrup et al.'s study (1998) contrast with that of Van Zyl et al. (1996). Two reasons suggested for the distinct differences in findings are, firstly, the severe gastrointestinal distress reported by the participants in Jeukendrup et al.'s (1998) study, whereas Van Zyl et al. (1996) reported no distress. Secondly, the difference in duration of the performance test (~1 h for van Zyl, ~15 min for Jeukendrup) may have led to the discrepancy. That is, the 15 minute performance bout used by Jeukendrup and colleagues may not have been long enough to observe a performance benefit with MCT ingestion.

Following the conflicting results of Van Zyl (1996) and Jeukendrup's (1998) studies, Goedecke et al. (1999) investigated the effects of ingesting different quantities of MCT on GI symptoms, CHO oxidation during exercise, and performance. Nine male cyclists rode for 2 h at 55% peak power output, immediately followed by a simulated 40-km time trial in three successive experimental conditions. During exercise, participants consumed a supplement of either a CHO only solution, CHO+LoMCT emulsion, or CHO+HiMCT emulsion. Total MCTs ingested over the course of exercise were 27.5 g in the LoMCT and 55 g in the HiMCT condition. No change in the rate of total CHO and fat oxidation was observed during the constant load exercise, despite increased β -hydroxybutyric acid concentrations when ingesting MCT. In keeping with other studies, MCT ingestion did not improve cycling performance; in fact, a trend toward worse performance was evident. Also in accordance with previous studies, participants experienced GI distress (belching, bloating, and nausea) during exercise, especially in the HiMCT condition. However, the authors reported that the GI distress did not contribute to any decrement in performance, as rated by participants on a scale of 0 (no symptoms) to 5 (severe enough to interfere with performance). It was suggested that the large CHO-rich meal given to the participants 2 h before exercise could have eliminated any glycogen-sparing effect of the MCT supplements, and could therefore have eliminated any performance benefit of MCT ingestion. However, the HiMCT condition only fed ~55 g throughout exercise, 30 g less than that fed by Van Zyl (1996), which is the only study thus far to have reported a performance benefit with MCT ingestion. Therefore the participants may not have ingested enough MCT during the 2 h steady state ride to induce a performance benefit. It may have been more beneficial to add a third MCT-feeding condition in which ~85

g of MCT was supplemented during the steady state ride, as this is a dose that would have been comparable to Van Zyl's study (1996), and would have allowed a direct comparison of MCT oxidation, GI distress and performance with low, medium, and high doses of MCT.

Chronic MCT Ingestion

There is evidence that a period of adaptation (>7 days) to a high-fat diet causes metabolic adaptations that substantially enhance fat oxidation during exercise and result in glycogen sparing (Burke & Hawley, 2002). Fushiki et al. (1995) reported a metabolic adaptation to 6 weeks of MCT supplementation in both trained and untrained mice. Mice fed a diet containing MCTs had increased plasma β -hydroxybutyrate concentrations, resulting in an increase of >20% in the activity of 3-keto-acyl-CoA transferase, an enzyme responsible for muscle oxidation of plasma ketones. These adaptations were associated with 20% greater muscle glycogen concentration following 30 min of forced swimming and a 10% longer swim to exhaustion compared to control mice.

Based on the findings of Fushiki et al. (1995), Misell et al. (2001) investigated the effects of a 2-week dietary adaptation period to an MCT supplement. It was hypothesised that the chronic ingestion of MCT would induce metabolic adaptation and cause performance enhancement. Twelve endurance-trained runners completed two 2-week dietary trials ingesting supplements made with either MCT or LCT (corn) oil emulsions. Participants were instructed to consume two drink supplements per day. In total, the participants consumed $60 \text{ g}\cdot\text{d}^{-1}$ of MCT oil in the MCT condition. Participants experienced GI symptoms when on the MCT supplement (diarrhoea, flatulence, cramping), but reported that these symptoms diminished or ceased throughout the 2-week supplementation period. Following the 2-week adaptation, participants completed a performance test in the lab, consisting of a 30-min run at 85% $\dot{V}O_{2\text{max}}$, after which the intensity was reduced to 75% $\dot{V}O_{2\text{max}}$ until voluntary exhaustion. In contrast to Fushiki et al. (1995), no performance benefit was found following the MCT adaptation period. However, this may be because the last supplement was given approximately 11-h before the performance test the next morning. The theory behind MCT supplementation is that it is rapidly absorbed and oxidised for energy, it is therefore likely that most or all of the MCT supplement ingested the night before had been used before or within the first few minutes of exercise, and would not contribute appreciably to the performance as a whole. Unfortunately, no direct measures of exogenous MCT oxidation were taken throughout the exercise bout, so the contribution of MCT oxidation to energy expenditure during exercise could not be calculated.

In a similar study to Misell et al. (2001), Ööpik et al. (2001) recruited seven male endurance-trained runners to supplement their diet with $\sim 34 \text{ g}\cdot\text{d}^{-1}$ MCT or placebo (flavoured commercial cooking oil) for 1 week. At the end of the week, participants completed a performance test, which consisted of a run to voluntary exhaustion on a treadmill at $\sim 80\text{-}85\%$ $\dot{V}O_{2\text{max}}$ ($\sim 1 \text{ h}$). In agreement with the results of Misell et al. (2001), Ööpik et al. found no performance benefit with dietary adaptation to MCTs in comparison to a pre-adaptation performance trial, and post-placebo adaptation performance. Five of the seven participants experienced mild to severe GI problems during the MCT supplementation period. Two of the five that experienced GI distress found that the symptoms decreased over the adaptation period. The results from these two studies indicate that some people may not be able to tolerate MCT ingestion as well as others, and also that an adaptation period may increase an individuals' tolerance level. If this is the case, had the adaptation period been longer, the other three participants may also have experienced reduced GI distress, as was found by Misell et al. (2001). In addition, the authors do not state that any supplement was given during exercise, which may explain the lack of performance enhancement.

Co-ingestion of Long and Medium-Chain Fats: structured triacylglycerols

Medium- and long-chain fatty acids can be chemically or enzymatically esterified to the same glycerol backbone, thereby forming a 'structured triacylglycerol'. When long chain fatty acids in the *sn*-1 and *sn*-3 positions are replaced with medium chain fatty acids, the resulting 'medium-long-medium' (MLM) structured triacylglycerol (TAG) is thought to combine the benefits of both the L- and MCFAs (Vistisen et al., 2003). That is, the MCTs provide a ready energy source due to their rapid absorption and oxidation, and the LCTs provide adequate intake of essential fatty acids and induce the normal GI response to fat ingestion (not produced by MCTs) (Mott et al., 1972; Iber, 1974; Bremer, 1980; Bach & Babayan, 1982). Supporting evidence for the beneficial effects of structured TAGs relative to LCT or MCT feedings alone comes from parenteral feeding of postoperative patients, which have shown a decrease in hyperlipidemia and ketosis, and an increase in whole body fat oxidation with ingestion of structured TAGs (Sandström et al., 1995; Lindgren et al., 2001). Therefore, supplementing MLM structured triacylglycerols could reduce the GI distress suffered by participants during and following exercise. Based on this theory, Vistisen et al. (2003) investigated the effects of ingesting MLM structured triacylglycerols on exercise performance, GI distress and plasma fatty acid species (chain length). Seven trained male cyclists participated in a crossover study, consuming either a CHO-only or CHO+MLM

supplement during 3 h of steady state cycling at 55% $\dot{V}O_{2\max}$ followed by a standardised 800 kJ self-paced time trial. A total of 62-85 g of MCT was ingested during the MLM condition. At 1-h into the steady state ride, a significantly lower RER was observed in the MLM condition in comparison to the CHO condition, thereby indicating higher overall fat oxidation in the MLM condition. However, this measurement does not distinguish between endogenous vs. exogenous fats, or LCT vs. MCT. Despite an increase in fat oxidation, no difference in performance was observed between the two conditions. Furthermore, although GI ratings were similar between the two conditions during exercise, participants experienced diarrhoea and vomiting in the hours following the MLM supplementation condition. However, this might not occur with dietary adaptation, as the body may become better at processing and absorbing structured TAGs and the resulting M- and LCFAs.

Methods

Experimental Procedures

Participants.

Nine well-trained male cyclists participated in this study. Participants were 37 ± 7.26 years, 81.36 ± 7.67 kg, training for at least 8-10 h per week and riding competitively. Mean $\dot{V}O_{2\max}$ and peak power output were 4.84 ± 0.46 L \cdot min⁻¹ and 357.33 ± 20.55 W respectively. Two to three participants came in to the lab at one time for testing. After reading the study information sheet, all participants were screened for precluding health conditions with a General Health Questionnaire (Appendix 1) and signed a consent form before beginning experimentation. Participants were also informed of their right to drop out of the study at any time for any reason, and of their right to privacy of their personal details and results. The experimental protocol of this study was approved by the Massey University Ethics Committee, Wellington Protocol number 03/1 43.

Experimental Design

General Design

All nine participants completed a double-blind, triple-crossover nutritional intervention protocol in pseudo-random order (Figure 4). The experimental conditions were: (a) *Placebo Condition* – LCFA-rich diet with experimental MCFA-rich esterified oil in exercise supplements (LC-MC); (b) *Intervention Condition* – MCFA-rich diet with experimental MCFA-rich esterified oil in exercise supplements (MC-MC); (c) *Control Condition* – LCFA-rich diet with carbohydrate control in exercise supplements (LC-CHO). Each experimental condition consisted of a 2-week dietary supplementation period, background trial, and performance test. Participants were given a 2-weekly training protocol to complete, including three-to-five supervised and workload-standardised lab sessions per 2 weeks where they ingested 500 ml of the dietary supplements during exercise. On the morning of each background and performance test, participants reported to the lab between 6-8 am in a fasted state, and were instructed not to drink any alcohol or perform exhausting exercise the day before.

Training and Diet

All participants began a standardised training regime 2 weeks before the baseline 3-h ride and performance test. The training protocol was individually based on what the participant would normally do in a 2-week block, and was repeated for each subsequent 2-week dietary intervention. In addition, participants were instructed not to eat any foods high in ^{13}C content beginning immediately following the baseline test in order to minimise the background ^{13}C signal. This included all sugar cane and corn-based foods.

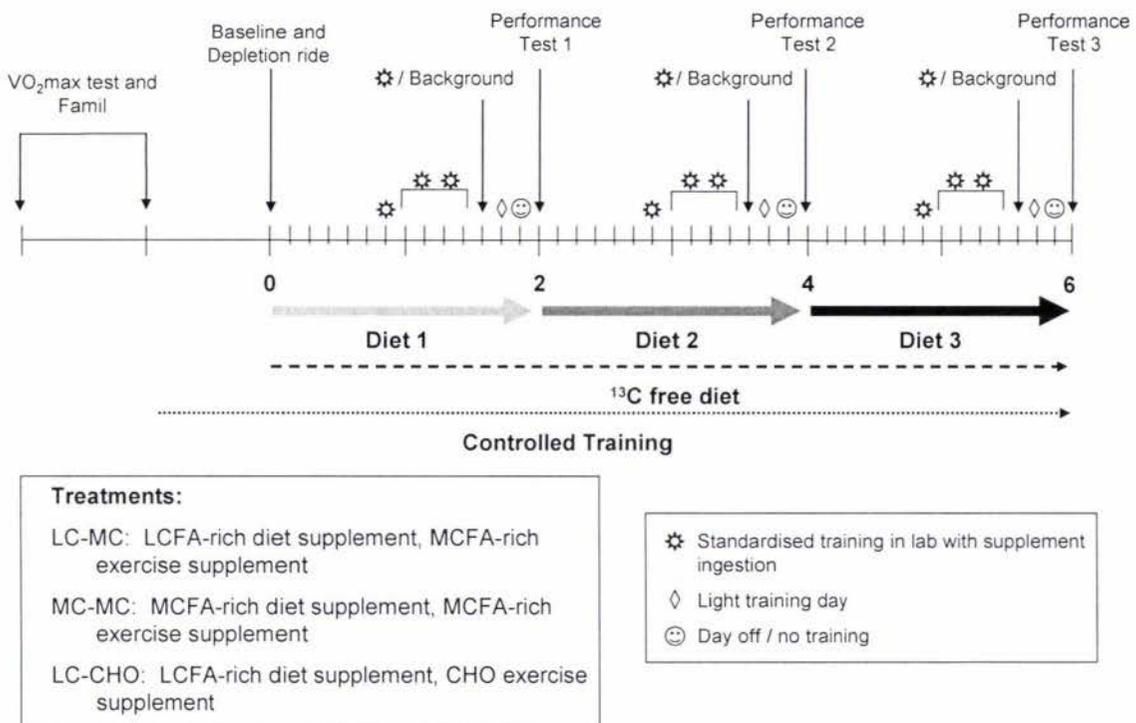


Figure 4. Study design. All nine participants completed a double-blind, randomised, triple crossover protocol consisting of three 2-week dietary supplement regimes followed by a performance test where they ingested either a treatment (MCFA-rich) or placebo (LCFA-rich) exercise supplement.

Protocols

Prior to each test, the parameters of the cycle ergometers were adjusted according to the subjects own racing cycle angles (seat height, handle bar height, seat-to-handlebar length).

$\dot{V}O_{2\max}$ Test. Maximal oxygen uptake ($\dot{V}O_{2\max}$) and Peak Power Output (PPO) were measured using a progressive exercise protocol on an electronically-braked cycle ergometer (VeloTron Racer Mate, Seattle, WA, USA). After a warm-up deemed sufficient by the participant, each subject started cycling at a workload of $3.33 \text{ W}\cdot\text{kg}^{-1}$ body mass for 150 s. After the first 150 s the load was increased by 50 W, and then by 25 W for every subsequent

150 s until exhaustion (Hawley & Noakes, 1992; Van Zyl et al., 1996). Exhaustion was determined by voluntary exhaustion, at which time the participant could no longer maintain a pedal cadence of 50 RPM after 3 warnings. Maximal oxygen uptake, using 20-s averages, was measured with a SensorMedics Vmax Spectra Series on-line gas analyser (SensorMedics Corp., Yorba Linda, CA, USA), and 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.

Familiarisation. Performed approximately one week after the $\dot{V}O_{2\max}$ test, the familiarisation session was a shortened version of the performance test, consisting of 2-h steady-state cycling at 50% PPO, plus 6 maximal-effort sprints interspersed with recovery periods at 40% PPO (Figure 5). The sprints and recovery periods were based on calories expended, the amount of which were determined by multiplying each participant's PPO by a factor of 0.125. Pilot trials determined that this would take the participants approximately 2-3 min to complete each sprint, and allow approximately 5-6 min recovery. Participants ingested a CHO-based solution every 20-min for familiarisation to what they would be required to ingest during the 3-h post-treatment test and performance sprints.

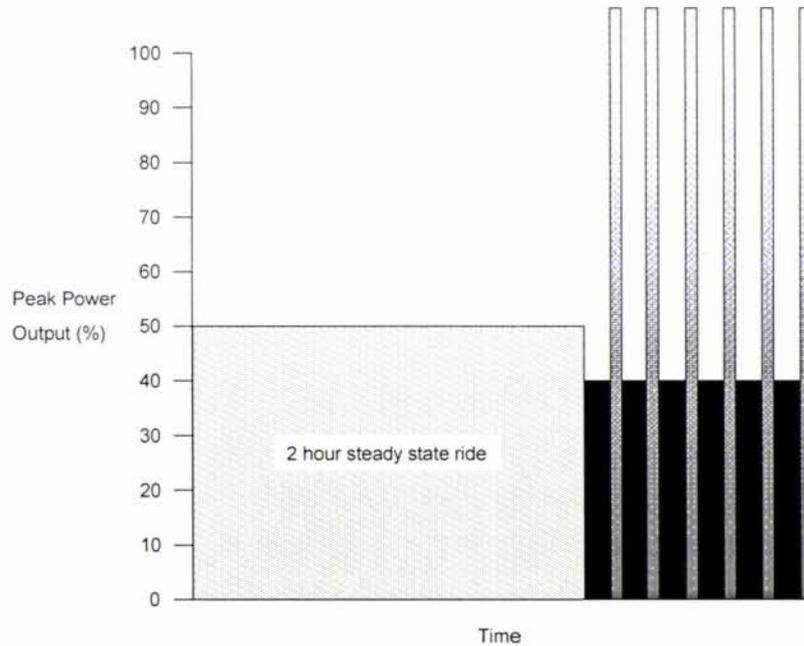


Figure 5. Familiarisation protocol. Shortened performance test consisting of a 2-h steady-state ride at 50% PPO plus 6 maximal effort intervals interspersed with 40% PPO recovery periods.

Baseline Exercise Test. One week after familiarisation, the participants began experimentation with a Baseline test which acted as a second, full, familiarisation (3-h steady

state at 50% PPO plus 10 maximal sprints; Figure 6), a ride to deplete endogenous glycogen stores to lower the natural breath ^{13}C background (^{13}C -depletion ride), and an initial naive exposure to MCFA-rich esterified oil ingestion. An exercise supplement consisting of a CHO + fat (randomised structured MCFA + LCFA triacylglycerol) emulsion was ingested every 20-min (see Exercise Supplements below for further information).

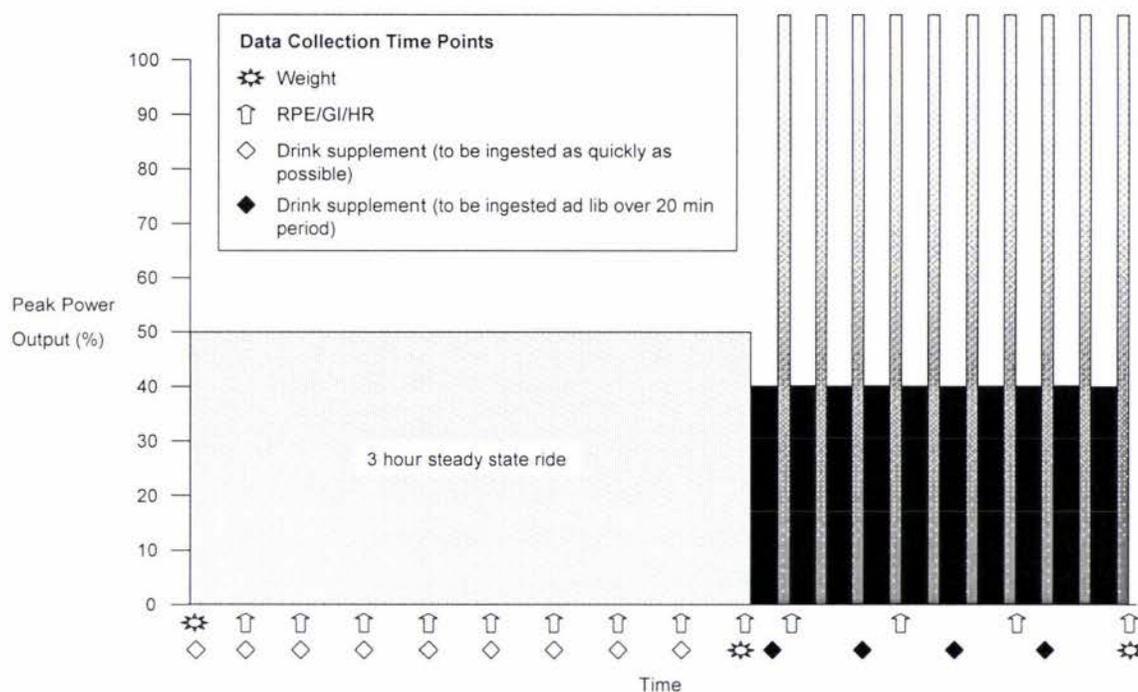


Figure 6. Baseline test protocol. Full performance test consisting of a 3-hour steady-state ride followed by 10 self-paced maximal effort sprints.

Immediately before the start of exercise, participants were weighed, and ingested a double quantity of supplement. Every 20 min throughout the 3-h steady-state ride, the participants filled out GI distress and RPE scales before ingesting the next bolus of supplement. Heart rate was also recorded at these time points. During the sprints, participants ingested each drink supplement throughout the 20 min as they liked, and reported GI and RPE immediately after sprints 1, 4, 7 and 10.

Dietary Supplementation. Immediately following the baseline test participants started their first 2-week dietary supplementation period (LC-MC, MC-MC or LC-CHO). The amount of dietary supplement food ingested by each participant was determined by body mass, relative to a model 75-kg male cyclist ingesting 90 g of supplement fats per day.

Background Test. A 3-h steady-state (50% PPO) background test was performed on day 11 of each 2-week supplementation block (Figure 7). The purpose of this test was to

determine the background ^{13}C -enrichment of the participant's breath at each sample point. Upon coming in to the lab, participants were asked to toilet and were then weighed. Participants then mounted their assigned ergometers and were asked to breathe naturally through a mouthpiece connected to a 2-way valve (Hans Rudolph Inc., Wyandotte, Kansas City, USA) and 1-m outflow tube, which was connected to a 5-L mixing chamber. Expired air was passed through the mixing chamber for 2 min, after which time triplicate breath samples were collected into 10-ml Labco Exetainer tubes (Labco Ltd, High, Wycombe, UK) via a 20-gauge vacutainer system needle positioned near the outlet of the mixing chamber. Participants then ingested their first drink supplement (double quantity) before starting exercise. Every 20-min during the 3-h ride participants marked GI and RPE scales, a 4 min respiratory gas sample was collected (SensorMedics Corp., Yorba Linda, CA, USA), a 1-min mixing chamber breath sample was collected, and participants ingested a drink supplement, following this order. The drink supplements for the background test consisted of the treatment drinks minus the MCFA-rich and LCFA-rich oils (Appendix 2).

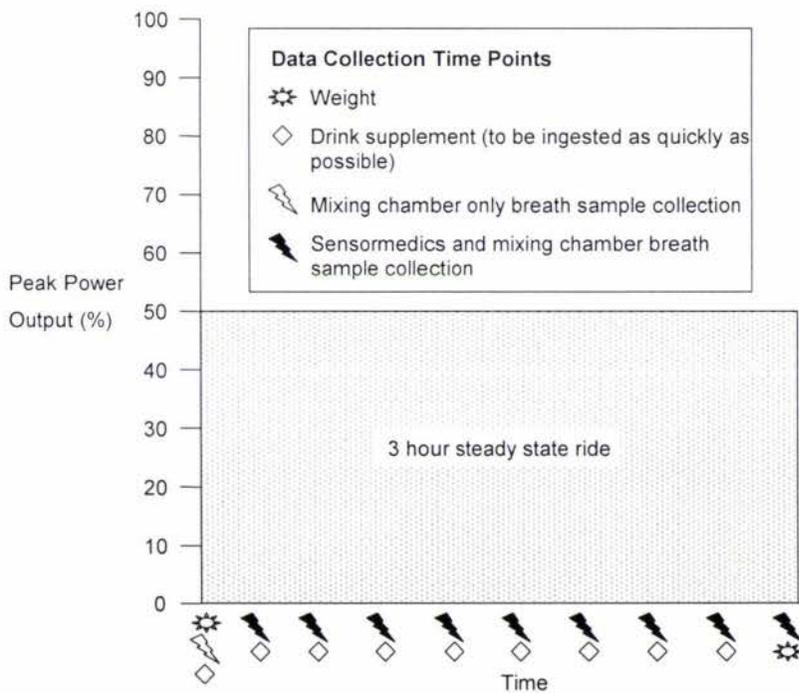


Figure 7. Background protocol.

Post-treatment Exercise and Performance Test. On day 14 of each 2-week supplementation period participants completed an exercise test consisting of a 3-h steady-state ride using the VeloTrons constant power mode set at 50% PPO, primarily for assessment of the physiological responses to the 2-week dietary interventions, but also as a long

preload/depletion ride. The preload was followed by 10 maximal-effort sprints interspersed with 40% PPO recovery periods (Figure 8). The VeloTron was changed to self-selected resistance mode during the sprints, which simulated gearing as if the participant was riding on the road. The power output of the Velotron during both constant power and self-selected resistance modes was verified against 12-gauge SRM cranks (Rudolph Schulten, Jülich, Germany). Power was between 0-1.5% of the SRM over the range of workloads tested (50-700 W). Participants were instructed not to exercise at all the day before a performance test, and to come in to the lab in a fasted state.

A performance test consisting of sprints was chosen over a time trial or cycle to exhaustion because this more accurately simulates a cycle race that would occur in the field, and would provide an assessment of the GI response during all-out cycling efforts. Repeated sprints have been shown by Watt et al. (2002) to have a between-trial mean power CV of 1.2 to 2.8%. In addition, Schabort et al. (1998) have shown that well-trained cyclists are able to reproduce race times during a prolonged bout of exercise with the inclusion of regular sprint intervals, with a CV of 0.61 to 4.1%.

Upon coming in to the lab, participants were weighed before having a cannula (BD Insyte 20GA, 1.2 x 25 mm cannula, Becton Dickinson Medical Pte Ltd, Singapore) inserted into the antecubital vein of their right forearm with a 2-way stop-cock valve (Connecta Plus 3, Becton Dickinson Medical Pte Ltd, Singapore). A 15-ml blood sample was taken immediately following cannulae placement and transferred into 10-ml and 5-ml EDTA Vacutainers, and a 2-ml Lithium Heparin Vacutainer (Becton Dickinson & Co., Franklin Lakes, USA). A resting mixing chamber breath sample for ^{13}C analysis followed the blood sample, which was then collected in triplicate into 10 ml Labco Exetainers (Labco Ltd., High Wycombe). A Polar Heart Rate strap and monitor (Polar Heart Rate Monitors, Carnegie, VIC) was worn by each participant during the 3-h and sprint tests. The monitor was started in conjunction with exercise and recorded heart rate at 5-s intervals. Immediately before exercise began, participants consumed a double bolus of drink supplement (either $1\text{-}^{13}\text{C}_1$ -enriched MCFA-rich drink, or CHO placebo).

Every 20-min throughout exercise several data variables were collected in the order of RPE and GI scales, 4-min SensorMedics breath sample (SensorMedics Corp., Yorba Linda, CA, USA), 1-min mixing chamber breath sample, and blood sample (15 ml every hour, 5 ml every intervening 20 min). Immediately following data collection participants ingested another drink supplement. Weight was recorded following the 3-h steady-state ride, and participants were allowed to toilet and stretch to ready themselves for the performance test.

Weight was recorded again before beginning the sprints. Each recovery and sprint interval was based on calories burnt, calculated as $0.125 \times \text{PPO}$, which was determined by pilot trials to take participants approximately 2-3 min to complete each sprint, and 5-6 min to complete each recovery interval. Participants completed 20 intervals in total, 10 recovery intervals at 40% PPO and 10 maximal effort sprints. Because no breath data was collected during the performance test, participants ingested either non- ^{13}C -enriched MCFA-rich supplements or a CHO placebo every 20-min throughout the test, which the participants could drink ad lib with the condition that they finish before the next supplement was due. Immediately after sprints 1, 4, 7 and 10, RPE and GI distress data were collected. Blood samples (15 ml) were also taken from six of the nine participants after sprints 1, 4, 7, and 10. A final weight measurement was made after sprint 10.

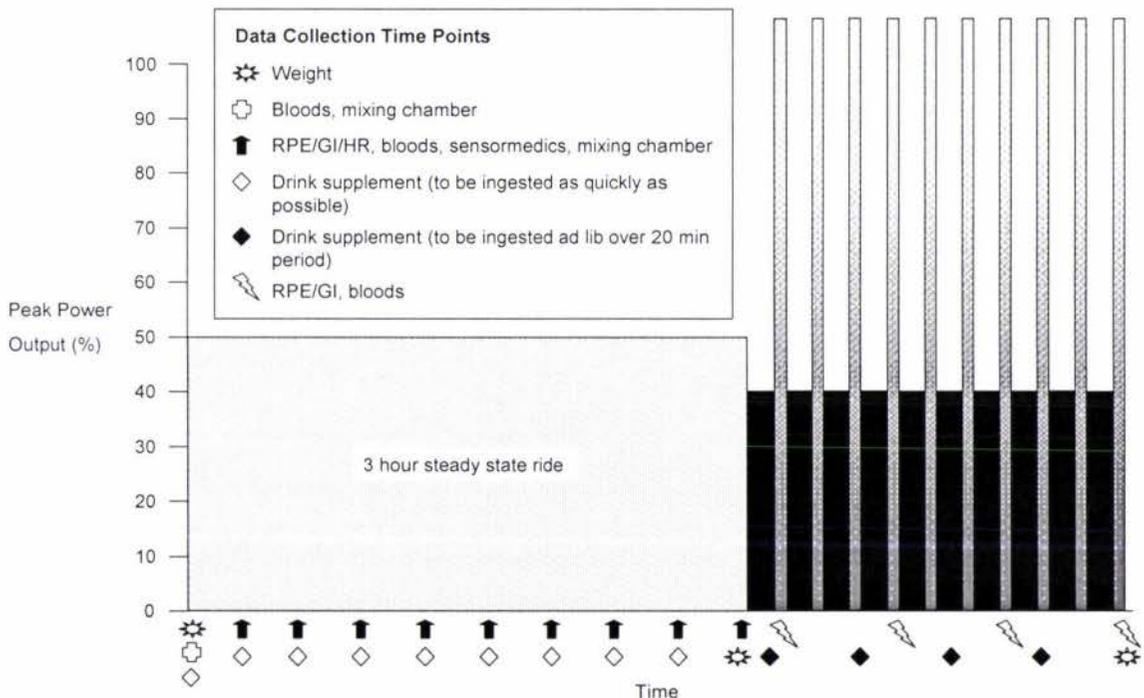


Figure 8. Exercise test and performance protocol.

Diet and Exercise Supplements

Exercise Supplements

During the performance tests, participants consumed one of two different drink supplements. In the LC-MC and MC-MC conditions participants ingested an exercise supplement containing randomised structured lipids (see Appendix 3 for production details), carbohydrate (wheat-derived dextrose and maltodextrin – natural ^{13}C -enrichment), milk

protein (sodium caseinate), salt, colours, chocolate and vanilla flavouring. The supplements for the 3-h steady state ride contained a 1- $^{13}\text{C}_1$ octanoic acid tracer, which was incorporated into the randomised oil (see Appendix 4 for production details). In the LC-CHO condition participants ingested an equi-carbohydrate supplement containing the same ingredients as above, minus the randomised oils, and with addition of a milk powder to emulate colour and taste of the MCFA-rich supplement (see Appendix 2 for recipe details). The supplement for the background test was the same for all conditions, consisting of a drink identical to the MCFA-rich supplement minus the randomised oil and ^{13}C tracer.

The quantity of supplement to be ingested was based on PPO, with a reference of 220 ml·20 min $^{-1}$ for a person with a PPO of 400 W. This quantity was determined from pilot trial evidence that this was the maximum amount of MCFA tolerated by participants without development of severe GI distress e.g. vomiting, severe nausea, diarrhoea. Scaling supplement ingestion to PPO was employed as a standardisation measure to accommodate size and fitness of the participant, which corresponds to sweat rate and energy utilisation, and therefore requirement for fluid and fuel replacement. Each 100 ml of exercise supplement contained ~6 g randomised structured oil (4 g MCFA). The mean quantities of randomised structured oil ingested were 112 ± 6.49 g (75 ± 4.33 g MCFA) during the 3-h steady-state ride, and 44 ± 5.20 g (29 ± 3.47 g MCFA) during the performance test.

Dietary Supplements

The randomised structured lipids used for the MC-MC diet foods were made with 1:3 molar ratio LCFA (canola) and MCFA (trioctanoin) oils, whereas LC-MC and LC-CHO diet foods were made with LCFA (canola) oil only. Foods included muffins, chocolate flavoured sports fudge bars, curries, bolognese and milk-like drinks (see Appedix 5 for recipe details). The mean amount of fat ingested was 99.7 ± 10.97 g·d $^{-1}$ (66.5 ± 7.32 g MCFA·d $^{-1}$). Participants were also instructed not to eat any foods high in ^{13}C content (cane sugar and maize products), but to otherwise maintain their normal diet. Jeukendrup et al. (1995, 1996) have shown that instructing participants in this way is effective in reducing background shifts (change in $^{13}\text{CO}_2$) from endogenous substrate stores. In addition to eating these supplements during the day, participants were instructed to ingest the sports bars and drinks during training sessions with the aim of further decreasing GI distress.

Psychological and Physiological Measurements

RPE and GI Scales

Several Rating of Perceived Exertion (tiredness, soreness of legs, ability to sprint, effort of cycling) and Gastrointestinal Distress (fullness/bloatedness, reflux/burping, stomach cramp, nausea, urge to vomit) markers were measured throughout both the 3-h steady-state ride and the performance tests. These factors were measured using scales modelled on Borg's CR10 scale (1970, 1985, 1988) (see Appendix 6 for original and modified Borg CR10 scales). The CR10 scale is based on evidence that perceptual magnitude grows in a positively accelerating way, and can be described by a power function with an exponent of 1.6 (Borg, 1998, 2001).

For this experiment, participants were asked to make a pen mark on a scale with linearly spaced verbal anchors (nothing at all, extremely weak, very weak, weak, moderate, strong, very strong, extremely strong, absolute maximum) how they rated their perceptions. For example, a question on their rating of perceived exertion was "how fatigued did you feel during the last sprint?", and a question on their rating of gastrointestinal distress was "how much discomfort do you feel in your stomach with respect to fullness/bloatedness?" Participants were instructed to treat the scale as continuous, i.e. to mark anywhere on the line, not just at the verbal anchors. The numerical value for each verbal anchor was not displayed on the scale charts, so as not to distract the participant from their rating, as the numerical value increased factorially in accordance with the CR10 scale by $x^{1.6}$.

Tracer Methodology

A 1- $^{13}\text{C}_1$ -octanoic acid tracer (Cambridge Isotope Labs, Andover, MA) was incorporated into the Randomised Structured oils (Appendix 4) and added to the MCFA-rich drink supplement for the Control and Intervention 3-h post-treatment exercise tests. Six batches of ^{13}C -enriched oil were produced with a mean oil enrichment of $84 \pm 30 \delta \cdot \text{mil}^{-1}$ vs. PDB (0.006854 $^{13}\text{C}/^{12}\text{C}$ ratio), which was confirmed to yield a detectable breath signal by pilot trials. Breath samples were taken during the 3-h steady state background test for each condition as a baseline ^{13}C reference for each time-point in the 3-h component of the post-treatment exercise test. The 3-h background test also functioned as an effective control exercise 3-d out from the performance test.

Calculations:

Isotopic enrichment of expired air is expressed as the delta per million difference between $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and a known laboratory reference standard (Pee Dee Balemental; PDB) according to the formula:

$$\delta^{13}\text{C} = \left(\left(\frac{^{13}\text{C}/^{12}\text{C sample}}{^{13}\text{C}/^{12}\text{C standard}} \right) - 1 \right) \cdot 10^3$$

$$^{13}\text{C}/^{12}\text{C standard} = 0.0112372$$

The amount of octanoic acid oxidised is then calculated according to the formula:

$$\text{Exogenous octanoic acid oxidation (g}\cdot\text{min}^{-1}) = \text{VCO}_2 \cdot \left(\frac{\delta_{\text{Exp}} - \delta_{\text{ref}}}{\delta_{\text{Ing}} - \delta_{\text{ref}}} \right) \cdot \left(\frac{1}{k} \right)$$

in which δ_{ref} is the ^{13}C enrichment of expired air in the 3-h background test, δ_{Exp} is the ^{13}C enrichment of expired air during the 3-h exercise with randomly esterified structured oil ingestion, δ_{Ing} is the ^{13}C enrichment of the MCT oil in the ingested exercise supplement, and k is the amount of CO_2 (litres) produced via oxidation of 1 g octanoic acid on a glycerol backbone ($k = 1.2369 \text{ L CO}_2\cdot\text{gMCT}^{-1}$) (Jeukendrup et al., 1995). A conversion factor of $34.19 \text{ kJ}\cdot\text{g}^{-1}$ was used to estimate MCFA contribution to energy expenditure (Livesey, 1988).

Indirect Calorimetry

From VCO_2 and VO_2 carbohydrate and fat oxidation rates were calculated using the non-protein respiratory quotient

$$\text{Carbohydrate oxidation (g}\cdot\text{min}^{-1}) = 4.210 \cdot \text{VCO}_2 - 2.962 \cdot \text{VO}_2$$

$$\text{Fat oxidation (g}\cdot\text{min}^{-1}) = 1.695 \cdot \text{VO}_2 - 1.701 \cdot \text{VCO}_2$$

These calculations are based on the assumptions that RER adequately reflects RQ, O_2 consumption and CO_2 production come solely from oxidative processes, and gas composition in expired breath reflects gas exchange from fuel metabolism at the tissue level. Muscle glycogen and plasma glucose are also assumed to contribute 80% and 20% to carbohydrate oxidation at moderate-high exercise intensities (Jeukendrup & Wallis, 2005). Conversion factors of $15.64 \text{ kJ}\cdot\text{g}^{-1}$ (Ferrannini, 1988) for CHO, and $40.81 \text{ kJ}\cdot\text{g}^{-1}$ (Péronnet & Massicotte, 1991) for fat oxidation was used to estimate contribution to energy expenditure.

Calibration Procedures

Blood Gas Analyser

The electronic signal from a sensor was adjusted in response to the known concentration from two different reagents. Each of the two reagents defined one point on the calibration curve (y-intercept offset and slope) to which the electronic signal was compared. Performance of one-point calibrations adjusted either the offset or slope drift for a parameter by measuring one of the reagents of known concentration. Two-point calibrations adjusted both the offset and slope drift for a parameter by measuring two reagents of known concentration.

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 PO₂, PCO₂, glucose and lactate were performed every 3 samples.

In addition, before beginning sample analysis on each day, a Quality Control test was run to evaluate the system for imprecision and inaccuracy, and to ensure that results of samples were accurate and reliable. Quality control samples are substances that have known expected values that cover clinically significant ranges for each parameter.

SensorMedics Vmax

The flow sensor was calibrated before testing each morning via a 3-L syringe (SensorMedics Corp., Yorba Linda, CA, USA) attached to tubing and a mouthpiece which was attached to the flow sensor. Room air was ejected from the sensor and tubing with two syringe strokes. The mass flow sensor was then automatically calibrated to zero gas flow, and temperature and barometric pressure were entered into the system. Several inspiratory and expiratory strokes from the syringe were performed for target flow rates: 0.0-0.6 LPS, 0.9-1.6 LPS, 2.4-5.5 LPS, and 7.0-12.0 LPS. Verification of flow volume was then performed by five full syringe strokes with at least one at 0.5 LPS, one at 0.5-1.5 LPS and one at 3.0 LPS. The flow volume was verified, and the flow sensor re-calibrated every hour during testing.

Oxygen and carbon dioxide gases were calibrated before testing each morning, and every hour throughout. The gas sample line was connected to the calibration fitting on the Pneumatics Module, and the calibration gases were turned on (16.02 O₂ and 4.02 CO₂; Alpha standard, BOC Special Gases, New Zealand). The calibration was then run automatically by the SensorMedics Vmax software.

Following testing, breath sample results were adjusted for drift of the sensor/medics sensors. Any error in the initial and verification calibrations measures were first adjusted to known flow rates and gas concentrations. Any drift between the initial and verification calibrations was assumed to be linear, and results were adjusted according to known flow rates and gas concentrations for each measurement time point.

VeloTron Ergometers

The AccuWatt calibration test for the VeloTron ergometers tested how much drift in watts there had been since factory calibration. The ergometers were accelerated to between 22-23 MPH and then allowed to decelerate. When the speed reached 22 MPH load was applied to the wheel and the software calculated the deviation from factory calibration, and also successive deviation from the last calibration run. The ergometers were tested twice during the testing period. The wattage deviation was consistently 0.5 to 1.5% from factory calibration over the course of the study.

Analyses

Breath ¹³C enrichment

Oxidation rates and contribution of each substrate (exogenous MCFA, endogenous fat, total CHO) to energy expenditure were calculated using stable ¹³CO₂/¹²CO₂ isotope GC continuous flow IRMS analysis (Dr. Jeukendrup, University of Birmingham, England) and indirect calorimetry. Enrichment of the randomised oil (added to exercise supplements) was measured using Elemental Analyser IRMS (EA-IRMS) (Iso-Analytical Ltd, Sandbach, Cheshire) (see Appendix 7 for details).

Plasma

After collection, blood was immediately centrifuged (Heraeus Sepatech Medifuge, West Germany) at 2000 G for 12 min. Plasma was then aspirated into eppendorf tubes (Neptune 1.6 ml flat snap cap micro centrifuge tubes, USA) and snap-frozen in liquid nitrogen before being stored in a -80°C freezer until analysis. The plasma aspirated from the lithium heparin tubes was analysed using a Bayer Rapidlab 800 system Blood Gas Analyser (Ciba Corning Diagnostics Corp., Medfield, MA, USA) for lactate and glucose concentrations, and the concentrations of PO₂, PCO₂ and acid-base variables. Plasma aspirated from the EDTA tubes was not used for the purposes of this thesis, but was sent to Dr. Bodil Vistisen (August Krogh Institutet, Universitetsparken 13, 2.sal, 2100 Copenhagen O, Denmark) for analysis of the

^{13}C -enrichment of plasma fatty acids, phospholipids, and ketones to provide evidence on the metabolic fate of the ingested ^{13}C -labelled octanoic acid.

Sprint Data

Each sprint and recovery interval was timed using stopwatches based on the calculation of calories expended, which was displayed by the velotron software (Velotron Coaching Software version 1.5, Seattle, WA). Mean power ($\text{J}\cdot\text{s}^{-1}$) for each interval was then calculated using the equation:

$$\text{kcal} \times 4.186 \times 1000 \times 0.25 / t$$

where kcal is the calories burnt by the participant during the sprint, 4.186 is the conversion factor from kilocalories to kilojoules, 0.25 is the efficiency factor of an exercising person used by the velotron software, and t is the time it took the participant to complete the sprint in seconds. The efficiency and kJ conversion factors were taken from velotron software calculations (Roger Moore, Velotron, Seattle, WA, personal communication).

Statistical Analysis

General Method. The effects of diet condition on the measured outcome variables were estimated with mixed modelling using Proc Mixed in the Statistical Analysis System (Vers8.2, SAS Institute, Cary, NC). For measures of performance, metabolism, and other dependent physiological variables, the effects of the three diets were compared in a three-way model, whereas the analysis of psychometric variables also included the Baseline exercise test responses. Independent repeated-measures analyses were conducted for the data at rest before exercise, during the 3-h exercise, and during the sprint procedure. In addition, the overall average effect was derived for each independent analytical data set. The modelled fixed effects were test order, treatment (diet condition) and sample time (or sprint) as the treatment*sample interaction. In addition to the normal between-cyclist variance, cyclist*xvarLC-MC and cyclist*xvarMC-MC (and cyclist*xvarBaseline in the psychometric analysis) were specified as random effects, which allowed for the determination of the extra variance associated with moving from the control condition to each of the two treatment diet conditions. The within-cyclist variance (associated with each sample) was calculated from the residual variance. For the performance and psychometric analysis, a term (order*treatment*sample) was included in the model to account for any familiarisation or fatigue effect between consecutive trials; in addition, the magnitude of these practice effects were also determined independent of treatment effect. When presenting order effects, test 1

refers to Baseline (GI variables only), and tests 2-4 refer to experimental conditions (MC-MC, LC-MC and LC-CHO).

Measures of centrality and spread for participant descriptive and dietary variables are raw means and standard deviations. Most dependent variables were analyzed after log transformation, to reduce or eliminate effects of non-uniformity of error; the exceptions were variables where the unit or expression is as a percentage (error normalised), data sets containing numerals <0 or $<0>$, and slope coefficients derived from within-subject polynomial modelling. Means derived from the analysis of log-transformed variables are back log-transformed least-squares means, with the associated spread represented by percentage standard deviations or factor standard deviations (\times/\div). For example, for a hormone concentration of $40 \text{ mmol}\cdot\text{L}^{-1}$ with a between-subject standard deviation of 20%, the typical variation is $40 \div 1.20$ to 40×1.20 , or 33 to $48 \text{ mmol}\cdot\text{L}^{-1}$. Between-variable comparisons are presented as the estimated result for one condition times less or higher than the estimated result for another condition. For example, if an estimated result of a condition is 0.82 times less than or 1.18 times higher a reference condition result of 1.0, then the result of the first condition is 0.82 or 1.18 respectively. Performance and metabolic data in graphs and text are shown as least-squares means to eliminate the artefactual variation that would otherwise be apparent with raw means when there are missing values for some levels of a repeated measure. Precision of the estimates is shown as 95% confidence limits (CL) or the \pm confidence interval with corresponding p-values (P). Reliability of sprint performance within the present intervention experiment is expressed as the within-cyclist error calculated from the residual variance derived from the repeated-measures analyses.

In order to help categorise the large volume of quantitative estimates and outcome statistics generated into a manageable description of effects likely to be meaningful for the population, outcomes with a p-value of ≤ 0.15 for the performance and metabolic measures and ≤ 0.10 for the psychometric outcome measures were considered to represent strong trends toward a clear directional population effect of the specified estimated magnitude. The more conservative approach toward psychometric measures was chosen due to the large within-cyclist variability associated with this measurement. Difference comparisons for psychometric units are expressed as multiples or fractions of a scale unit. Data are summarised in the results section as overall averages (averaged across all sample time-points for that test), for 3-h post-treatment and sprint tests unless otherwise stated.

Polynomial Analysis of Power Output and Nausea. The trends in mean power output and Nausea ratings during the sprint procedure were compared using within-subject modelling. For each cyclist and experimental condition, a polynomial with linear and quadratic components was fitted to the natural log of the variables for measurement. The derived positional, linear, and quadratic slope coefficients were compared using an appropriate repeated-measures analysis in Proc Mixed.

Mechanisms Analysis. The positional and linear slope effects derived from the polynomial analysis for nausea (there was no evidence for a quadratic effect) were included as covariates in separate repeated-measures analyses of the effects of treatment on the positional and linear slope effects for mean power. Reduction of the treatment effect by the covariate indicates the extent to which changes in sprint mean power were attributable to change in the covariate.

Results

Performance

Sprints

Mean power was 10.4% (95% CI \pm 5.5%, p-value = 0.0004) and 6.8% (\pm 2.8%, $<$ 0.0001) higher in LC-CHO compared to LC-MC and MC-MC conditions respectively (Figure 9). Separately, mean power for sprints 1-9 in the LC-CHO condition was 7-14% (\pm 7.6-7.7%, 0.0001-0.04) higher than LC-MC, but the effect for sprint 10 was less clear (6% \pm 8%, 0.08). Relative to MC-MC, mean power for sprints 1 and 3-9 in the LC-CHO condition were 5.3-9.4% (\pm 5.6-5.7%, 0.0006-0.05; 0.002-0.05). Sprint 2 mean power tended to be higher (4.3% \pm 5.6%, 0.12) in LC-CHO, but there was no clear difference between LC-CHO and MC-MC for sprint 10 (3.8% \pm 5.6%, 0.16) (Figure 9). No clear difference was evident for mean power between MC-MC relative to LC-MC conditions (3.4% \pm 5.9%, 0.25). Sprint mean power did not change with test order (Table A3, Appendix 8).

Recovery

Mean power was 1.7% (\pm 1.6%, 0.04) and 0.7% (\pm 2.3%, 0.57) lower relative to LC-CHO in the LC-MC and MC-MC conditions respectively (Figure 10). No clear difference was evident for mean power between MC-MC relative to LC-MC conditions (1.0% \pm 2.6%, 0.44). Separately, mean power between LC-CHO and LC-MC began to diverge from recovery 5, becoming clearly different for recovery 8 (3.8% \pm 3.5%, 0.03), and showing trends for recoveries 6, 7, and 9 (2.7-2.9% \pm 3.5-3.6%, 0.10-0.12). Recovery mean power did not change with test order (Table A3, Appendix 8).

Mechanism Correlation

An analysis of mechanisms revealed a negative relationship between nausea rating and sprint mean power. For every 1 unit increase in the position of the nausea curve, the position of the mean power curve decreased by 6 W (\pm 3.8, 0.004). Relative to LC-CHO, the position of the mean power quadratic without the covariate nausea was 26.8 W (\pm 21.1, 0.02) and 16.9 W (\pm 19.7, 0.09) lower in the LC-MC and MC-MC conditions, respectively; mean power was 9.9 W (\pm 20.7, 0.32) higher in the MC-MC condition relative to LC-MC. With the position of the nausea curve added as the covariate in the polynomial analysis, the decrease in mean power relative to LC-CHO was made unclear at 11.5 W (\pm 26.9, 0.37) and 9.9 W (\pm 20.3,

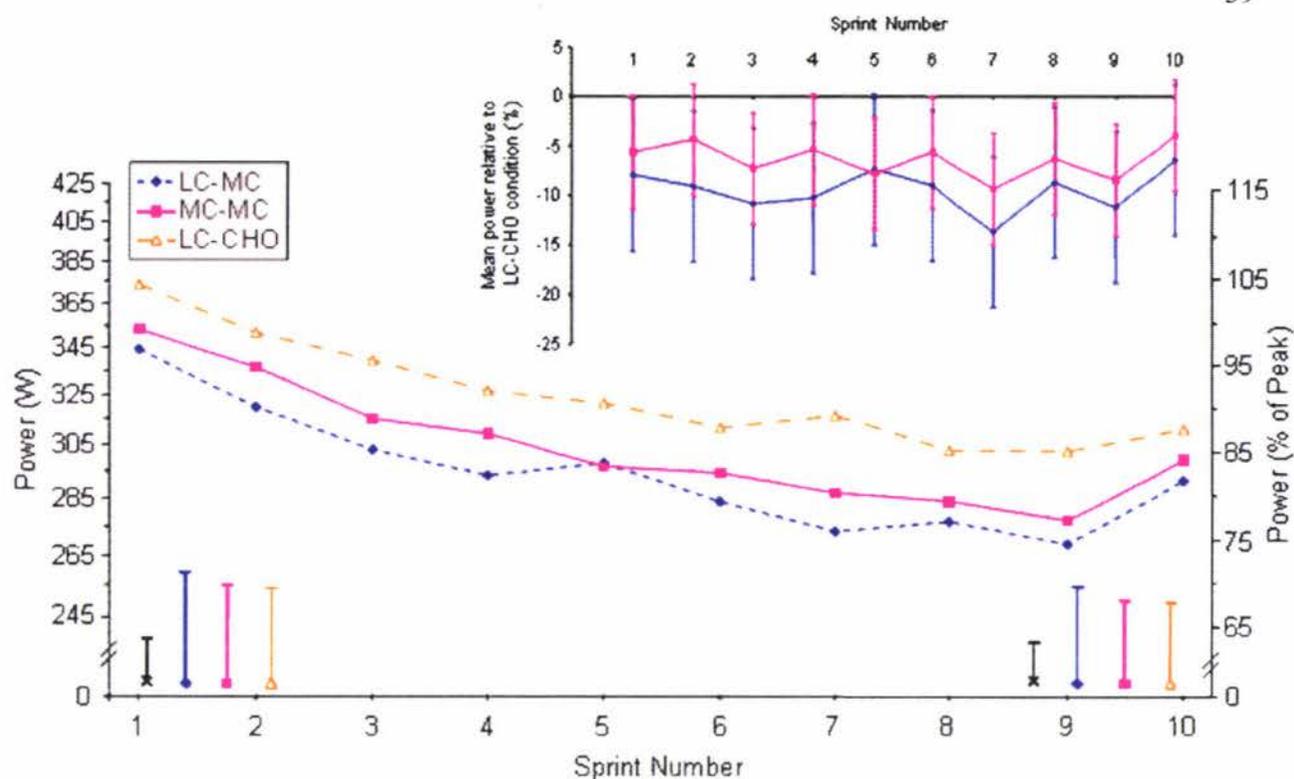


Figure 9. Performance test sprint intervals. Mean power for each sprint interval, and mean power as a percentage of peak power for LC-MC, MC-MC and LC-CHO conditions. Data are presented on a logarithmic y-axis as mean power \pm between-subject SD, and on a linear y-axis as mean power as a percentage of peak power output \pm between-subject SD. Within-subject \pm SDs are represented by the black bars marked with an x. The inset graph shows mean power for LC-MC and MC-MC conditions as a percentage of LC-CHO mean power \pm CI

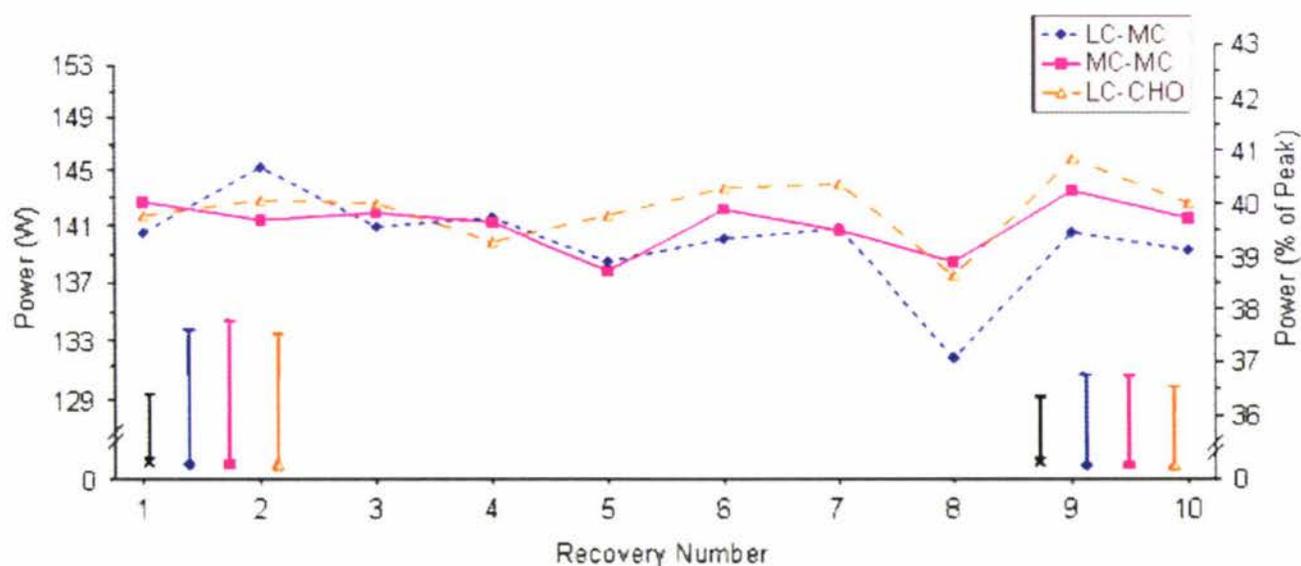


Figure 10. Performance test recovery intervals. Mean power for each recovery interval, and mean power as a percentage of peak power for LC-MC, MC-MC and LC-CHO conditions. Data are presented on a logarithmic y-axis as mean power \pm between-subject SD, and on a linear y-axis as mean power as a percentage of peak power output \pm between-subject SD. Within-subject \pm SDs are represented by the black bars marked with an x.

0.31) lower than in the LC-MC and MC-MC conditions respectively; and 1.5 W ($\pm 21.9, 0.88$) higher in the MC-MC relative to the LC-MC condition. Therefore, nausea had a moderate-to-strong influence on sprint mean power.

For every 1 unit·sprint⁻¹ increase in the slope of the nausea curve, there was a 2.8 W·sprint⁻¹ ($\pm 2.2, 0.18$) increase in the slope of the mean power curve. The slope of the mean power quadratic without the covariate nausea was 4.5 W·sprint⁻¹ ($\pm 10.7, 0.38$) and 0.1 W·sprint⁻¹ ($\pm 10.0, 0.98$) higher than LC-CHO in the LC-MC and MC-MC conditions respectively. Mean power slope was 4.4 W·sprint⁻¹ ($\pm 10.5, 0.38$) lower in the MC-MC condition relative to LC-MC. With the slope of the nausea curve added as the covariate in the polynomial analysis, the increase in mean power slope relative to LC-CHO was reduced to 1.7 W·sprint⁻¹ ($\pm 12.9, 0.77$) and -0.3 W·sprint⁻¹ ($\pm 10.1, 0.95$) in the LC-MC and MC-MC conditions respectively; and -2.0 W·sprint⁻¹ ($\pm 12.0, 0.72$) in the MC-MC condition relative to LC-MC. Therefore, despite the fact that there was initially no difference between slopes, addition of the nausea curve as a covariate still had a small influence the slope of the mean power curve. There was no evidence for an effect of treatment on the quadratic component of polynomial (curvature).

Substrate Metabolism

No clear difference was evident in MCFA-oxidation rate following MC-MC (1.11 \times/\div 1.13, 0.18) compared with the LC-MC condition. Exogenous MCFA-oxidation rates increased throughout the 3-h post-treatment test, reaching a maximum of 0.38 g·min⁻¹ (\times/\div 1.23; 13.04 kJ·min⁻¹), and 0.43 g·min⁻¹ (\times/\div 1.42, 0.21; 14.61 kJ·min⁻¹) in the MC-MC and LC-MC conditions respectively (Figures 11, 12 and 13a), contributing 25.3% and 26.6% to total energy expenditure respectively (Figure 14). Oxidation of endogenous fat stores was 0.49 (\times/\div 1.15, <0.0001) and 0.57 (\times/\div 1.35, 0.0002) times lower relative to LC-CHO in the LC-MC and MC-MC conditions, respectively. Endogenous fat-oxidation rates decreased throughout the 3-h post-treatment test from 0.54 g·min⁻¹ (22.14 kJ·min⁻¹) to 0.25 g·min⁻¹ (10.18 kJ·min⁻¹) in the LC-MC condition, and from 0.61 g·min⁻¹ (24.91 kJ·min⁻¹) to 0.21 g·min⁻¹ (8.42 kJ·min⁻¹) in the MC-MC condition. However, oxidation rates stayed approximately the same throughout the 3-h post-treatment test for the LC-CHO condition (0.63 \pm 0.04 g·min⁻¹, 25.80 \pm 1.53 kJ·min⁻¹) (Figure 13b). Endogenous fat stores contributed on average 24.1%, 28.7%, and 45.4% to total energy expenditure in LC-MC, MC-MC and LC-CHO conditions respectively (Figure 14). No difference in CHO oxidation was observed

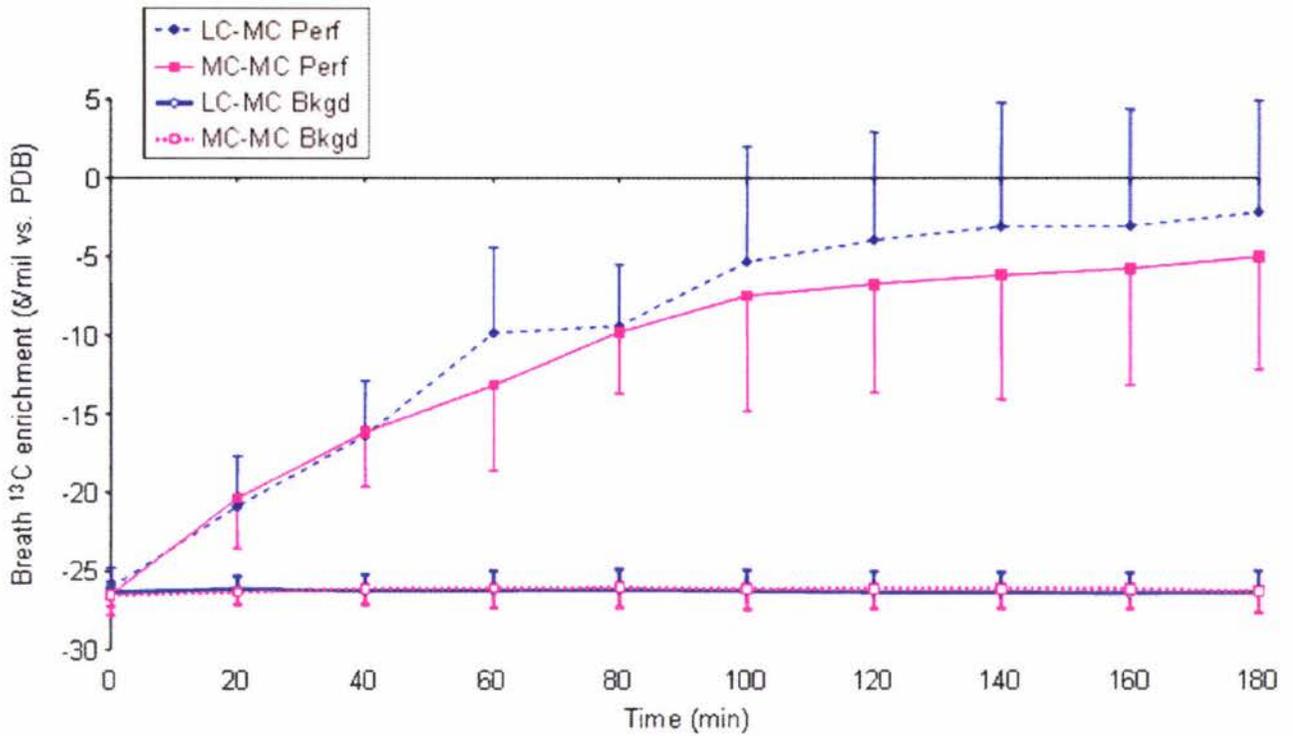


Figure 11. Breath ¹³CO₂-enrichment for LC-MC and MC-MC background (Bkgd) and performance (Perf) tests. Data are presented on a linear y-axis as least-squares means \pm SD.

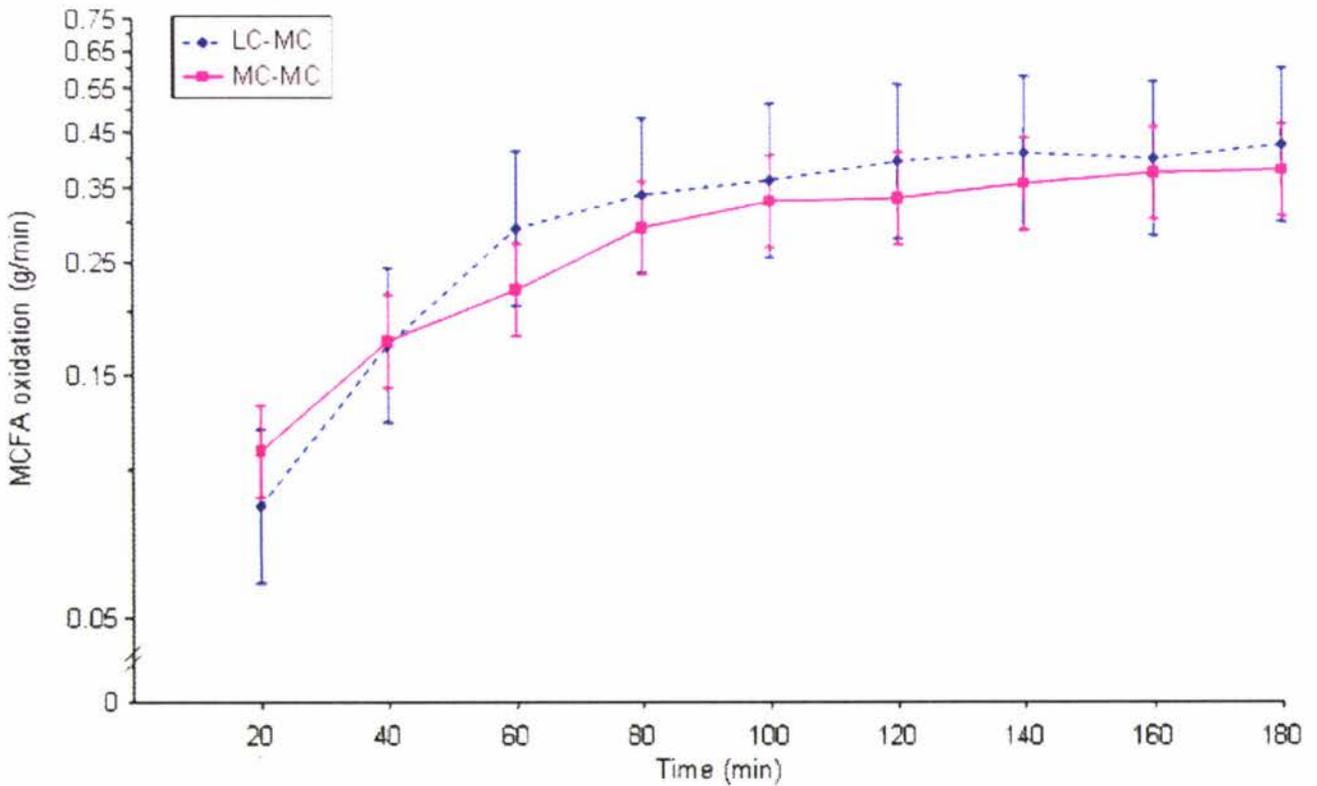


Figure 12. Exogenous octanoic-acid oxidation calculated from breath ¹³CO₂-enrichment and exercise supplement ¹³C-enriched MCFAs for the LC-MC and MC-MC conditions. Data are presented on a logarithmic y-axis as means \pm SD.

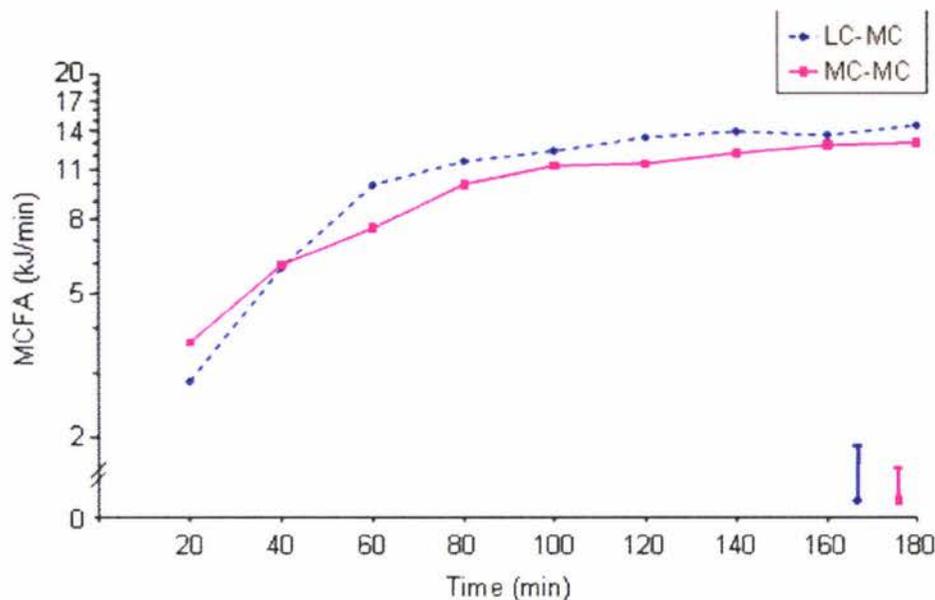


Figure 13a. Absolute contribution of MCFA oxidation to energy expenditure. Data are presented on a logarithmic y-axis as means \pm SD.

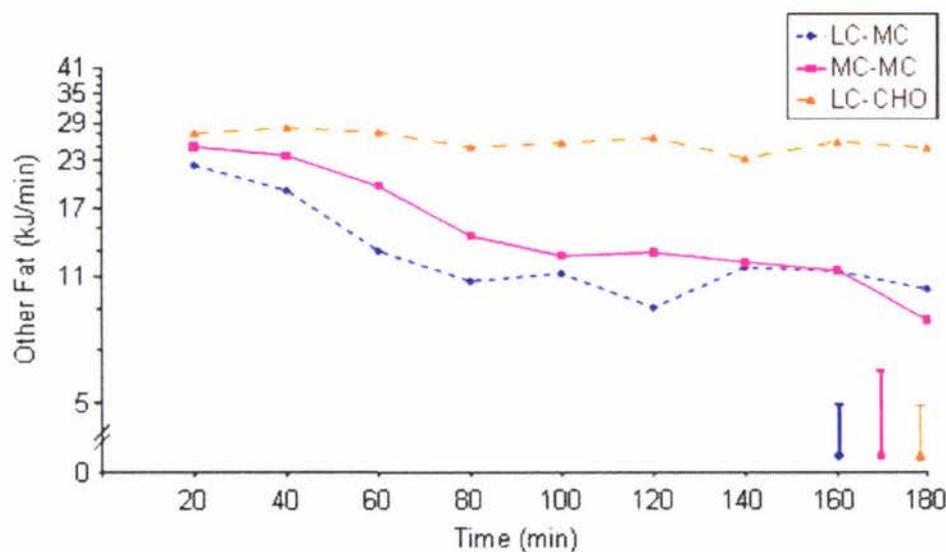


Figure 13b. Absolute contribution of fat (excluding exogenous MCFA) oxidation to energy expenditure. Data are presented on a logarithmic y-axis as

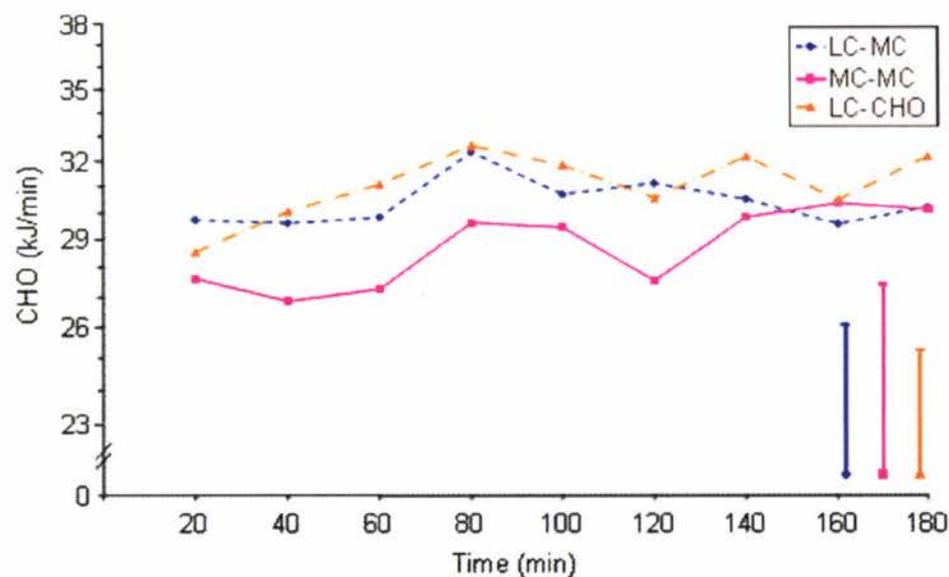


Figure 13c. Absolute contribution of CHO oxidation to energy expenditure. Data are presented on a logarithmic y-axis as means \pm SD.

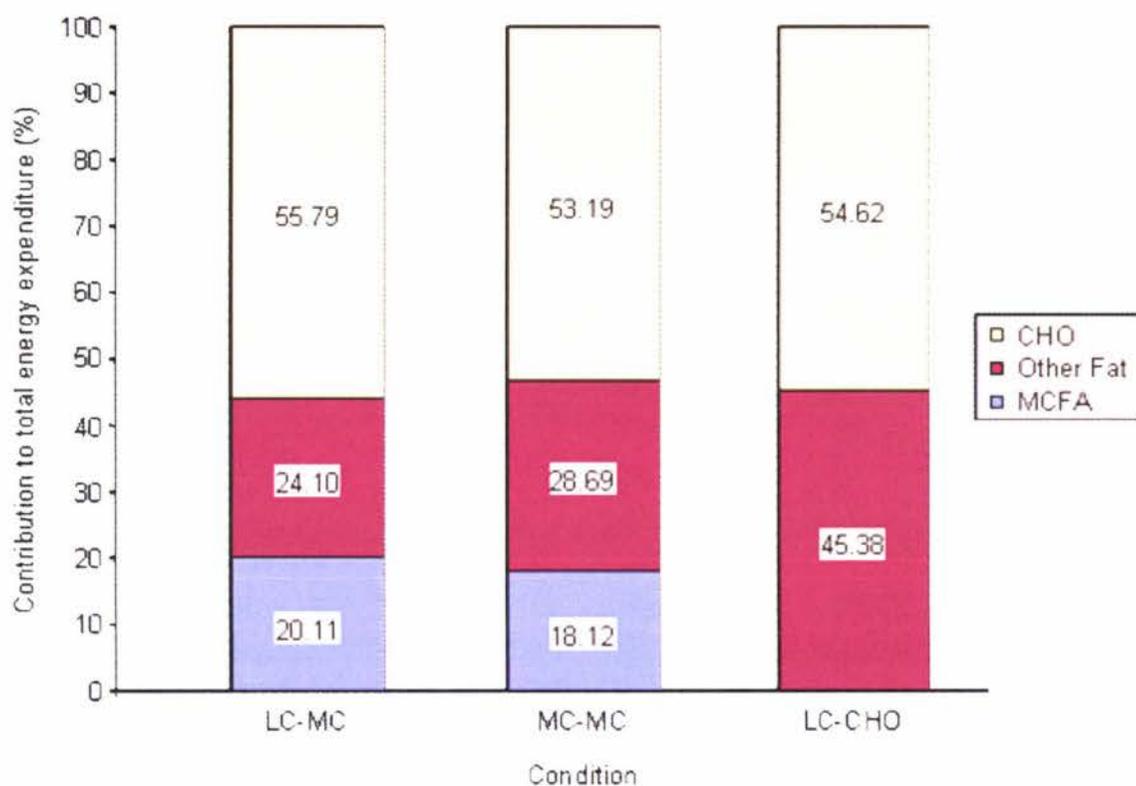


Figure 14. Overall contribution of exogenous MCFA, other fats (exogenous and endogenous), and CHO oxidation to total energy expenditure. Data are presented as mean contribution to total energy expenditure (%), calculated from relative kJ expenditure.

between LC-MC ($0.98 \times \div 1.08, 0.58$) and LC-CHO, MC-MC ($0.93 \times \div 1.13, 0.21$) and LC-CHO, or MC-MC ($0.95 \times \div 1.15, 0.42$) and LC-MC conditions. Total CHO-oxidation rates stayed approximately the same throughout the 3-h post-treatment test at $1.94 \pm 0.06 \text{ g}\cdot\text{min}^{-1}$ ($30.40 \pm 0.91 \text{ kJ}\cdot\text{min}^{-1}$), $1.84 \pm 0.09 \text{ g}\cdot\text{min}^{-1}$ ($28.74 \pm 1.39 \text{ kJ}\cdot\text{min}^{-1}$), and $1.99 \pm 0.08 \text{ g}\cdot\text{min}^{-1}$ ($31.06 \pm 1.30 \text{ kJ}\cdot\text{min}^{-1}$), contributing on average 55.8%, 53.2%, and 54.6% to total energy expenditure for the LC-MC, MC-MC and LC-CHO conditions respectively (Figures 13c and 14).

Psychological Parameters

RPE

Tiredness (Figure 15a; Table 1; Table A1 Appendix 6). Participant ratings of tiredness were 1.37 ($\times \div 1.39, 0.06$) and 1.27 ($\times \div 1.18, 0.006$) times greater in the LC-MC condition relative to LC-CHO for the 3-h post-treatment and sprint performance tests respectively. Participants also felt 0.82 ($\times \div 1.16, 0.009$) times less tired in the MC-MC condition relative to LC-MC during the sprint test. Participant ratings of tiredness decreased with test order (Table A3, Appendix 8).

Leg Soreness (Figure 15b; Table 1; Table A1 Appendix 6). No differences were observed during the 3-h post-treatment test, ratings of leg soreness were 1.32 ($\times \div 1.51, 0.19$) and 1.28 ($\times \div 1.63, 0.31$) times higher relative LC-CHO in the LC-MC and MC-MC conditions respectively; and MC-MC ratings were 0.97 ($\times \div 1.85, 0.93$) times lower than LC-MC. Ratings for the performance test were 1.48 ($\times \div 1.32, 0.007$) and 1.36 ($\times \div 1.44, 0.10$) times higher relative to LC-CHO in the LC-MC and MC-MC conditions respectively; and MC-MC ratings were 1.09 ($\times \div 1.50, 0.67$) times higher relative to LC-MC. Ratings of leg soreness decreased with test order for the 3-h post-treatment test, and tended to decrease with test order for the performance test (Table A3, Appendix 8)

Strong/Ability to Sprint (Figure 15c; Table 1; Table A1 Appendix 6). No differences were observed during the 3-h post-treatment test, ratings of strength/ability to sprint were 0.91 ($\times \div 1.29, 0.49$) times lower and 1.08 ($\times \div 1.57, 0.75$) times higher relative to LC-CHO in LC-MC and MC-MC conditions respectively; and 1.18 ($\times \div 1.64, 0.52$) times higher in the MC-MC condition relative to LC-MC. Ratings for the sprint performance test were 0.70 ($\times \div 1.25, 0.002$), and 0.61 ($\times \div 1.37, 0.003$) times lower relative to LC-CHO for the MC-MC and LC-MC conditions respectively; and MC-MC ratings were 0.88 ($\times \div 1.34, 0.40$) times lower than

Table 1. Within-subject CV for RPE and GI scales. Data are presented as mean CV \pm CI (% of a likert scale). For example, a CV mean of 3 scale units \pm 91% equals a range between 2.73-3.30 scale units.

Test	Within-Subject CV (%)								
	Tired	Leg Soreness	Strength	Effort	Bloated	Reflux	Stomach Cramp	Nausea	Urge to Vomit
3-h post-treatment	56.4 \pm 5.1%	61.7 \pm 5.5%	51.6 \pm 4.6%	48.0 \pm 4.4%	77.9 \pm 7.2%	84.7 \pm 7.7%	92.2 \pm 10.2%	91.1 \pm 9.2%	95.4 \pm 10.3%
Sprint	35.5 \pm 6.2%	45.1 \pm 7.9%	66.3 \pm 9.9%	21.8 \pm 3.8%	74.7 \pm 12.1%	69.4 \pm 11.1%	83.3 \pm 14.2%	93.1 \pm 17.2%	77.2 \pm 13.9%

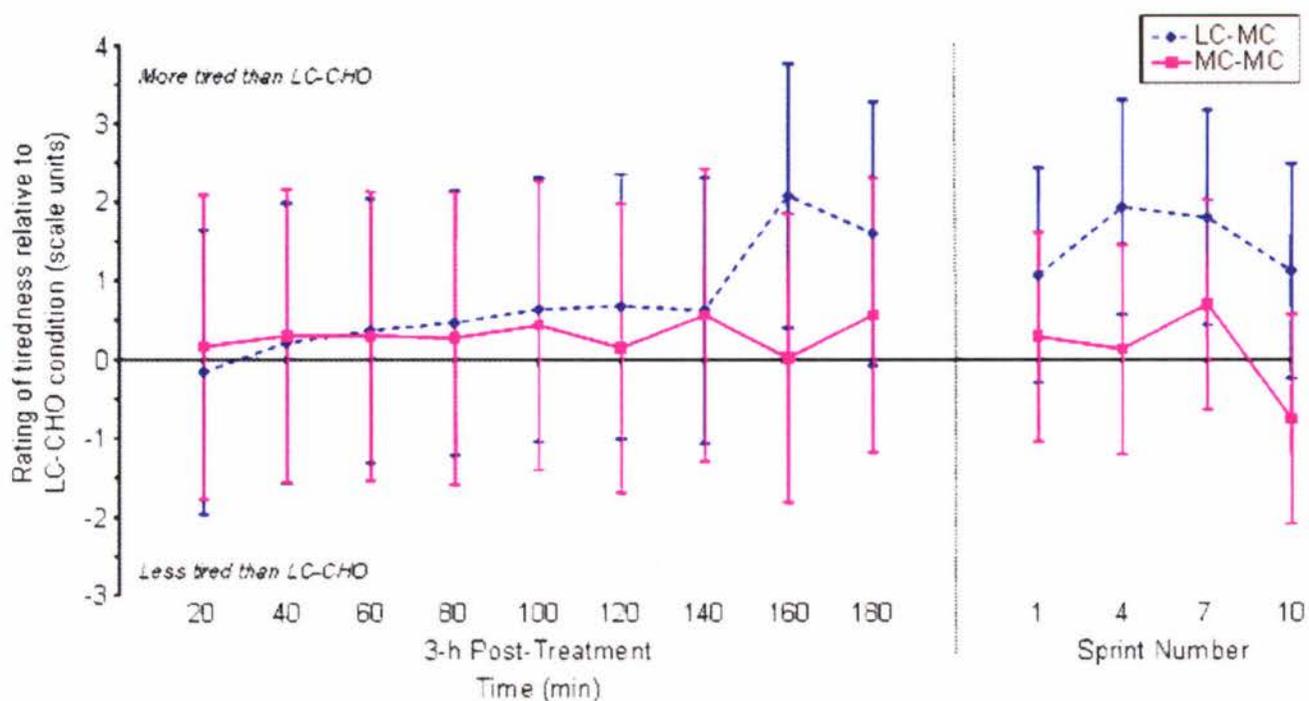


Figure 15a. Mean ratings of tiredness relative to LC-CHO condition ratings during cycling (3-h post-treatment and performance tests). Data are presented on a linear y-axis as means \pm CI, where zero equals mean ratings of tiredness for LC-CHO condition.

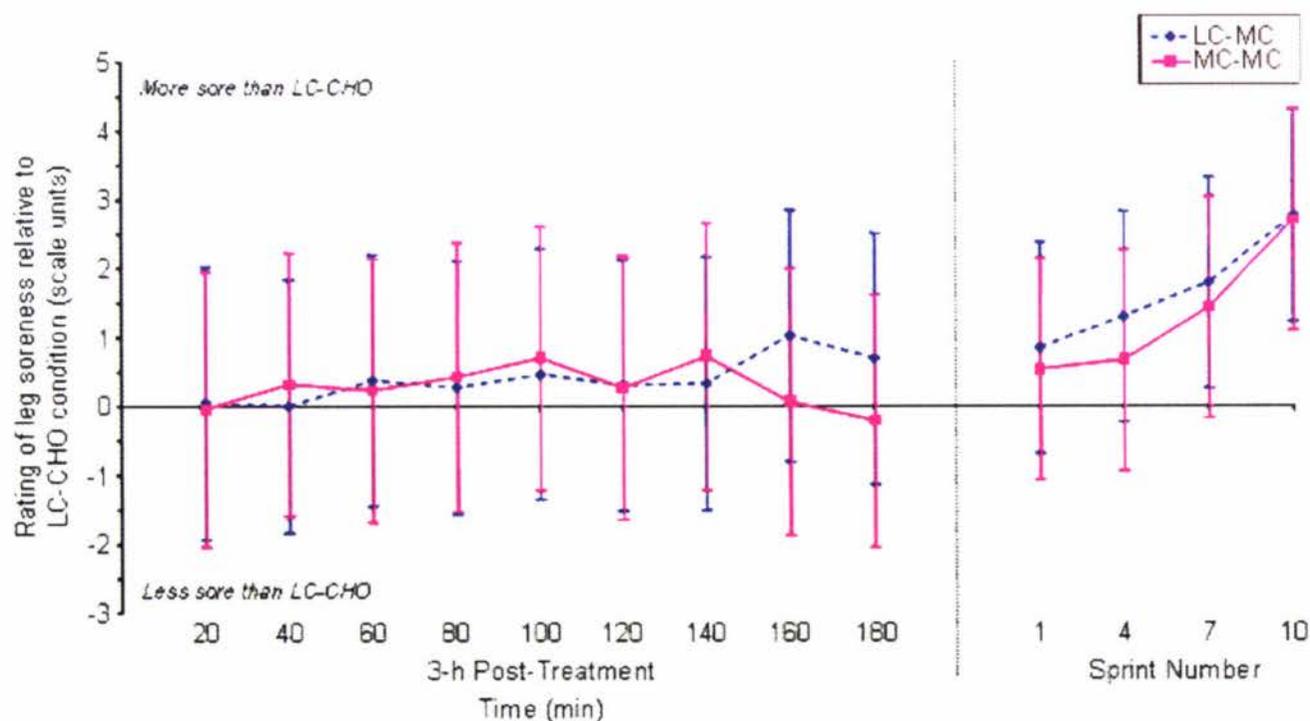


Figure 15b. Mean ratings of leg soreness relative to LC-CHO condition ratings during cycling (3-h post-treatment and performance tests). Data are presented on a linear y-axis as means \pm CI, where zero equals mean ratings of leg soreness for LC-CHO condition.

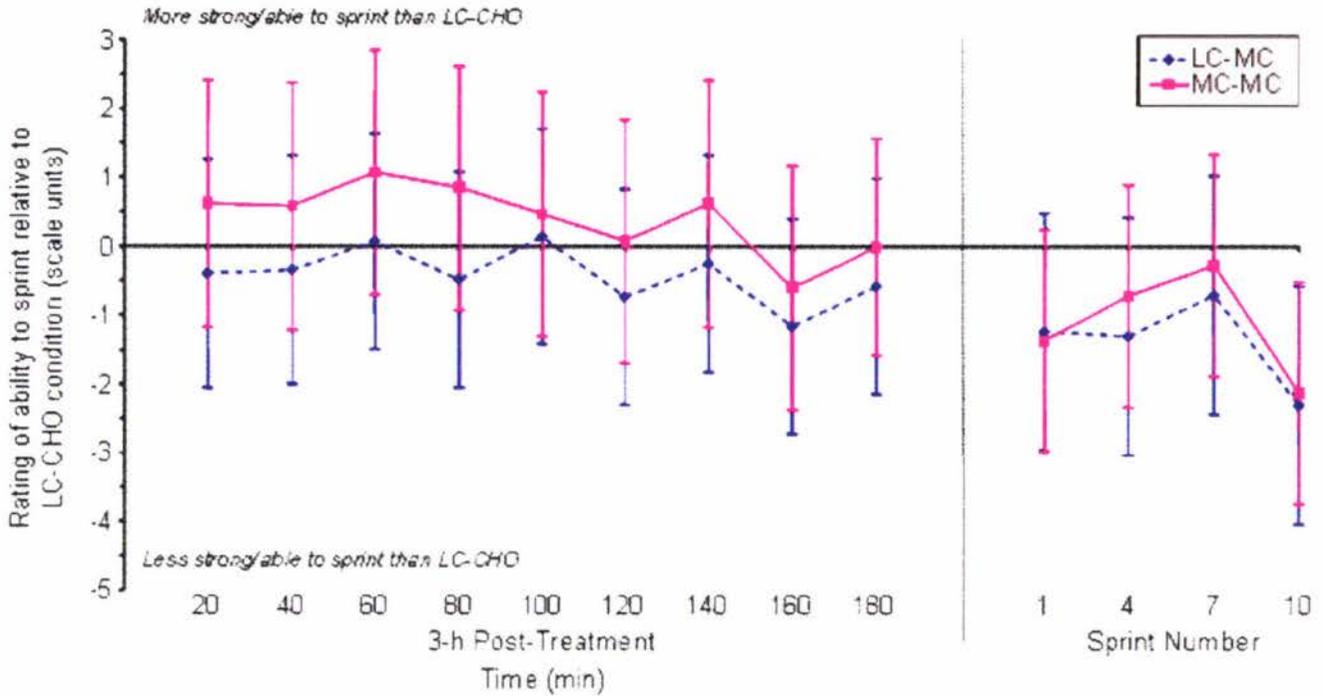


Figure 15c. Mean ratings of strength/ability to sprint relative to LC-CHO condition ratings during cycling (3-h post-treatment and performance tests). Data are presented on a linear y-axis as means \pm CI, where zero equals mean ratings of strength/ability to sprint for LC-CHO condition.

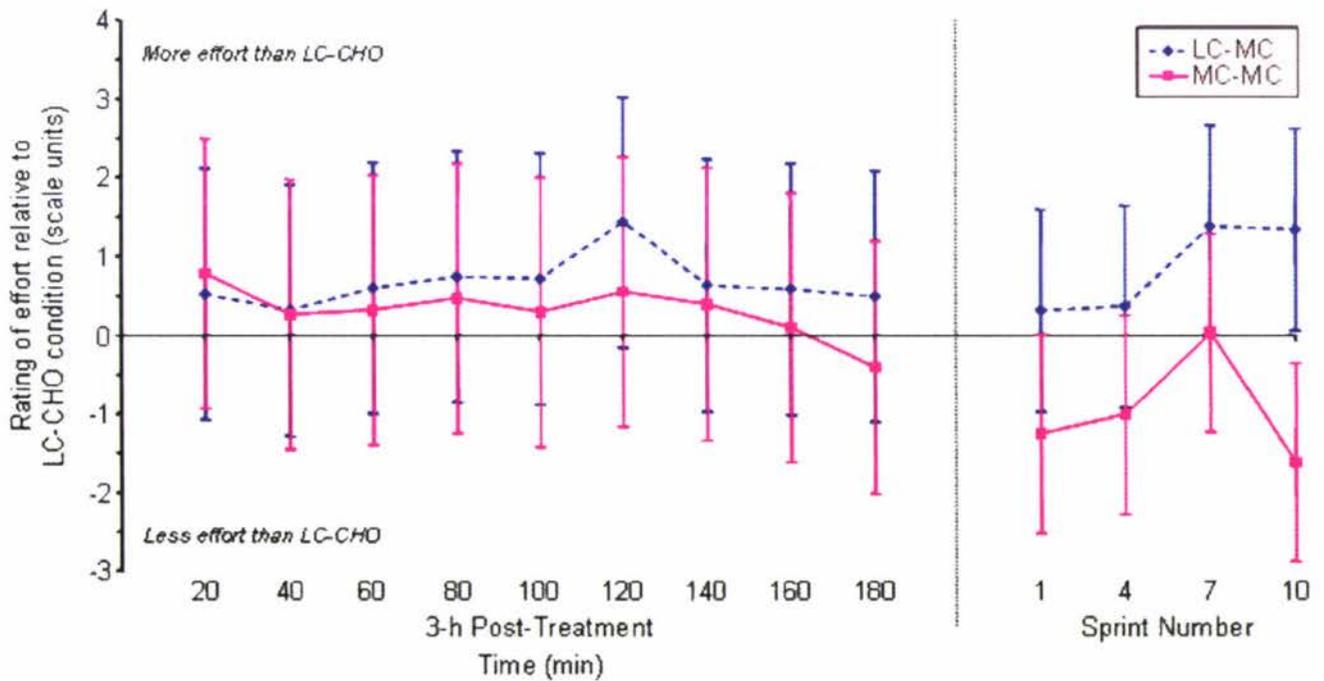


Figure 15d. Mean ratings of effort relative to LC-CHO condition ratings during cycling (3-h post-treatment and performance tests). Data are presented on a linear y-axis as means \pm CI, where zero equals mean ratings of effort for LC-CHO condition.

LC-MC. Ratings of strength/ability to sprint did not change with test order (Table A3, Appendix 8).

Effort (Figure 15d; Table 1; Table A1 Appendix 6). Participants felt they had to exercise 1.52 (x/\div 1.35, 0.007) times harder during the 3-h post-treatment test for the LC-MC condition relative to LC-CHO. MC-MC was not clearly different at 0.85 (x/\div 1.63, 0.50) times lower and 1.29 (x/\div 1.51, 0.22) times higher than the LC-MC and LC-CHO conditions, respectively. Ratings of effort for the performance test were 1.25 (x/\div 1.19, 0.01) and 1.10 (x/\div 1.20, 0.30) times higher for LC-MC relative to the MC-MC and LC-CHO conditions respectively. No clear difference was evident for the MC-MC condition at 0.88 (x/\div 1.18, 0.13) times lower than the LC-CHO condition. Ratings of effort decreased with test order for the 3-h post-treatment and performance tests (Table A3, Appendix 8).

In summary, participants felt more tired during the both the 3-h post-treatment and performance tests in the LC-MC and MC-MC conditions relative LC-CHO. Participants also felt less strong and their legs more sore during the performance test in the LC-MC and MC-MC conditions relative to LC-CHO. Despite feeling less tired during the performance tests in the MC-MC condition, participants put in less effort than in the LC-MC condition.

GI Distress

Fullness/Bloatedness (Figure 16a; Table 1; Table A2 Appendix 6). Participants ratings of fullness/bloatedness were 1.89 (x/\div 1.99, 0.007), 1.82 (x/\div 2.27, 0.15) and 2.00 (x/\div 1.66, 0.008) times higher relative to LC-CHO for Baseline, LC-MC and MC-MC conditions respectively in the 3-h post-treatment test. Ratings in LC-MC (0.96 x/\div 2.83, 0.94) and MC-MC (1.05 x/\div 2.26, 0.90) were not different to Baseline, nor was MC-MC (1.10 x/\div 1.66, 0.84) different to LC-MC. During the performance test, participants felt 1.94 (x/\div 1.45, 0.0007), 1.99 (x/\div 1.62, 0.006) and 2.78 (x/\div 1.26, <0.0001) times more full/bloated in the Baseline, MC-MC and LC-MC conditions relative to LC-CHO, and 1.43 (x/\div 1.39, 0.03) times more bloated in the LC-MC condition relative to Baseline. There was no clear difference between MC-MC (1.03 x/\div 1.72, 0.93) and Baseline, or MC-MC (0.72 x/\div 1.58, 0.15) and LC-MC conditions. Ratings of fullness/bloatedness decreased with test order for the 3-h post-treatment test, but did not change for the performance test (Table A3, Appendix 8).

Reflux (Figure 16b; Table 1; Table A2 Appendix 6). Participants rated their experience of reflux 1.91 (x/\div 1.89, 0.05) and 1.86 (x/\div 1.48, 0.002) times higher in Baseline and MC-MC

conditions relative to LC-CHO for the 3-h post-treatment test. No clear difference was evident between LC-MC (1.79 \times/\div 2.18, 0.14) and LC-CHO, LC-MC (0.96 \times/\div 2.29, 0.93) and MC-MC, or MC-MC (0.97 \times/\div 2.02, 0.94) and Baseline conditions. Ratings for the performance test were 1.72 (\times/\div 1.39, 0.002), 2.20 (\times/\div 1.34, <0.0001) and 1.44 (\times/\div 1.52, 0.08) times higher relative to LC-CHO in the Baseline, LC-MC and MC-MC conditions respectively. Participants also rated their reflux 1.52 (\times/\div 1.53, 0.05) times higher in the LC-MC condition relative to MC-MC. No clear difference was evident between LC-MC (1.28 \times/\div 1.41, 0.16) or MC-MC (0.84 \times/\div 1.58, 0.45) conditions relative to Baseline. Ratings of reflux decreased with test order between tests 1, 2 and 3 for the 3-h post-treatment, and tests 1 and 2 for the performance test (Table A3, Appendix 8).

Stomach Cramp (Figure 16c; Table 1; Table A2 Appendix 6). Participants rated their stomach cramp 1.88 (\times/\div 1.56, 0.006), 1.66 (\times/\div 1.60, 0.03) and 1.77 (\times/\div 1.59, 0.02) times higher relative to LC-CHO in Baseline, MC-MC and LC-MC conditions respectively for the 3-h post-treatment test. No clear difference was evident between LC-MC (0.94 \times/\div 1.73, 0.83) and Baseline, MC-MC (0.89 \times/\div 1.75, 0.67) and Baseline, or MC-MC (0.94 \times/\div 1.78, 0.83) and LC-MC conditions. Participant ratings for the performance test were 1.54 (\times/\div 1.46, 0.03) times higher in the LC-MC condition relative to LC-CHO. No clear differences were observed between the other conditions for the performance test. Ratings for the MC-MC condition were 0.90 (\times/\div 1.96, 0.75) and 0.83 (\times/\div 1.84, 0.53) times lower than Baseline and LC-MC conditions respectively, and 1.27 (\times/\div 1.84, 0.43) times higher than LC-CHO. No clear differences were evident between LC-MC (1.09 \times/\div 1.60, 0.72) and LC-CHO (0.71 \times/\div 1.61, 0.15) relative to Baseline. Ratings of stomach cramp tended to decrease with test order for the 3-h post-treatment test (Table A3, Appendix 8).

Nausea (Figure 16d; Table 1; Table A2 Appendix 6). Participant ratings of nausea were 3.26 (\times/\div 2.32, 0.006), 3.21 (\times/\div 2.40, 0.009), and 3.15 (\times/\div 2.39, 0.01) times higher relative to LC-CHO in the Baseline, LC-MC and MC-MC conditions respectively. No clear difference was evident between LC-MC (0.99 \times/\div 2.30, 0.97) and Baseline, MC-MC (0.97 \times/\div 2.28, 0.94) and Baseline, or MC-MC (0.98 \times/\div 2.36, 0.96) and LC-MC conditions. Participants also rated their feelings of nausea 2.89 (\times/\div 1.63, <0.0001), 3.59 (\times/\div 1.54, <0.0001) and 2.71 (\times/\div 1.54, <0.0001) times higher relative to LC-CHO in the Baseline, LC-MC and MC-MC conditions respectively during the performance test. No differences were observed between LC-MC (1.24 \times/\div 1.68, 0.41) and Baseline, MC-MC (0.94 \times/\div 1.68, 0.80)

and Baseline, or MC-MC (0.76 \times/\div 1.58, 0.23) and LC-MC conditions. Ratings of nausea decreased with test order for the 3-h performance test, but only decreased from tests 1-2 for the performance test (Table A3, Appendix 8).

Urge to Vomit (Figure 16e; Table 1; Table A2 Appendix 6). Participants rated their urge to vomit 2.30 (\times/\div 1.82, 0.0007) and 2.97 (\times/\div 1.38, <0.0001) times higher in the Baseline and MC-MC conditions respectively relative to LC-CHO for the 3-h post-treatment test. No differences were evident between LC-MC (1.95 \times/\div 3.07, 0.24) and LC-CHO, LC-MC (0.85 \times/\div 3.38, 0.79) and Baseline, MC-MC (1.29 \times/\div 1.78, 0.38) and Baseline, or MC-MC (1.53 \times/\div 3.04, 0.45) and LC-MC. For the performance test, participants rated their urge to vomit 1.86 (\times/\div 1.57, 0.008), 1.99 (\times/\div 1.44, 0.0004) and 2.87 (\times/\div 1.873, 0.001) times higher relative to LC-CHO for Baseline, MC-MC and LC-MC conditions respectively. No difference was evident between LC-MC (1.55 \times/\div 1.94, 0.19) and Baseline, MC-MC (1.07 \times/\div 1.54, 0.75) and Baseline, or MC-MC (0.69 \times/\div 1.82, 0.22) and LC-MC conditions. Ratings of urge to vomit tended to decrease with test order (Table A3, Appendix 8).

In summary, during the 3-h post-treatment test, participants rated all their GI distress markers higher in the Baseline and MC-MC conditions relative to LC-CHO; and rated their experience of stomach cramp and nausea higher in the LC-MC condition relative to LC-CHO. During the performance tests, participants rated their experience of bloatedness, reflux, nausea, and urge to vomit higher in Baseline, MC-MC and LC-MC conditions relative to LC-CHO, but only ratings for the LC-MC condition were higher than LC-CHO for the GI distress marker stomach cramp. In addition, participant ratings of bloatedness during the performance test were higher for the LC-MC condition relative to Baseline; and ratings of reflux were higher in the LC-MC condition relative to MC-MC.

Plasma

Electrolyte Status

pH (Figure 17a). No differences were observed at rest between LC-MC (1.00 \times/\div 1.02, 0.68) and LC-CHO, MC-MC (1.00 \times/\div 1.02, 0.99) and LC-CHO, or MC-MC (1.00 \times/\div 1.02, 0.68) and LC-MC conditions. No clear differences were evident between LC-MC (1.00 \times/\div 1.01, 0.96) and LC-CHO, MC-MC (1.00 \times/\div 1.01, 0.43) and LC-CHO, or MC-MC (1.00 \times/\div 1.01, 0.56) and LC-MC during the 3-h post-treatment test. During the performance test, plasma pH concentrations were 1.01 (\times/\div 1.01, 0.006) times higher in MC-MC relative to LC-

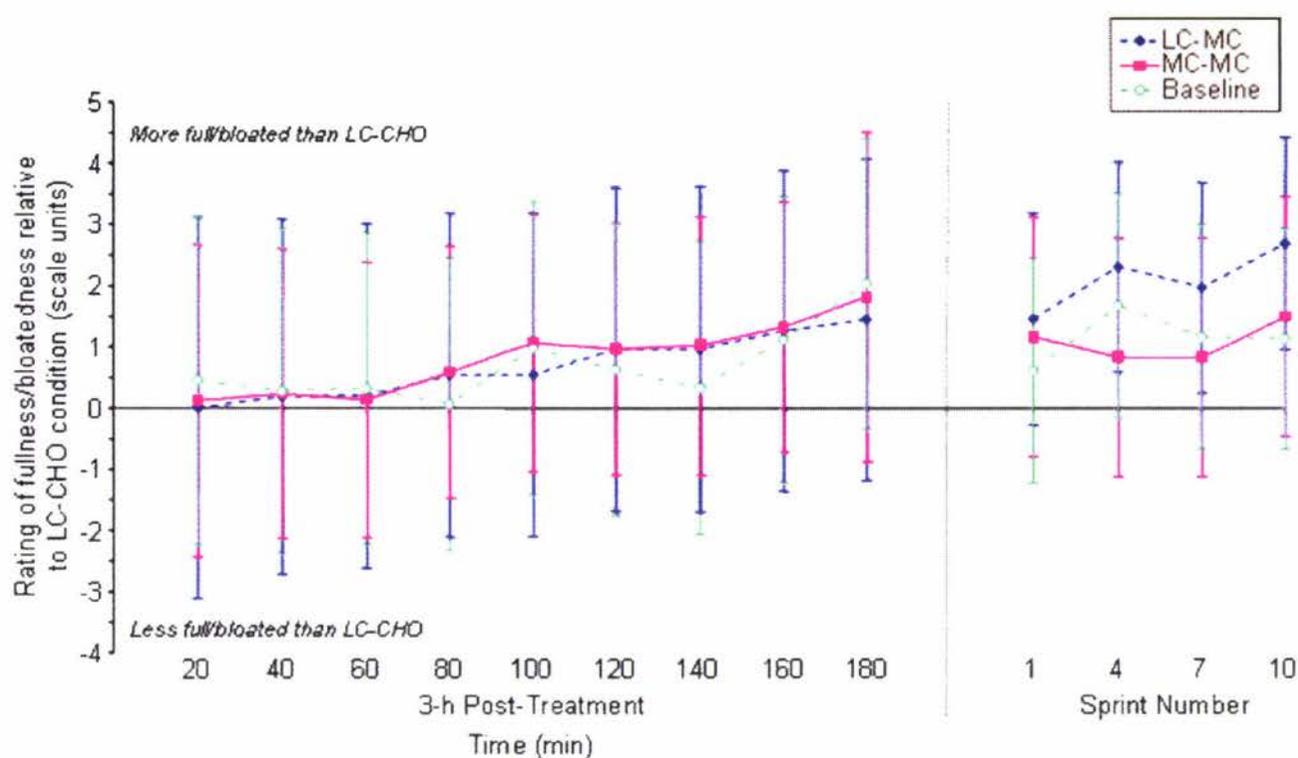


Figure 16a. Mean ratings of fullness/bloatedness relative to LC-CHO condition ratings during cycling (3-h post-treatment and performance tests). Data are presented on a linear y-axis as means \pm CI, where zero equals mean ratings of fullness/bloatedness for LC-CHO condition.

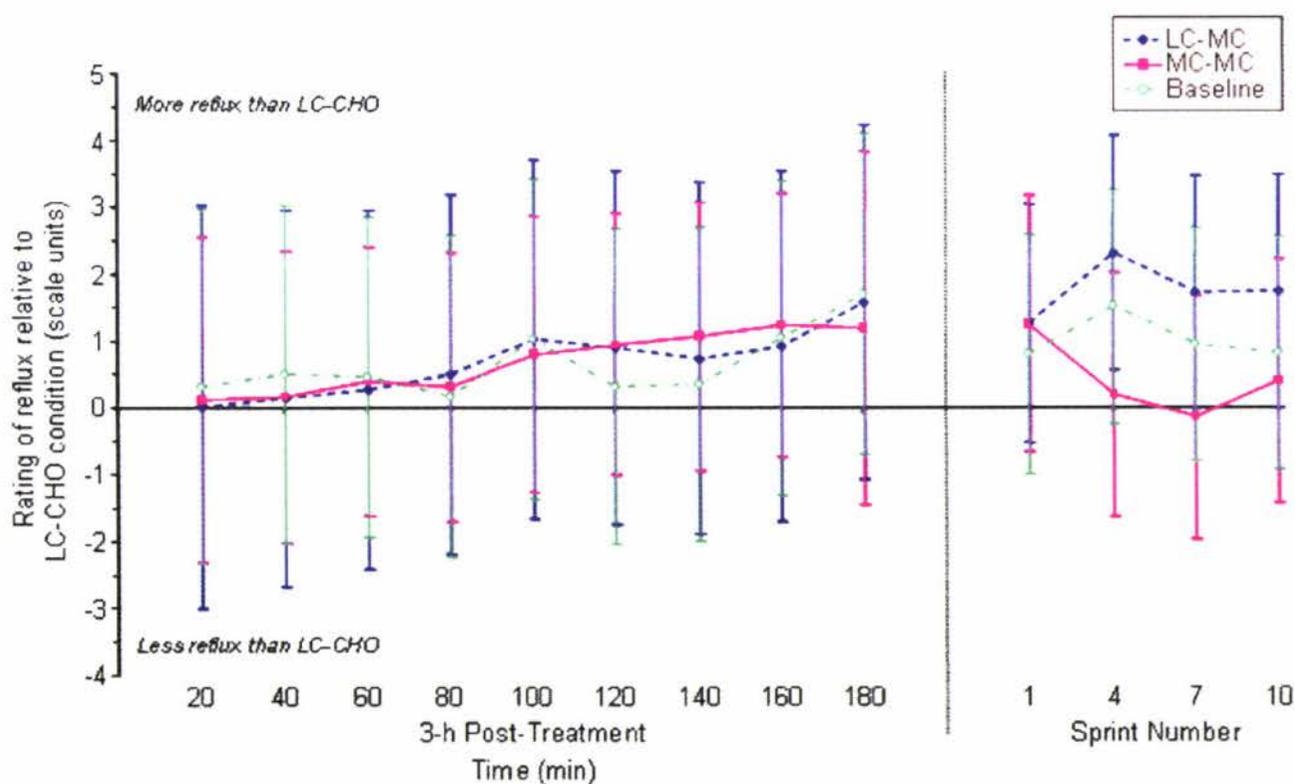


Figure 16b. Mean ratings of reflux relative to LC-CHO condition ratings during cycling (3-h post-treatment and performance tests). Data are presented on a linear y-axis as means \pm CI, where zero equals mean ratings of reflux for LC-CHO condition.

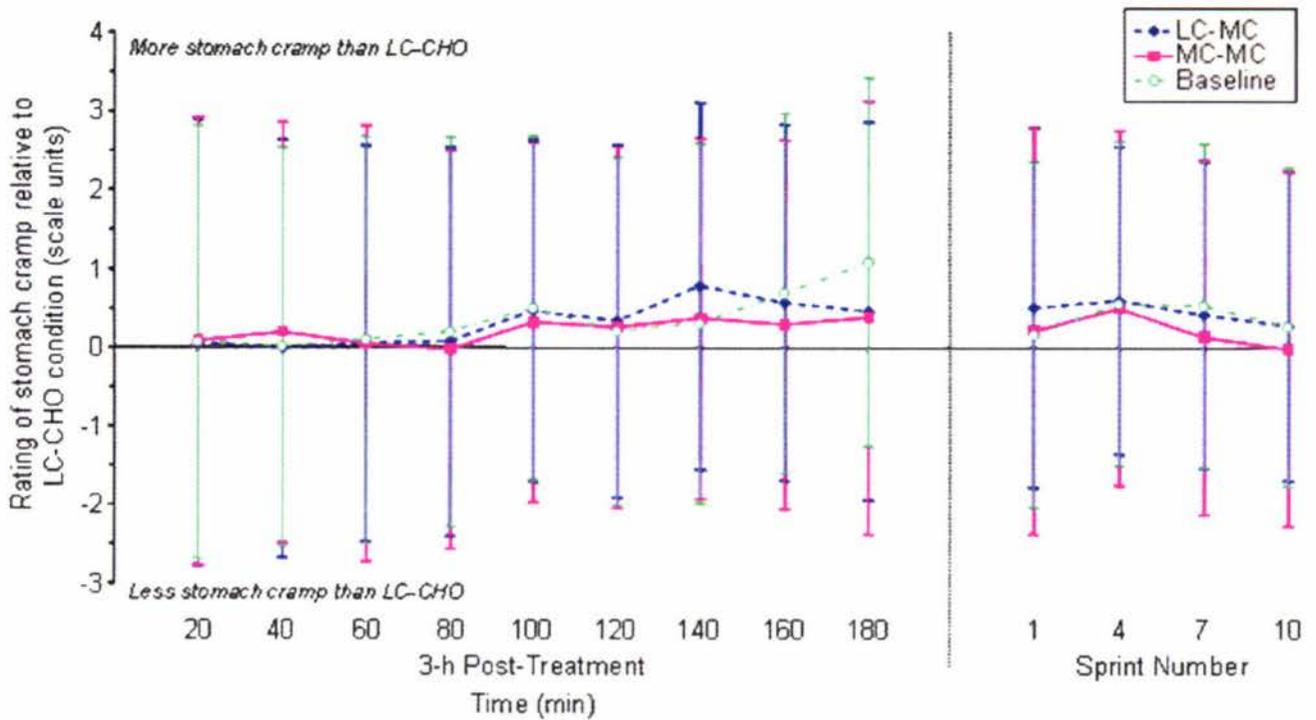


Figure 16c. Mean ratings of stomach cramp relative to LC-CHO condition ratings during cycling (3-h post-treatment and performance tests). Data are presented on a linear y-axis as means \pm CI, where zero equals mean ratings of stomach cramp for LC-CHO condition.

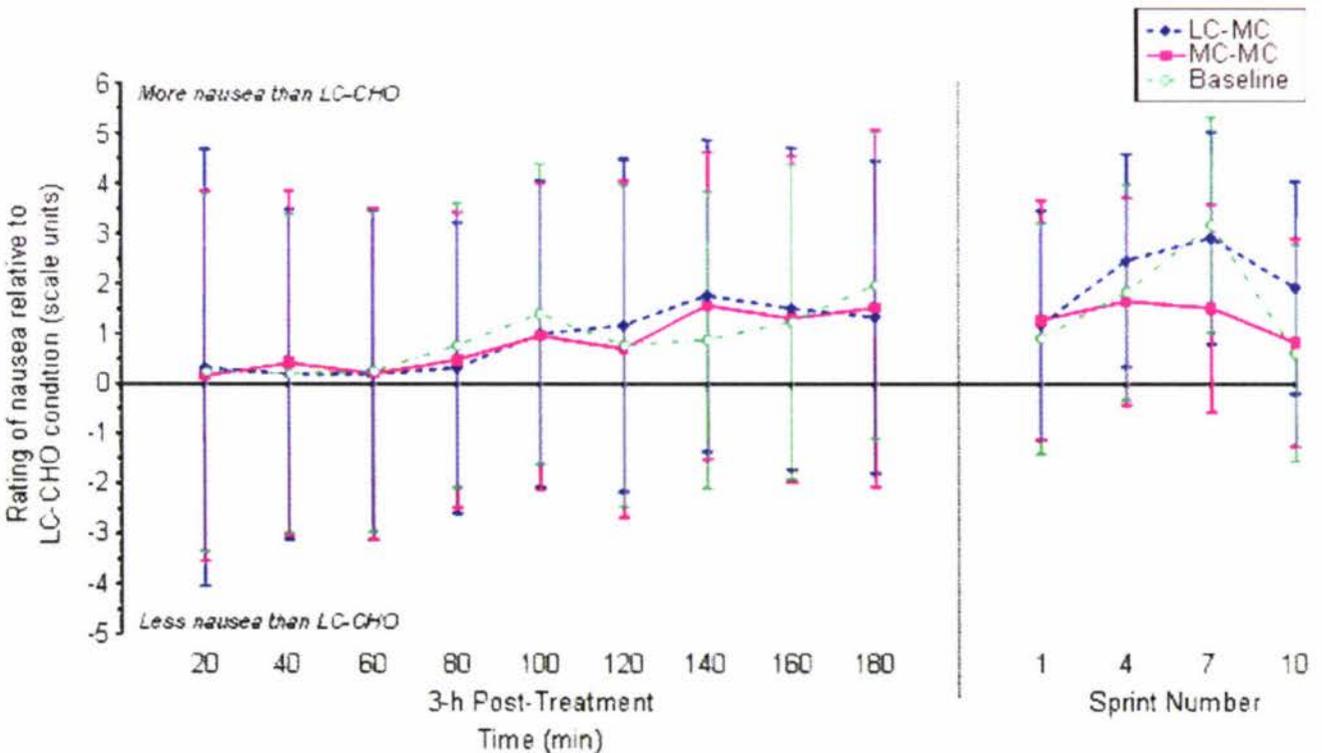


Figure 16d. Mean ratings of nausea relative to LC-CHO condition ratings during cycling (3-h post-treatment and performance tests). Data are presented on a linear y-axis as means \pm CI, where zero equals mean ratings of nausea for LC-CHO condition.

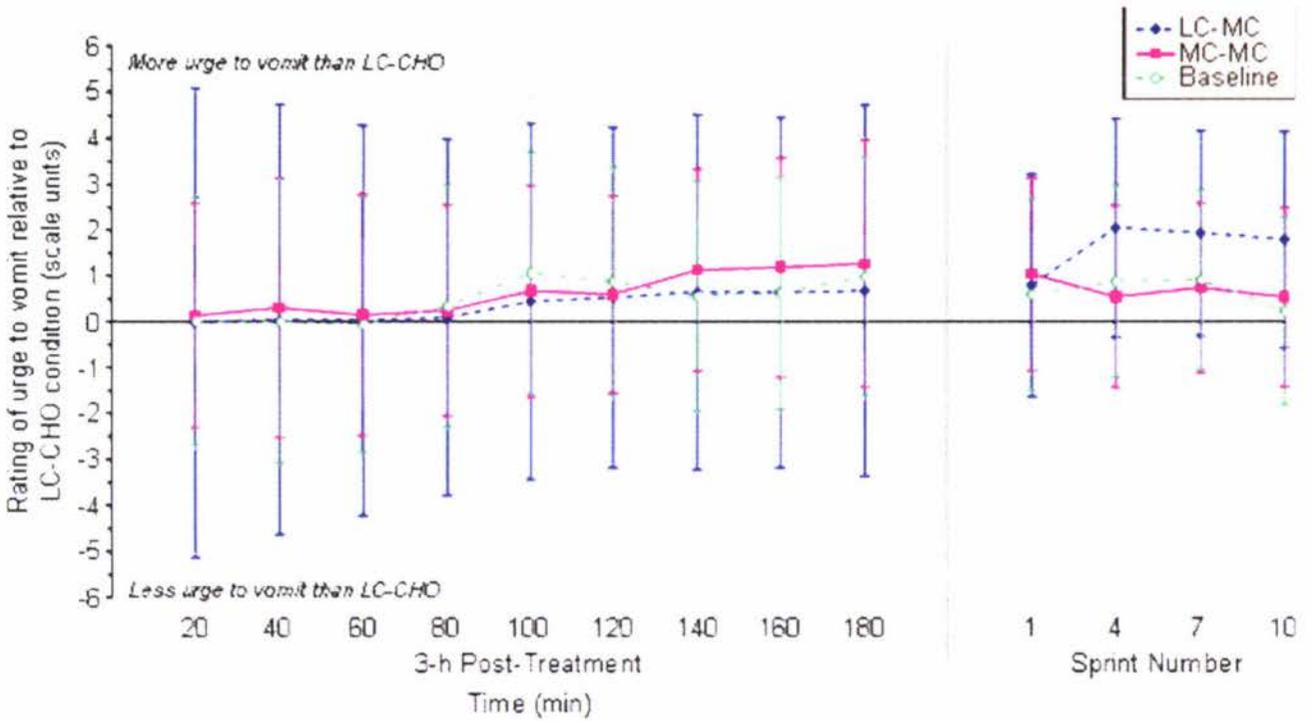


Figure 16e. Mean ratings of urge to vomit relative to LC-CHO condition ratings during cycling (3-h post-treatment and performance tests). Data are presented on a linear y-axis as means \pm CI, where zero equals mean ratings of urge to vomit for LC-CHO condition.

CHO. There were no other clear differences between LC-MC (1.01 \times/\div 1.01, 0.19) and LC-CHO, or MC-MC (1.01 \times/\div 1.01, 0.25) and LC-MC conditions during the performance test.

Standard Bicarbonate (Figure 17b). No differences were observed between LC-MC (1.07 \times/\div 1.13, 0.24) and LC-CHO, MC-MC (1.03 \times/\div 1.14, 0.59) and LC-CHO, or MC-MC (0.96 \times/\div 1.14, 0.53) and LC-MC conditions at rest. Plasma stdHCO_3^- during the 3-h post-treatment test was 0.96 (\times/\div 1.04, 0.04) and 0.96 (\times/\div 1.06, 0.14) times lower in the LC-MC and MC-MC conditions respectively relative to LC-CHO, but no clear difference was evident between MC-MC (0.99 \times/\div 1.07, 0.86) and LC-MC conditions. No differences were observed between conditions during the performance test. Plasma stdHCO_3^- concentrations were 1.00 (\times/\div 1.07, 0.90) times lower relative to LC-CHO in the LC-MC condition, and 1.04 (\times/\div 1.07, 0.27) and 1.04 (\times/\div 1.09, 0.30) times higher in the MC-MC condition relative to LC-CHO and LC-MC conditions respectively.

In summary, no difference in electrolyte status was observed at rest. However, stdHCO_3^- was lower in the LC-MC condition and tended to be lower in the MC-MC condition relative to LC-CHO during the 3-h post-treatment test. During the performance test, plasma pH was lower in the LC-CHO condition relative to MC-MC.

Electrolytes

Potassium (Figure 18a). No clear differences were observed between LC-MC (0.97 \times/\div 1.07, 0.35) and LC-CHO, MC-MC (0.97 \times/\div 1.07, 0.32) and LC-CHO, or MC-MC (1.00 \times/\div 1.07, 0.95) and LC-MC conditions at rest. Plasma K^+ concentrations were 0.97 (\times/\div 1.03, 0.03) and 0.96 (\times/\div 1.02, <0.0001) times lower relative to LC-CHO in the LC-MC and MC-MC conditions respectively for the 3-h post-treatment test. No clear difference was evident between MC-MC (0.99 \times/\div 1.03, 0.52) and LC-MC conditions. During the performance test, there was a trend for MC-MC (0.95 \times/\div 1.06, 0.08) plasma K^+ concentrations to be lower than LC-CHO, but no clear differences were observed between LC-MC (0.95 \times/\div 1.10, 0.33) and LC-CHO, or MC-MC (1.00 \times/\div 1.10, 0.99) and LC-MC conditions.

Calcium (Figure 18b). No clear differences were observed between LC-MC (0.98 \times/\div 1.11, 0.61) and LC-CHO, MC-MC (1.03 \times/\div 1.11, 0.54) and LC-CHO, or MC-MC (1.06 \times/\div 1.11, 0.27) conditions at rest. Plasma MC-MC Ca^{++} concentrations during the 3-h post-treatment test were 1.02 (\times/\div 1.03, 0.04) and 1.05 (\times/\div 1.05, 0.06) times higher than LC-CHO and LC-MC conditions respectively. No clear difference was evident between LC-MC (0.98

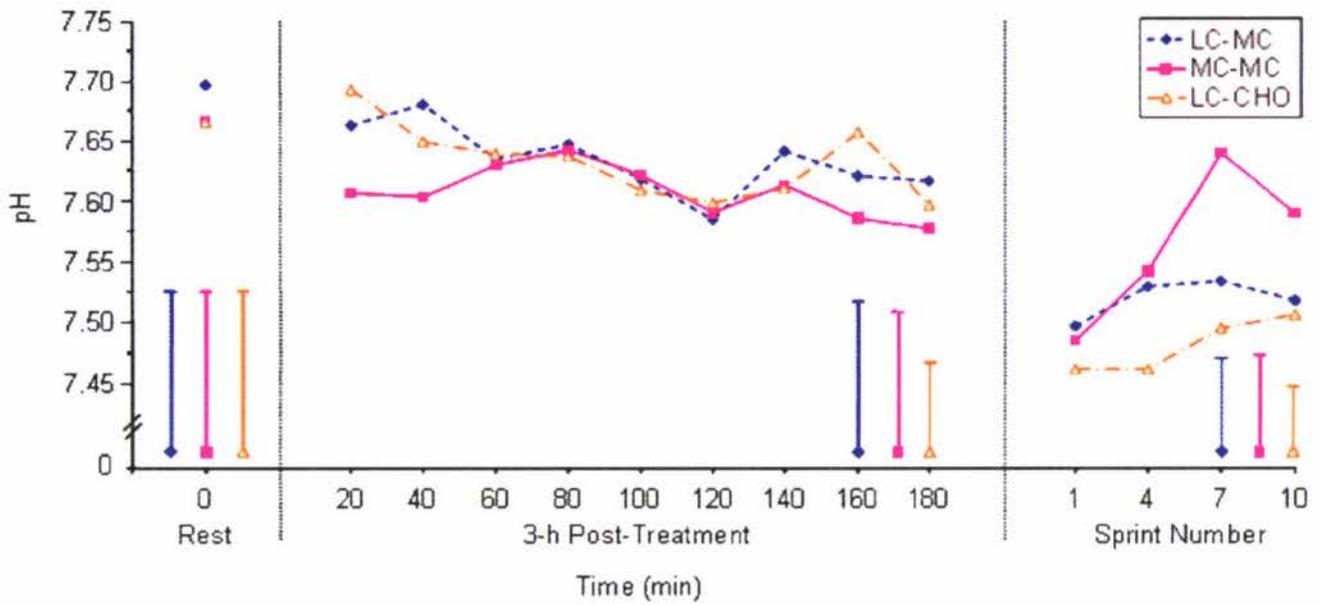


Figure 17a. Mean plasma pH concentrations at rest and during cycling (3-h post-treatment and performance tests). Data are presented on a logarithmic y-axis as means \pm SD.

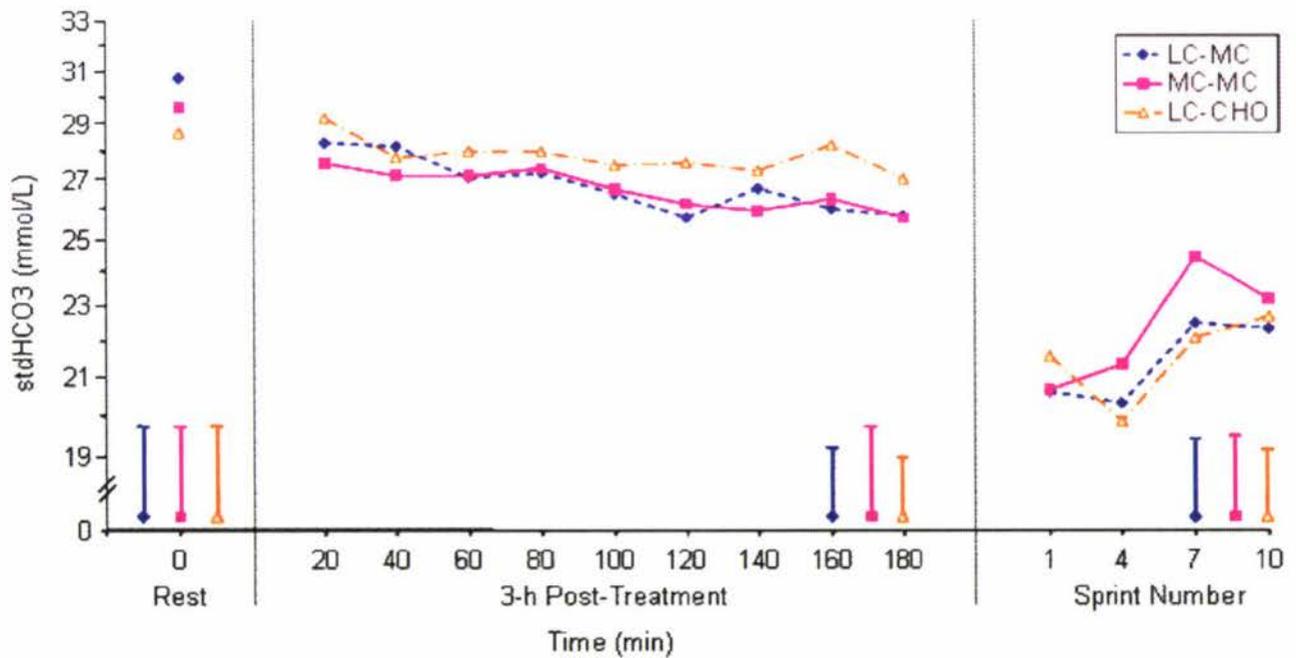


Figure 17b. Mean plasma standard bicarbonate concentrations at rest and during cycling (3-h post-treatment and performance tests). Data are presented on a logarithmic y-axis as means \pm SD.

x/\div 1.05, 0.41) and LC-CHO conditions. Plasma Ca^{++} concentrations during the performance test were 0.93 (x/\div 1.08, 0.06) and 0.94 (x/\div 1.39, 0.001) times lower relative to LC-CHO in the LC-MC and MC-MC conditions respectively. There was no clear difference between MC-MC (1.01 x/\div 1.08, 0.85) and LC-MC conditions.

Sodium (Figure 18c). No clear difference in plasma Na^+ concentrations were observed between conditions at rest or during exercise. At rest, LC-MC and MC-MC conditions respectively were 0.98 (x/\div 1.05, 0.47) and 1.00 (x/\div 1.05, 0.84) times lower relative to LC-CHO, and MC-MC was 1.01 (x/\div 1.05, 0.60) times higher relative to LC-MC. No clear differences were observed during the 3-h post-treatment test. Plasma Na^+ concentrations were 1.00 (x/\div 1.02, 0.62) times lower in the LC-MC condition relative to LC-CHO, and 1.01 (x/\div 1.01, 0.23) and 1.01 (x/\div 1.02, 0.18) times higher in the MC-MC condition relative to LC-CHO and LC-MC respectively. No differences were observed during the performance test either. Plasma concentrations were 1.00 (x/\div 1.06, 0.24) and 0.99 (x/\div 1.04, 0.51) times higher in the LC-MC and MC-MC conditions relative to LC-CHO, respectively; and MC-MC plasma concentrations were 1.02 (x/\div 1.06, 0.46) times higher relative to LC-MC.

Chlorine (Figure 18d). No clear difference in plasma Cl^- concentrations were observed between conditions at rest or during exercise. At rest, MC-MC plasma Cl^- concentrations were 1.01 (x/\div 1.05, 0.61) and 1.01 (x/\div 1.05, 0.54) times higher relative to LC-CHO and LC-MC conditions respectively, and LC-MC plasma concentrations were 1.00 (x/\div 1.05, 0.91) times lower relative to LC-CHO. During the 3-h test, MC-MC plasma concentrations were 1.01 (x/\div 1.01, 0.21) and 1.00 (x/\div 1.02, 0.89) times higher relative to LC-CHO and LC-MC respectively, and LC-MC concentrations were 1.01 (x/\div 1.02, 0.39) times higher relative to LC-CHO. During the performance test, plasma Cl^- concentrations were 0.98 (x/\div 1.05, 0.39) and 1.00 (x/\div 1.05, 0.89) times lower relative to LC-CHO in LC-MC and MC-MC conditions respectively. And concentrations in the MC-MC condition were 1.02 (x/\div 1.05, 0.47) times higher relative to LC-MC.

In summary, plasma electrolyte concentrations were similar between conditions at rest and no difference in Na^+ and Cl^- plasma concentrations were observed during exercise. However, plasma K^+ concentrations in the 3-h post-treatment test were lower in the LC-MC and MC-MC conditions relative to LC-CHO, and tended to be lower during the performance test for the MC-MC condition relative to LC-CHO. Plasma Ca^{++} concentrations during the 3-h post-treatment test were higher in the MC-MC condition, and tended to be higher in the LC-MC

condition relative to LC-CHO, but were higher in the LC-CHO condition relative to MC-MC and LC-MC during the performance test.

Metabolites

Glucose (Figure 19a). No clear differences were observed between LC-MC (1.03 \times/\div 1.11, 0.62) and LC-CHO, MC-MC (0.98, 1.11, 0.66) and LC-CHO, or MC-MC (0.95 \times/\div 1.11, 0.35) and LC-MC conditions at rest. During the 3-h ride, plasma glucose concentrations were 0.92 (\times/\div 1.07, 0.01) and 0.88 (\times/\div 1.04, <0.0001) times lower relative to LC-CHO in the LC-MC and MC-MC conditions respectively. No clear difference was evident between MC-MC (0.96 \times/\div 1.07, 0.29) and LC-MC conditions. Plasma glucose concentrations during the performance test were 0.91 (\times/\div 1.09, 0.02) and 0.88 (\times/\div 1.09, 0.006) times lower than LC-CHO in the LC-MC and MC-MC conditions respectively. No clear difference was evident between MC-MC (0.97 \times/\div 1.09, 0.52) and LC-MC conditions.

Lactate (Figure 19b). Plasma lactate concentrations at rest were 0.84 (\times/\div 1.20, 0.05) and 0.87 (\times/\div 1.20, 0.12) times lower in the MC-MC condition relative to LC-CHO and LC-MC conditions respectively. No clear differences were observed between LC-MC (0.96 \times/\div 1.20, 0.66) and LC-CHO conditions. During the 3-h post-treatment test, plasma concentrations were 0.90 (\times/\div 1.14, 0.10) and 0.83 (\times/\div 1.14, 0.005) times lower relative to LC-CHO in the LC-MC and MC-MC conditions respectively. No clear difference was evident between the MC-MC (0.93 \times/\div 1.19, 0.42) and LC-MC conditions. Plasma concentrations during the performance test were 0.71 (\times/\div 1.44, 0.06) and 0.73 (\times/\div 1.20, 0.001) times lower relative to LC-CHO in the LC-MC and MC-MC conditions respectively. Again, no clear difference was evident between the MC-MC (1.03 \times/\div 1.44, 0.89) and LC-MC conditions.

In summary, no difference in plasma glucose concentration was observed between conditions at rest. However, both LC-MC and MC-MC plasma glucose concentrations were lower relative to LC-CHO during exercise. Plasma lactate concentrations were lower in the MC-MC condition relative to LC-CHO at rest and during exercise, but were only lower in the LC-MC condition relative to LC-CHO during exercise.

Heart Rate

Overall, mean HR in the MC-MC condition was 1.5% (\pm 1.7%, 0.001) and 1.1% (\pm 1%, 0.006) lower than LC-MC and LC-CHO respectively. No clear difference in heart rate was evident between LC-MC (0.4% \pm 0.8%, 0.24) and LC-CHO conditions.

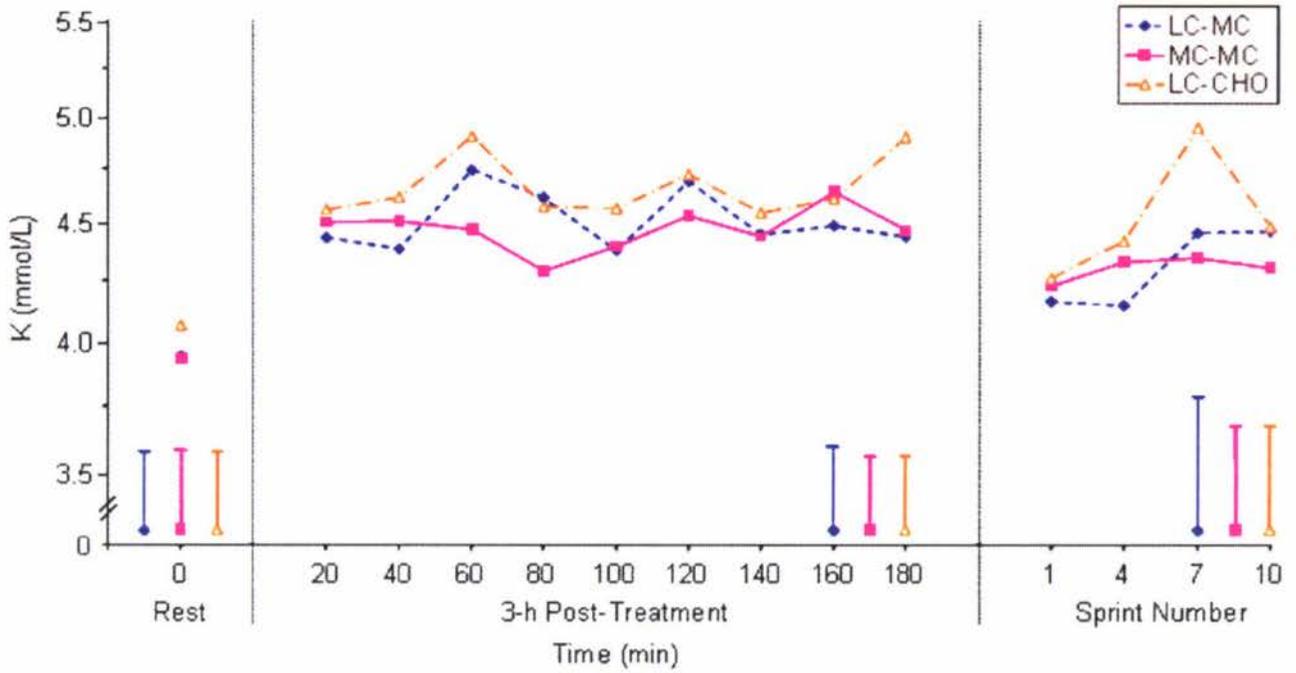


Figure 18a. Mean plasma potassium concentrations at rest and during cycling (3-h post-treatment and performance tests). Data are presented on a logarithmic y-axis as means \pm SD.

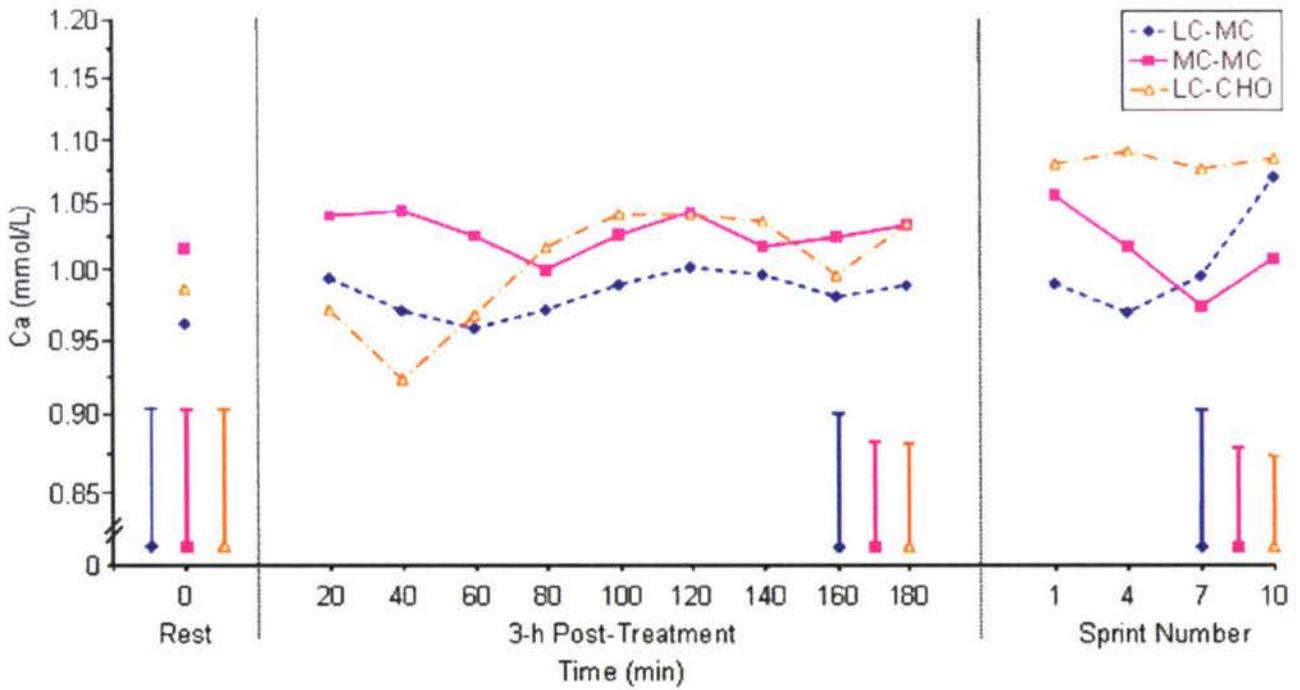


Figure 18b. Mean plasma calcium concentrations at rest and during cycling (3-h post-treatment and performance tests). Data are presented on a logarithmic y-axis as means \pm SD.

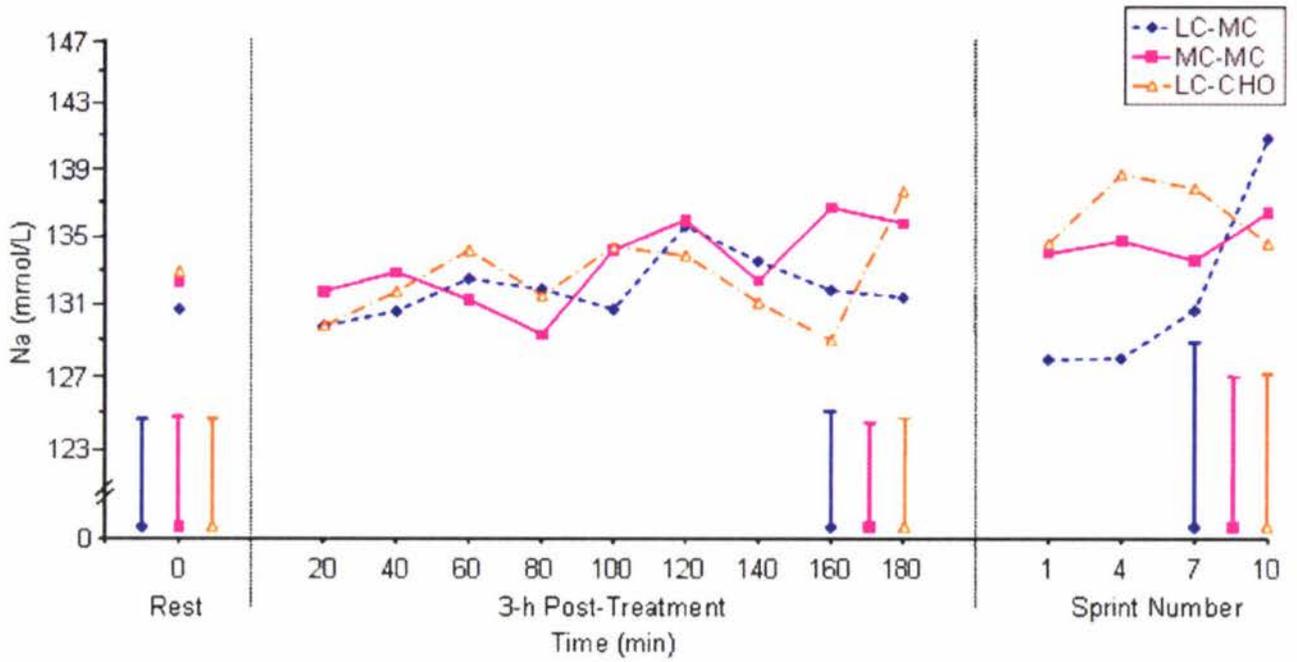


Figure 18c. Mean plasma sodium concentrations at rest and during cycling (3-h post-treatment and performance tests). Data are presented on a logarithmic y-axis as means \pm SD.

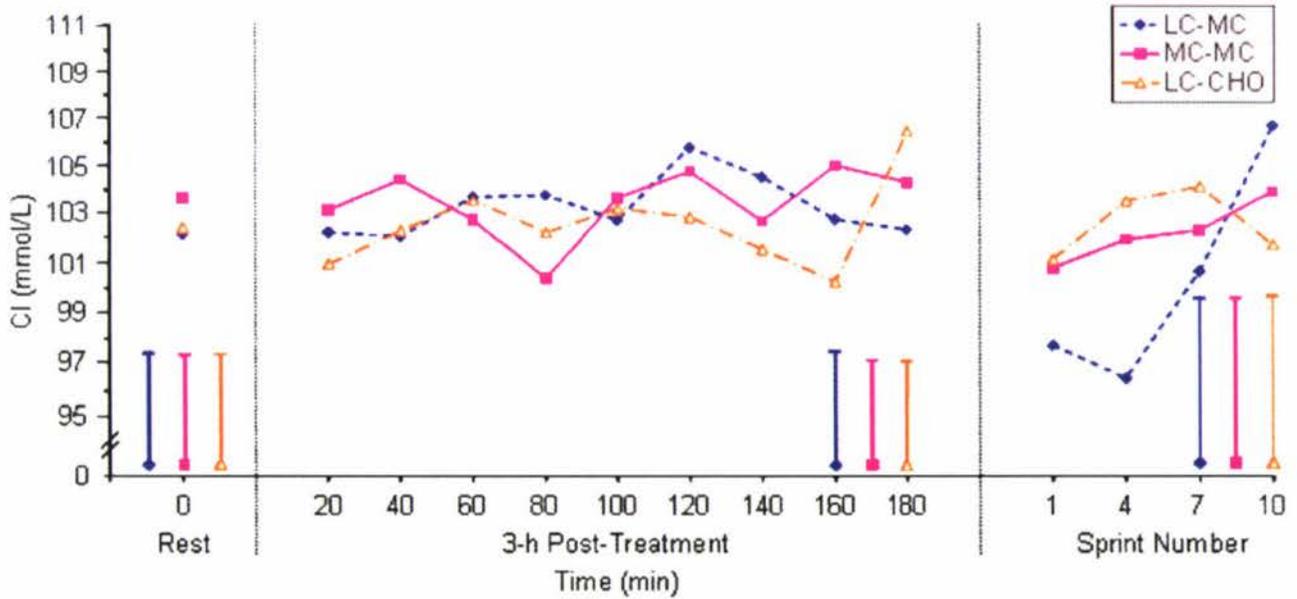


Figure 18d. Mean plasma chlorine concentrations at rest and during cycling (3-h post-treatment and performance tests). Data are presented on a logarithmic y-axis as means \pm SD.

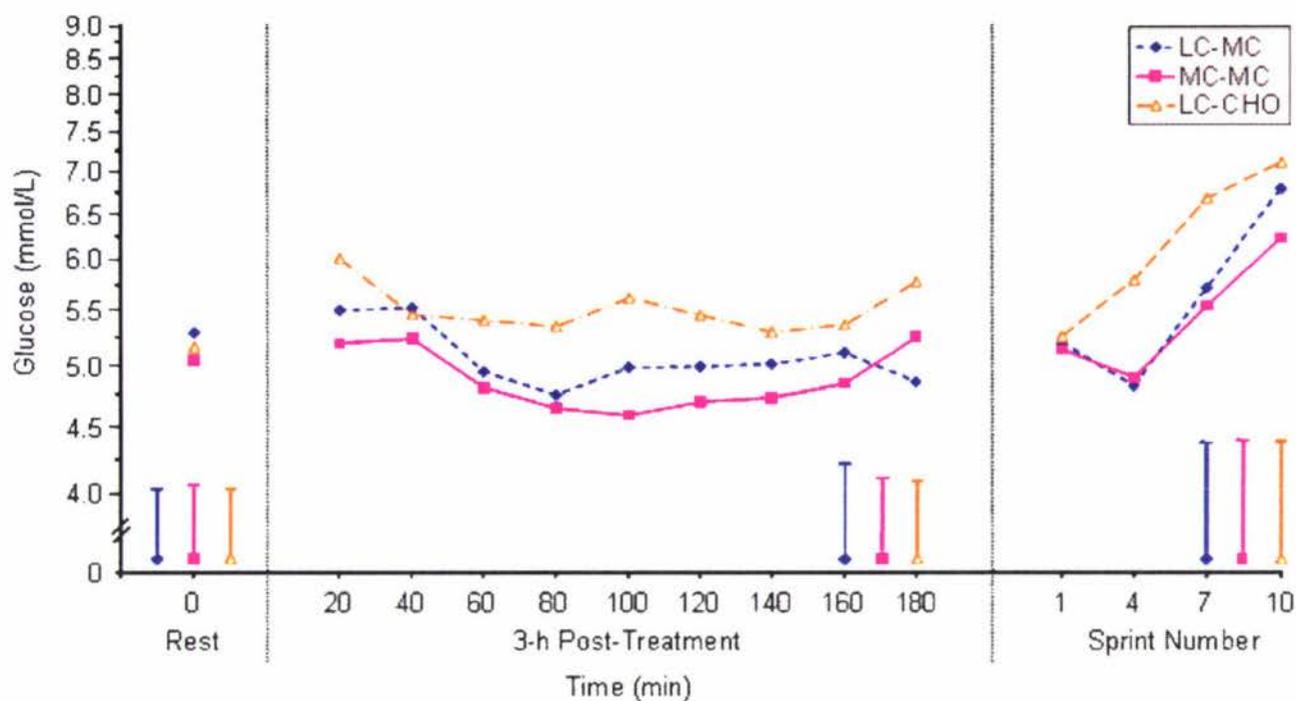


Figure 19a. Mean plasma glucose concentrations at rest and during cycling (3-h post-treatment and performance tests). Data are presented on a logarithmic y-axis as means \pm SD.

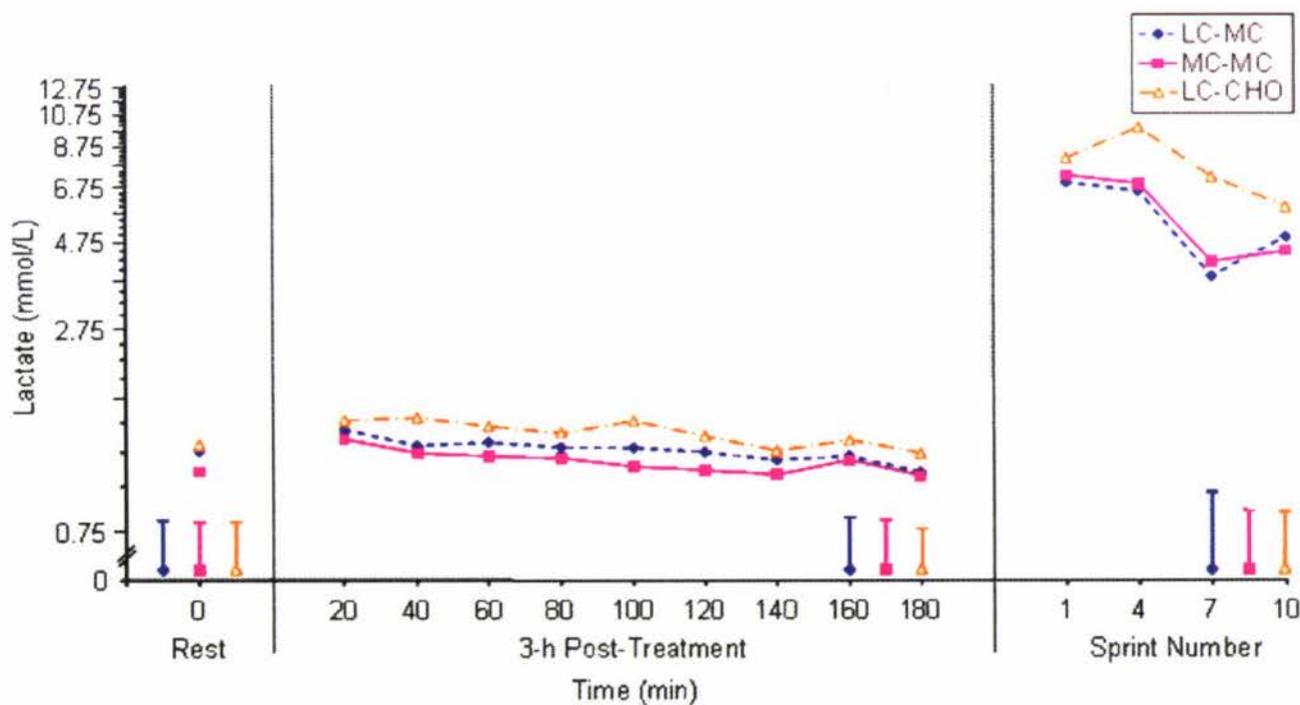


Figure 19b. Mean plasma lactate concentrations at rest and during cycling (3-h post-treatment and performance tests). Data are presented on a logarithmic y-axis as means \pm SD.

Discussion

The main purpose of this study was to investigate the effects of a 2-week high-MCFA dietary adaptation period on GI distress, MCFA oxidation rate and performance while ingesting an MCFA-rich exercise supplement during endurance cycling. Contrary to expected outcomes, dietary MCFA supplementation did not have a substantial or clear lowering effect on GI distress or accentuating effect on MCFA oxidation during exercise in comparison to the LCFA supplement control. However, MCFA oxidation rates were almost three-fold what has been previously reported, reaching a maximum of $0.43 \text{ g}\cdot\text{min}^{-1}$. Despite the high oxidation rates, ingestion of the MCFA-rich supplement during exercise was detrimental to cycling performance relative to a CHO placebo. Relative to the LC-CHO control condition, sprint times were 10% and 7% slower in the LC-MC and MC-MC conditions, respectively.

Previous studies in this research area have used submaximal constant-work performance tests lasting up to 1 h. Reliability studies investigating changes in mean power for constant-work tests have established a CV of approximately 2% (Hopkins et al., 2001). Repeated sprint tests have been shown by Watt et al. (2002) to have a between-trial mean power CV of 1.2 to 2.8%. In addition, Schabert et al. (1998) have shown that well-trained cyclists are able to reproduce race times during a prolonged bout of exercise, with the inclusion of regular sprint intervals, with a CV of 0.61 to 4.1%. The performance test used in this study was developed using sprint intervals to more closely simulate the variable-intensity nature of a road cycle race. The within-subject standard deviation for mean power in the performance test was 5.3%. This standard deviation is derived from the residual variance of the repeated-measures analysis and is analogous to the CV% obtained in a reliability trial (W.G. Hopkins, personal communication, 2005). Relative to other performance tests, the present model for cycling performance appeared to have a relatively poor reliability. However, the magnitude of the effect was approximately 1.5 to 2-fold greater than the typical within-test error, which indicates that the test has sufficient sensitivity to detect effects important to cyclists. Interestingly, recovery 8 of the performance test was much slower for the LC-MC and LC-CHO conditions, and recoveries 5 and 8 tended to be slower for the MC-MC condition. This is probably due to blood sampling following sprints 4 and 7, as there were occasional problems with blockage of the cannulae that appeared to cause participants to reduce their workload.

Sprint performance was worse in the MC-MC condition relative to LC-CHO, despite more total calories being ingested in the MCFA-rich exercise supplements relative to the

CHO-rich supplement. This is in contrast with previous studies (Decombaz et al., 1983; Jeukendrup, 1998; Goedecke, 1999; Oopik, 2001; Misell, 2001; Vistisen et al., 2003) in which no clear difference in performance was observed when MCFA-rich exercise supplements were ingested in comparison to CHO-only. It has been suggested that the lack of glycogen sparing and performance benefit in these studies can be attributed, at least partially, either to the low doses of MCT ingested during exercise, or the moderate-to-high GI distress experienced by participants with higher doses (Van Zyl et al., 1996; Jeukendrup et al., 1998, Goedecke et al., 1999). However, exogenous MCFAs may not reach the systemic circulation as MCFAs. After the ingestion of large amounts of C8:0, Vistisen et al. (1996) did not detect any C8:0 in plasma triacylglycerols, fatty acids or phospholipids. This indicates that medium-chain fats may not reach the systemic circulation after passing the liver, which would exclude utilisation of their potentially beneficial properties (rapid absorption by the intestine, more efficient uptake and oxidation in the muscle mitochondria). There are several possibilities for the fate of MCFAs other than oxidation in muscle mitochondria. Firstly, the majority of exogenous MCFAs may be elongated in the liver before being released into the systemic circulation for use as muscle substrate, in which case their ability to enter the muscle mitochondria free of the rate-limiting carnitine transport system would be nullified (Vistisen et al., 2003; Pakula et al., 1997). Alternatively, MCFAs can be converted into ketone bodies because of the rapid build-up of acetyl-CoA in the mitochondria. Ketone bodies are then either recycled back into energy substrate by the body, or can be excreted through the urine and breath (Fery & Balasse, 1985; Laffel, 1999). It is also possible that not all the exogenous MCFAs are absorbed by the gut, and may be excreted in the faeces.

Despite the unknown fate of MCFAs, it does appear that some are oxidised for use as energy, either in the form of MCFAs, elongated fats or ketone bodies. This has been demonstrated by the use of ^{13}C -enriched MCFA-rich supplements, and subsequent measurement of $^{13}\text{CO}_2$ in expired breath. Up to the first hour of $^{13}\text{CO}_2$ results in expired breath must be reviewed critically due to trapping of exogenous $^{13}\text{CO}_2$ in the bicarbonate pool (Coggan, 1993; Jeukendrup et al., 1995). This is a large, slowly exchanging pool in which a certain amount of CO_2 arising from decarboxylation of energy substrates is temporarily trapped. However, during exercise, CO_2 production increases to an extent where a steady-state occurs and $^{13}\text{CO}_2$ in expired air equilibrates with the bicarbonate pool. Dilution of $^{13}\text{CO}_2$ becomes negligible, and recovery in the breath approaches 100% after about 60 min of exercise (Coggan, 1993; Jeukendrup et al., 1995). It has previously been shown that MCFAs can be oxidised up to a rate of $\sim 0.15 \text{ g}\cdot\text{min}^{-1}$ (Jeukendrup et al., 1996) when co-ingested with

CHO. However, the maximum mean oxidation rate in this study was almost 3-fold greater at $0.43 \text{ g}\cdot\text{min}^{-1}$, contributing $\sim 17\%$ to total energy expenditure.

No metabolic adaptation occurred with high dietary MCFA ingestion in this study, as equally high rates of MCFA oxidation during exercise were observed in both the MC-MC and LC-MC conditions. This result is evidence against an intestinal MCFA transporter, but suggests that there was a unique quality of our supplements that allowed such high absorption and oxidation rates. The digestion of fats is normally slow because TAGs are insoluble in water-mediums and therefore aggregate into large lipid droplets in the small intestine. This slows the action of *pancreatic lipase* because its digestive action takes place only at the surface of a lipid droplet. However, the fats in our supplement were suspended in an emulsion, which distributes lipids into smaller droplets and prevents them from re-aggregating, thereby increasing their surface area and accessibility to pancreatic lipase (Vander et al., 1994). Also, we fed our participants MCFAs and LCFAs in the form of structured TAGs, which may have enhanced MCFA absorption due to the increase in pancreatic secretion that is stimulated by LCFA ingestion (but not by MCFAs) (Mott et al., 1972). Therefore, more extensive hydrolysis of the structured TAGs would have occurred in comparison to MCT solutions. Higher absorption rates from the small intestine would then allow higher oxidation rates due to greater availability in the blood and muscle.

The structures of the TAGs in this study are similar to that which Vistisen et al. (2003) fed their participants. However, ours were randomly re-esterified, creating any of 8 possible combinations of M- and LCFAs on the sn-1, -2 and -3 positions of the glycerol backbone, whereas the TAGs in the Vistisen et al (2003) study were in the specific form of MLM (medium-long-medium) structured TAGs. Structured TAGs in the form of MLM are more readily broken down and absorbed by the gut due to the actions of *acid lipases* in the stomach and small intestine. Specifically, *pancreatic lipase* preferentially cleaves fats from the sn-1 and sn-3 positions of the glycerol backbone, allowing the fast absorption of MCFAs without slowing of digestion by LCFAs (Bach & Babayan, 1982; Bendixen et al., 2002). Unfortunately, Vistisen et al (2003) did not quantify MCFA oxidation rates, therefore we cannot make any oxidation comparisons between the random structured TAGs used in this study and MLM structured TAGs used by Vistisen et al. (2003).

Despite similarities in exercise supplements between this study and that of Vistisen et al (2003) (structured TAGs and emulsification of lipids), the performance outcomes were quite different. Co-ingestion of MCFAs and CHO in the present study caused a performance detriment in comparison to CHO-only supplements, which contrasts with the findings of

Vistisen et al (2003), and previous studies in which no change in performance was observed with MCFA and CHO co-ingestion. However, performance detriments have been observed when participants ingested MCT-only supplements during exercise (Jeukendrup et al., 1998; Van Zyl et al., 1996). The decrease in performance in this study with MCFA and CHO co-ingestion may have been due to GI distress suffered when ingesting the MCFA-rich supplement. Because participants were required to complete 10 maximal sprints, GI distress may have hindered their performance more than in previous studies which have used lower-intensity time-trial tests. A decrease in performance due to GI distress is supported in this study by the moderately-strong negative statistical relationship between nausea and sprint mean power. It is thought that this is the first time that the correlation between nausea and sprint performance has been quantified. The CR10 scale used to construct the GI and RPE scales for this study has previously been shown to be highly reliable and valid (Borg, 2001), and were also shown to have a within-subject variation of 21.8 to 95.4% (or $1.22-1.95 \times / \div$ a scale unit; Table 1). For example, if a participant rated their perception of nausea '5', a CV of 95.4% would give a rating range of 4.56 to 5.47 scale units.

The purpose of the dietary intervention was to decrease GI distress, and therefore allow the potential metabolic and performance benefits to be revealed in the absence of effective GI distress. In addition to supplementing their daily diet regime, participants were also instructed to ingest MCFA-rich supplements during training to try and induce further metabolic changes during exercise. The structured TAGs were also emulsified in the exercise supplements to prevent the oils from sitting on top of the stomach contents and causing a large amount of oil to enter the small intestine at once, which is likely to cause high GI distress. Distress was measured subjectively by participant ratings of specific GI markers (bloating, reflux, stomach cramps, nausea, and urge to vomit). There was approximately a 2-fold decrease in GI distress with test order from the initial naïve exposure to MCFA ingestion in the Baseline test, to that of the second exposure in either the LC-MC or MC-MC condition, suggesting some sort of initial adaptation. In addition, there were some emerging trends evident between GI distress in the MC-MC and LC-MC conditions. Though ratings of reflux were the only significantly lower variable in the MC-MC condition relative to LC-MC (1.34 scale units lower; 95% CI 0.88-3.14 scale units lower; p-value = 0.05), trends were also emerging for bloating (1.03; 0.74-2.27; 0.15), nausea (0.81; 0.62-1.69; 0.23) and urge to vomit (0.93; 0.64-2.45; 0.22) variables. However, participants still experienced higher distress overall in the MC-MC and LC-MC conditions (mild to moderate/high) relative to LC-

CHO (extremely mild to mild/moderate; $p < 0.05$). Therefore, though the GI distress observed with MCFA ingestion was only on average mild-moderate, it was still higher than that reported during the LC-CHO placebo condition and could have interfered with performance, hence, longer-term adaptation may be required to further decrease GI distress during exercise when ingesting esterified oil emulsions.

The only group of researchers thus far to observe a performance benefit with MCT ingestion relative to CHO in a human study was Van Zyl et al. (1996). After 120 min of submaximal exercise at 60% $\dot{V}O_{2\max}$, their participants performed a simulated 40-km time trial. Performance decreased by 7.5% with MCT-only ingestion, but increased by 2.5% ($p < 0.05$) with CHO + MCT ingestion relative to CHO-only. No GI distress was reported by Van Zyl et al., despite feeding participants 86 g of MCT, which is in contrast to other studies feeding >30 g of MCT, including the present study where participants ingested on average 104 g of MCT. Van Zyl et al. (1996) suggested that the increase in performance in their study was due to an increase in availability of plasma fatty acids and subsequent sparing of muscle glycogen. This result is in contrast to the present findings, in which an increase in MCFA oxidation had no effect on CHO oxidation, and appeared to spare only endogenous-fat stores. Other studies have also failed to demonstrate a glycogen-sparing effect of MCT ingestion. In three studies in which the effects of MCT ingestion on substrate utilisation and performance were investigated, no difference in CHO or total fat oxidation in CHO-only and CHO+MCT conditions were observed (Jeukendrup et al., 1995, 1996, 1998). Similarly, Goedecke et al (1999) found no difference in CHO or fat oxidation rates when investigating CHO-only, low-MCT and high-MCT ingestion. These four studies, like the present study, suggest that MCT ingestion during exercise spares endogenous fat stores rather than endogenous glycogen stores, and therefore would not contribute to any performance benefit. The results of Van Zyl et al. (1996) should be interpreted with care, as only six participants were tested, making it more likely that a type I error could occur (i.e. a false positive result).

It is worth noting here that, in the present study, participants put significantly less effort in to their sprints in the MC-MC relative to the LC-MC condition, as rated on the RPE scales after sprints 1, 4, 7 and 10. This result is supported by lower overall mean heart rates during the sprints in the MC-MC condition, and could indicate that performance in the MC-MC condition should be closer to LC-CHO, which would be consistent with previous studies co-ingesting MCFAs and CHO. However, it does not explain the decrease in performance for the LC-MC condition because GI distress, MCFA oxidation rates, and plasma variables were

similar relative to MC-MC conditions, but ratings of effort were significantly higher (LC-MC = very high to extremely high/absolute maximum; MC-MC = high to very high/extremely high; p -value = 0.01).

Plasma pH levels overall were higher (more alkaline) in the MC-MC condition during the performance test relative to LC-CHO (Figure 17a), which is consistent with higher plasma-lactate concentrations in LC-CHO (Figure 19b). Plasma lactate levels were probably higher in the LC-CHO condition because the participants were exercising at a higher intensity, which would increase glycolytic flux above the capacity of the Krebs cycle and cause the production of lactate from pyruvate. The main buffer for lactate (Lac^-) in the extracellular fluid is bicarbonate (HCO_3^-). Bicarbonate maintains electrical neutrality in the extracellular compartment, and is therefore also influenced by other strong ions (K^+ , Na^+ , Cl^-). Bicarbonate is an effective buffer because it rapidly disassociates to CO_2 , which is expired by the lungs. By doing this, HCO_3^- buffers the majority of negative strong-ion influx into the system, with the positive charge balance being accounted for by the addition of H^+ ions from the H_2O disassociation reaction (Stewart, 1983).

Plasma K^+ concentrations were higher in the LC-CHO condition relative to both the MC-MC and LC-MC conditions during the 3-h post-treatment test (Figure 18a). A possible mechanism for this occurrence is the increasing extracellular Ca^{++} concentrations in the LC-CHO condition, which may have caused Ca^{++} influx into cells and increased intracellular concentrations sufficiently to open Ca^{++} -sensitive K^+ channels, causing K^+ efflux into the extracellular fluid. However, this does not explain why extracellular K^+ concentrations were elevated from the first 20-min blood sample. Plasma K^+ concentrations were also higher in the LC-CHO condition during the performance test, probably due to the higher overall cycling intensity in this condition, as K^+ release from the intracellular space is proportional to exercise intensity (Lindiger, 1995; Vollestad et al., 1994).

As mentioned above, plasma Ca^{++} concentrations increased over the 3-h post-treatment test in the LC-CHO condition by $\sim 0.02 \text{ mmol}\cdot\text{L}\cdot\text{h}^{-1}$ (Figure 18b). Assuming that Ca^{++} is evenly distributed across the interstitial fluid ($\sim 10 \text{ L}$) and plasma ($\sim 3 \text{ L}$), this equates to an increase in $\sim 0.3 \text{ mmol}\cdot\text{h}^{-1}$ absolute ($0.023 \text{ mmol}\cdot\text{L}\cdot\text{h}^{-1}$). This increase in extracellular Ca^{++} in LC-CHO relative to LC-MC and MC-MC conditions can be attributed to the extra Ca^{++} ingested with added milk powder to the exercise supplement, which was done to emulate the colour and taste of the MCFA-rich supplements. Participants were therefore ingesting an extra $\sim 5 \text{ mmol}\cdot\text{h}^{-1}$ of Ca^{++} in the LC-CHO condition. Both intra- and extracellular Ca^{++} concentrations are tightly regulated in the body, and we must therefore account for some loss

of ingested Ca^{++} in the urine, sweat, flux into cells, and also in the gut (what has not been absorbed), which would explain the increase in plasma concentration of only $0.3 \text{ mmol}\cdot\text{h}^{-1}$ relative to the extra $5 \text{ mmol}\cdot\text{h}^{-1}$ ingestion rate.

There was no difference between conditions in plasma Na^+ and Cl^- concentration, and their concentration curves followed each other closely (Figures 18c & 18d). This is because Na^+ and Cl^- are tightly regulated in the extracellular fluid in order to maintain normal cross-membrane charges. The rapid rise in plasma concentration for both of these ions from sprints 4-7 and 7-10 probably indicates a delay in gastric emptying and absorption of NaCl from the exercise supplement due to diminished gastric blood flow, which is supported by a similar increase in plasma Ca^{++} and glucose concentrations.

Summary

The present study revealed a performance decrement with ingestion of randomised structured TAGs following 2-weeks of targeted adaptation, despite an almost three-fold increase in maximum MCFA oxidation relative to the findings of previous studies. Ingestion of an MCFA-rich exercise supplement caused a 10% and 7% decrease in sprint performance relative to CHO-only following a diet high in LCFAs and MCFAs, respectively. Instead of sparing endogenous glycogen, MCFA oxidation appeared to spare only endogenous-fat stores. However, 2-weeks of dietary adaptation did appear to slightly lower GI distress during exercise in the MC-MC condition relative to LC-MC, which was also reflected in the emerging trend toward higher sprint mean power in the MC-MC condition. The ingestion of MCFAs in the form of structured TAGs also appeared to be beneficial in terms of GI distress, as no participants in this study experienced diarrhoea or vomited during or after exercise, and most GI symptoms were rated as mild-to-moderate.

Future Research

The results of this study are fairly definitive in ruling out any potential performance benefit of MCT supplementation for male athletes. Even with a decrease in GI distress and increase in MCFA oxidation, no performance benefits were observed. This is consistent with most other studies in this area (Decombaz, 1983; Jeukendrup, 1995, 1996, 1998; Goedecke, 1999; Misell, 2001; Oopik, 2001; Vistisen, 2003) – bar one. However, the results of Van Zyl et al. (1996) study must be interpreted with care, as only six participants were tested, and a type I error may have occurred.

Investigation into the ingestion of MCFAs for female athletes may, however, be warranted. Females are known to burn more fat during the same relative-intensity exercise in comparison to males (Tarnopolsky, 2000; Cortright, 2000) and, therefore, may be able to spare endogenous glycogen in favour of MCFA oxidation more effectively than their male counterparts.

References

- Bach, A., & Babayan, V. (1982). Medium chain triglycerides: an update. *Am. J. Clin. Nutr.* **36**: 950-962.
- Bendixen, H., Flint, A., Raben, A., Høy, C., Mu, H., Xu, X., et al. (2002). Effect of 3 modified fats and a conventional fat on appetite, energy intake, energy expenditure, and substrate oxidation in healthy men. *Am. J. Clin. Nutr.* **75**(1): 47-56.
- Bergstrom, J., Hermansen, L., Hultman, E., & Stalin, B. (1967). Diet, muscle glycogen and physical performance. *Acta Physiologica Scandinavica* **71**: 140-150.
- Borg, G. (1998). *Borg's perceived exertion and pain scales*. Champaign, IL: Human Kinetics.
- Borg, G. (2001). Perceptual exertion. *Int. J. Sport Psychol.* **32**: 110-126.
- Bremer, J. (1980). Carnitine and its role in fatty acid metabolism. *Trends Biochem. Sci.* **2**: 207-209.
- Brouns, F., & Van der Vusse, G. (1998). Utilization of lipids during exercise in human subjects: Metabolic and dietary constraints. *Br. J. Nutr.* **79**: 117-128.
- Burke, L., & Hawley, J. (2002). Effects of short-term fat adaptation on metabolism and performance of prolonged exercise. *Med. Sci. Sports Exerc.* **34**(9): 1492-1498.
- Burke, L., Kiens, B., & Ivy, J. (2004). Carbohydrates and fat for training and recovery. *J. Sports Sci.* **22**: 15-30.
- Coggan, A. (1993). Underestimation of substrate oxidation during exercise due to failure to account for bicarbonate kinetics. *J. Appl. Physiol.* **75**(5): 2341-2343.
- Cohen, M., Morgan, R., & Hoffman, A. (1971). Libolytic activity of human gastric and duodenal juice against medium and long chain triglycerides. *Gastroenterology* **60**: 1-15.
- Cortright, R., & Koves, T. (2000). Sex differences in substrate metabolism and energy homeostasis. *Can. J. Appl. Physiol.* **25**(4): 288-311.
- Coyle, E., Coggan, A., & Hemmert, M. (1986). Muscle glycogen utilisation during prolonged strenuous exercise when fed carbohydrate. *J. Appl. Physiol.* **61**: 165-172.
- Decombaz, J., Arnaud, M., Milon, J., Moesch, H., Philippossian, G., Thélin, A., et al. (1983). Energy metabolism of medium-chain triglycerides versus carbohydrates during exercise. *Eur. J. Physiol.* **52**: 9-14.
- Dyck, D., Putman, C., Heigenhauser, G., Hultman, E., & Spriet, L. (1993). Regulation of fat-carbohydrate interaction in skeletal muscle during intense aerobic cycling. *Am. J. Physiol.* **265**: E852-E859.

- Ferrannini, E. (1988). The theoretical bases of indirect calorimetry: a review. *Metabolism* **37**(3): 287-301.
- Fery, F., & Balasse, E. (1985). Ketone body production and disposal in diabetic ketosis: a comparison with fasting ketosis. *Diabetes* **34**: 326-332.
- Fushiki, T., Matsumoto, K., Inoue, K., & Kawada, T. (1995). Swimming capacity of mice is increased by chronic consumption of medium-chain triacylglycerols. *J. Nutr.* **125**: 531-539.
- Goedecke, J., Elmer-English, R., Dennis, S., Schloss, I., Noakes, T., & Lambert, E. (1999). Effects of medium-chain triacylglycerol ingested with carbohydrate on metabolism and exercise performance. *Int. J. Sport Nutr.* **9**: 35-47.
- Greenberger, N., Rodgers, J., & Isselbacher, K. (1966). Absorption of medium and long chain triglycerides: Factors influencing their hydrolysis and transport. *J. Clin. Invest.* **45**: 217-227.
- Hargreaves, M., Hawley, J., & Jeukendrup, A. (2003). Pre-exercise carbohydrate and fat ingestion: effects on metabolism and performance. *J. Sport Sci.* **22**: 31-38.
- Hawley, J. (2002a). Symposium: Limits to fat oxidation by skeletal muscle during exercise - introduction. *Med. Sci. Sports Exerc.* **34**(9): 1475-1476.
- Hawley, J. (2002b). Effect of increased fat availability on metabolism and exercise capacity. *Med. Sci. Sports Exerc.* **34**(9): 1485-1491.
- Hawley, J., & Noakes, T. (1992). Peak sustained power output predicts VO₂max and performance time in trained cyclists. *Eur. J. Appl. Physiol. Occup. Physiol.* **65**: 79-83.
- Hopkins, W., Schabort, E., & Hawley, J. (2001). Reliability of power in physical performance tests. *Sports Med.* **31**(3): 211-234.
- Horowitz, J., & Klein, S. (2000). Lipid metabolism during endurance exercise. *Am. J. Clin. Nutr.* **72**(suppl): 558S-563S.
- Iber, F. (1974). Relative rates of metabolism MCT, LCT, and ethanol in man. *J. Nutr. Sci.* **17**(Suppl): 9-16.
- Jansson, E., & Kaijser, L. (1984). Leg citrate metabolism at rest and during exercise in relation to diet and substrate utilization in man. *Acta Physiologica Scandinavica* **122**(145-153).
- Jeukendrup, A. (1997b). General discussion. In *Aspects of carbohydrate and fat metabolism during exercise*. Holland: Uitgeverij De Vrieseborch.
- Jeukendrup, A. (2002). Regulation of fat metabolism in skeletal muscle. *Ann. N.Y. Acad. Sci.* **967**: 217-235.

- Jeukendrup, A., & Aldred, S. (2004). Fat supplementation, health and endurance performance. *Nutrition* **20**: 678-688.
- Jeukendrup, A., Brouns, E., Wagenmakers, A., & Saris, W. (1997a). Carbohydrate feedings improved 1-h time trial cycle performance. *Int. J. Sports Med.* **18**: 125-129.
- Jeukendrup, A., & Jentjens, R. (2000). Oxidation of carbohydrate feedings during prolonged exercise. *Sports Med.* **29**(6): 407-424.
- Jeukendrup, A., Saris, W., Brouns, F., & Kester, A. (1996a). A new validated performance test. *Med. Sci. Sports Exerc.* **28**(2): 266-270.
- Jeukendrup, A., Saris, W., Diesen, R. v., Brouns, F., & Wagenmakers, A. (1996b). Effect of endogenous carbohydrate availability on oral medium-chain triglyceride oxidation during prolonged exercise. *J. Appl. Physiol.* **80**: 949-954.
- Jeukendrup, A., Saris, W., Schrauwen, P., Brouns, F., & Wagenmakers, A. (1995). Metabolic availability of oral medium chain triglycerides co-ingested with carbohydrates during prolonged exercise. *J. Appl. Physiol.* **79**(3): 756-762.
- Jeukendrup, A., Thielen, J., Wagenmakers, A., Brouns, F., & Saris, W. (1998). Effect of medium-chain triacylglycerol and carbohydrate ingestion during exercise on substrate utilization and subsequent cycling performance. *Am. J. Clin. Nutr.* **67**: 397-404.
- Jeukendrup, A., & Wallis, G. (2005). Measurement of substrate oxidation during exercise by means of gas exchange measurements. *J. Int. Sports Med.* **26**(Suppl. 1): S1-S10.
- Johnson, N., Stannard, S., Mehalski, K., Trenell, M., Sachinwalla, T., Thompson, C., et al. (2003). Intramyocellular triacylglycerol in prolonged cycling with high- and low-carbohydrate availability. *J. Appl. Physiol.* **94**(1365-1372).
- Laffel, L. (1999). Ketone bodies: a review of physiology, pathophysiology and application of monitoring of diabetes. *Diabetes/Metabolism Research and Reviews* **15**(6): 412-426.
- Lindgren, B., Ruokonen, E., Magnusson-Borg, K., & Takala, J. (2001). Nitrogen sparing effect of structured triglycerides containing both medium- and long-chain fatty acids in critically ill patients: a double blind randomized controlled trial. *Clin. Nutr.* **20**: 43-48.
- Lindiger, M. (1995). Potassium regulation during exercise and recovery in humans: Implication for skeletal and cardiac muscle. *J. Mol. Cell. Cardiol.* **27**: 1011-1022.
- Livesey, G., & Marinos, E. (1988). Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: Evaluation of errors with special reference to the detailed composition of fuels. *Am. J. Clin. Nutr.* **47**: 608-628.

- McConnell, G., Fabris, S., & Proretto, J. (1994). Effect of carbohydrate ingestion on glucose kinetics during exercise. *J. Appl. Physiol.* **77**(3): 1537-1541.
- Misell, L., Lagomarcino, N., Schuster, V., & Kern, M. (2001). Chronic medium-chain triacylglycerol consumption and endurance performance in trained runners. *J. Sports Med. Phys. Fitness* **41**: 210-215.
- Mott, C., Sarles, H., & Tiscornia, O. (1972). Different action of short, medium, and long chain triglycerides on exocrine pancreatic secretion in man. *Biol. Gastro-enterol.* **5**(1): 79-84.
- Noakes, T., & Gibson, A. (2004). Logical limitations to the "catastrophe" models of fatigue during exercise in humans. *Br. J. Sports Med.* **38**: 1-30.
- Odland, L., Heigenhauser, G., Lopaschuk, G., & Spriet, L. (1996). Human skeletal muscle malonyl-CoA at rest and during prolonged submaximal exercise. *Am. J. Physiol.* **270**: E541-E544.
- Odland, L., Howlett, R., & Heigenhauser, G. (1998). Skeletal muscle malonyl-CoA content at the onset of exercise as varying power outputs in humans. *Am. J. Physiol.* **274**: E1080-E1085.
- Oopik, V., Timpmann, S., Megijainen, L., & Lemberg, H. (2001). Effects of daily medium-chain triglyceride ingestion on energy metabolism and endurance performance capacity in well-trained runners. *Nutrition Research* **21**: 1125-1135.
- Pakula, R., Rubin, M., Moser, A., Lichtenberg, D., & Tietz, A. (1997). Biosynthesis of medium-chain triglycerides and phospholipids by HepG-2 cells. *Lipids* **32**: 489-495.
- Péronnet, F., & Massicotte, D. (1991). Table of non-protein respiratory quotient: An update. *Can. J. Sport Sci.* **161**(1): 23-29.
- Pitsiladis, Y., & Maughan, R. (1999). The effects of exercise and diet manipulation on the capacity to perform prolonged exercise in the heat and in the cold in trained humans. *J. Physiol.* **517**: 919-930.
- Randle, P., Hales, C., Garland, P., & Newsholme, E. (1963). The glucose-fatty acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* **1**: 785-789.
- Romjin, J., Coyle, E., Sidossis, L., Zhang, X., & Wolfe, R. (1995). Relationship between fatty acid delivery and fatty acid oxidation during strenuous exercise. *J. Appl. Physiol.* **79**: 1939-1945.

- Rowlands, D., & Hopkins, W. (2002). Effect of high-fat, high-carbohydrate, and high-protein meals on metabolism and performance during endurance cycling. *Int. J. Sport Nutr. and Exerc. Metab.* **12**: 318-335.
- Sandström, R., Hyltander, A., Körner, U., & Lundholm, K. (1993). Structured triglycerides to postoperative patients: A safety and tolerance study. *J. Parent. Enteral Nutr.* **17**: 153-157.
- Sandström, R., Hyltander, A., Körner, U., & Lundholm, K. (1995). Structured triglycerides were well tolerated and induced increased whole body fat oxidation compared with long-chain triglycerides in postoperative patients. *J. Parenter. Enteral. Nutr.* **19**: 381-386.
- Sarritt, E., Howlett, R., Heigenhauser, G., & Sprite, L. (2000). Sensitivity of CPT I to malonyl CoA in trained and untrained human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **278**: E462-E468.
- Schabort, E., Hawley, J., Hopkins, W., Mujika, I., & Noakes, T. (1998). A new reliable laboratory test of endurance performance for road cyclists. *Med. Sci. Sports Exerc.* **30**(12): 1744-1750.
- Sidossis, L., Gastaldelli, A., Klein, S., & Wolfe, R. (1997). Regulation of plasma fatty acid oxidation during low- and high-intensity exercise. *Am. J. Physiol. Endocrinol. Metab.* **272**(6): E1065-E1070.
- Sidossis, L., Stuart, C., Shulman, G., Lopaschuk, G., & Wolfe, R. (1996). Glucose plus insulin regulate fat oxidation by controlling the rate of fatty acid entry into mitochondria. *J. Clin. Invest.* **98**(10): 2244-2250.
- Spriet, L. (2002). Regulation of skeletal muscle fat oxidation during exercise in humans. *Med. Sci. Sports Exerc.* **34**(9): 1477-1484.
- Stewart, P. (1983). Modern quantitative acid-base chemistry. *Can. J. Physiol. Pharmacol.* **61**: 1444-1461.
- Swift, L., Hill, J., Peters, J., & Greene, H. (1990). Medium-chain fatty acids: evidence for incorporation into chylomicron triglycerides in humans. *Am. J. Clin. Nutr.* **52**: 834-836.
- Tarnopolsky, N. (2000). Gender differences in substrate metabolism during endurance exercise. *Can. J. Appl. Physiol.* **25**(4): 312-327.
- Tso, P., & Crissinger, K. (2000). Digestion and absorption of lipids. In *Biochemical and Physiological Aspects of Human Nutrition*. Philadelphia: W.B. Saunders & Co.

- Vander, A., Sherman, J., & Luciano, D. (1994). *Human Physiology: The mechanisms of body function* (6th ed.). USA: McGraw-Hill Inc.
- VanLoon, L., Greenhaff, P., Constantin-Teodosiu, D., Saris, W., & Wagenmakers, A. (2001). The effects of increasing exercise intensity on muscle fuel utilisation in humans. *J. Physiol.* **536**(1): 295-304.
- VanZyl, C., Lambert, E., Hawley, J., Noakes, T., & Dennis, S. (1996). Effects of medium-chain triglyceride ingestion on fuel metabolism and cycling performance. *J. Appl. Physiol.* **80**(6): 2217-2225.
- Vistisen, B., Nybo, L., Xu, X., Høy, C., & Kiens, B. (2003). Minor amounts of plasma medium-chain fatty acids and no improved time trial performance after consuming lipids. *J. Appl. Physiol.* **95**: 2434-2443.
- Vollestad, N., Hallen, J., & Sejersed, O. (1994). Effect of exercise intensity on potassium balance in muscle and blood of man. *J. Physiol.* **475**: 359-368.
- Vukovich, M., Costill, D., Hickey, M., Trappe, S., Cole, K., & Fink, W. (1993). Effect of fat emulsion, infusion and fat feeding on muscle glycogen utilisation during exercise. *J. Appl. Physiol.* **75**: 1513-1518.
- Walker, J., Heigenhauser, G., Hultman, E., & Spriet, L. (2000). Dietary carbohydrate, muscle glycogen content, and endurance performance in well-trained women. *J. Appl. Physiol.* **88**: 2151-2158.
- Wallis, G., Rowlands, D., Shaw, C., Jentjens, R., & Jeukendrup, A. (2005). Oxidation of combined ingestion of maltodextrins and fructose during exercise. *Med. Sci. Sports Exerc.* **37**(3): 426-432.
- Watt, K., Hopkins, W., & Snow, R. (2002). Reliability of performance in repeated sprint cycling tests. *Science and Medicine in Sport* **5**(4): 354-361.
- Whitley, J., Humphreys, S., & Campbell, I. (1998). Metabolic and performance responses during exercise after high-fat and high-carbohydrate meals. *J. Appl. Physiol.* **85**: 418-424.
- Willis, W., Lencki, R., & Maragoni, A. (1998). Lipid modification strategies in the production of nutritionally functional fats and oils. *Critical Reviews in Food Sci. and Nutr.* **38**(8): 639-674.

Appendix 1
Massey University
Sport and Exercise Sciences

General Health Questionnaire

Name:

Address:

.....

.....

Phone:

.....

Name of the responsible investigator for the study:

.....

Please answer the following questions. If you have any doubts or difficulty with the questions, please ask the investigator for guidance. These questions are to determine whether the proposed exercise is appropriate for you. Your answers will be kept strictly confidential.

1.	You are.....	Male	Female
2.	What is your exact date of birth? Day..... Month..... Year..19..... So your age is..... Years		
3.	When did you last see your doctor? In the: Last week..... Last month..... Last six months..... Year..... More than a year.....		
4.	Are you currently taking any medication? (incl. contraceptives) Details:	YES	NO
5.	Has your doctor ever advised you not to partake in vigorous exercise?	YES	NO

6.	Has your doctor ever said you have "heart trouble"?	YES	NO
7.	Has your doctor ever said you have high blood pressure?	YES	NO
8.	Have you ever taken medication for blood pressure or your heart?	YES	NO
9.	Do you feel pain in your chest when you undertake physical activity?	YES	NO
10.	In the last month have you had pains in your chest when not doing any physical activity?	YES	NO
11.	Has your doctor (or anyone else) said that you have raised blood cholesterol?	YES	NO
12.	Have you had a cold or feverish illness in the last month?	YES	NO
13.	Do you ever lose balance because of dizziness, or do you ever lose consciousness?	YES	NO
14.	a) Do you suffer from back pain b) if so, does it ever prevent you from exercising?	YES YES	NO NO
15.	Do you suffer from asthma? Details: (severity, medication)	YES	NO
16.	Do you have any joint or bone problems which may be made worse by exercise?	YES	NO
17.	Has your doctor ever said you have diabetes?	YES	NO
18.	Have you ever had viral hepatitis?	YES	NO
19.	Do you know of any reason, not mentioned above, why you should not exercise?	YES	NO
20.	Are you accustomed to vigorous exercise (an hour or so a week)?	YES	NO

I have completed the questionnaire to the best of my knowledge and any questions I had have been answered to my full satisfaction.

Signed:

Date:

Source: School of Sport and Exercise Sciences, University of Birmingham

Appendix 2

Production of Exercise Supplements

Quantities of ingredients are presented as percentage by weight. All drink supplements contained a total of 8% carbohydrate made with carbohydrates containing the natural abundance of ^{13}C (wheat derived, Maldex 50, Tate & Lyle, Belgium). The exercise drinks were made by the author.

Background Test Supplement

Ingredient	Amount (%)
Water	90.056
Na casseinate	1.5
Maltodextrin	5.6
Dextrose	2.4
Structured triacylglycerol	0
^{13}C -enriched octanoic acid	0
Salt	0.08
Chocolate flavour	0.2
Vanilla flavour	0.15
Brown colour	0.007
Red colour	0.005
Yellow colour	0.002
Milk powder	0
Total	100

Carbohydrate Supplement (LC-CHO condition 3-h post-treatment test and sprint performance)

Ingredient	Amount (%)
Water	86.056
Na casseinate	1.5
Maltodextrin	5.6
Dextrose	2.4
Structured triacylglycerol	0
^{13}C -enriched octanoic acid	0
Salt	0.08
Chocolate flavour	0.2
Vanilla flavour	0.15
Brown colour	0.007
Red colour	0.005
Yellow colour	0.002
Milk powder	4.0
Total	100

Non- ^{13}C -enriched MCFA-rich Performance Supplement (LC-MC and MC-MC conditions 3-h post-treatment test)

Ingredient	Amount (%)
Water	84.356
Na casseinate	1.5
Maltodextrin	5.6
Dextrose	2.4
Structured triacylglycerol	5.7
^{13}C -enriched octanoic acid	0
Salt	0.08
Chocolate flavour	0.2
Vanilla flavour	0.15
Brown colour	0.007
Red colour	0.005
Yellow colour	0.002
Milk powder	0
Total	100

^{13}C -enriched MCFA-rich Performance Supplement (LC-MC and MC-MC conditions sprint performance test)

Ingredient	Amount (%)
Water	84.356
Na casseinate	1.5
Maltodextrin	5.6
Dextrose	2.4
Structured triacylglycerol	5.594
^{13}C -enriched octanoic acid	0.106
Salt	0.08
Chocolate flavour	0.2
Vanilla flavour	0.15
Brown colour	0.007
Red colour	0.005
Yellow colour	0.002
Milk powder	0
Total	100

Appendix 3

Production of Randomised Structured Lipids

The principle of chemical esterification to produce randomised structured lipids is illustrated in figure A1. Randomisation was conducted in a conventional 45-litre batch reactor, temperature of the oil was controlled by a jacket with steam and tap water. Stirring was done by an impeller stirrer. Vacuum and N₂ systems were connected to the vessel.

A blended oil mixture consisting of 1/3 canola (LCT) and 2/3 triolein (MCT) oil was loaded into the reactor and stirred at 230 rpm. After the vacuum reached 100 mbar, temperature of the mixture was increased to 90°C and maintained for 30 min to dry the oil. Following this, temperature was decreased to 60°C and 0.1 wt% sodium methoxide was added while stirring. Any air in the reactor was removed using the vacuum and N₂ systems. After 30 min, the reaction was stopped by addition of a 5 wt% citric acid water solution. The oil was then washed 3-4 times until pH was below 7, and dried again before deodorisation (Xu, Porsgaard, Zhang, Adler-Nissen & Høy, 2002).

Deodorisation of the randomised structured lipids was carried out in a 40 litre conventional batch deodoriser. Once lipid was fed into the deodoriser, 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 deodorisation, the oil was cooled by tap water circulation with N₂ protection, and stored in a -25°C freezer (Xu, Jacobsen, Nielsen, Heinrich & Zhou, 2002).

The oil was produced as a gift-in-collaboration by Xu Xuebing, Biocentrum-DTU, Technical University of Denmark, Building 221, DK-2800 Kgs, LYNGBY.

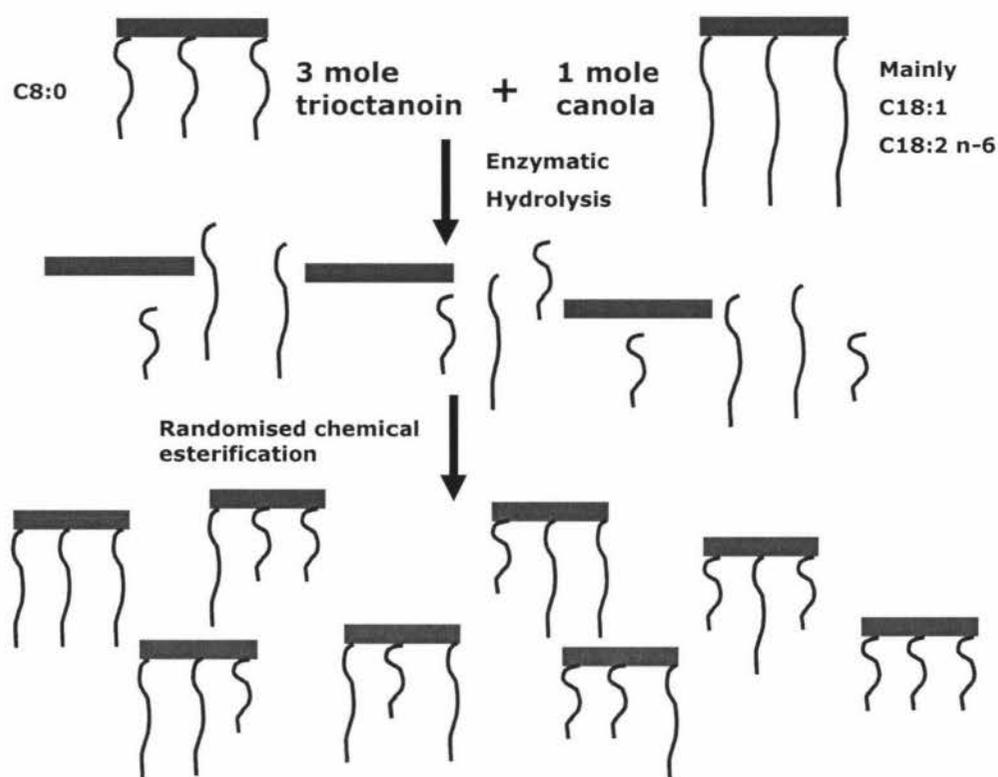


Figure A1. Random esterification method. Three mole of medium- and one mole of long-chain triacylglycerols were enzymatically hydrolysed to detach the fatty acids from their glycerol backbone. The fatty acids were then chemically esterified back on to the glycerol backbones in a random order. This could produce a possible eight different structured triglycerides: LLL, LLM, LML, MLL, MML, MLM, LMM and MMM (L = long- and M = medium-chain fatty acid).

Appendix 4

Production of 1-¹³C₁-Octanoic Acid Labelled Randomised Structured Lipids

The 1-¹³C₁-octanoic acid tracer was produced by esterification of ¹³C-labelled caprylic acid (C8:0), oleic acid (18:1n-9) and glycerol in the proportion of 3:1:1.6. The 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 Na₂SO₄ (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 ¹³C-enriched oil was made as a gift-in-collaboration by Dr. Bodil Vistisen, August Krogh Institutet, Universitetsparken 13, 2.sal, 2100 Copenhagen O, DENMARK.

Appendix 5

Production of Dietary Supplements*Carrot and Raisin Muffins*

<u>Ingredients:</u>	Makes 6	Makes 100
Eggs	1 nos	16 nos
Oil (MCT or LCT)	90 g	1440 g
Pure Honey	70 g	1120 g
Maltodextrin	40 g	640 g
Vanilla essence	2.5 ml	30 ml
Wholemeal flour	$\frac{3}{4}$ cup	12 cups
Salt	Pinch	Pinch
Baking soda	$\frac{3}{4}$ Tspn	12 Tspns
Cinnamon	$\frac{3}{4}$ Tspn	6 Tspns
Nutmeg	$\frac{1}{2}$ Tspn	3 Tspns
Milk	20 g	320 g
Grated carrots	1 cup	16 cups
Coconut	20 g	320 g
Raisins	65 g	1040 g
Walnuts (Roughly chopped)	20 g	320 g
Plain flour	20 g with MCT oil	320 g
	10 g with LCT oil	160 g

Method:

Beat eggs until thick, pale and creamy.

Add oil, vanilla essence, honey. Beat well.

Sift flours, maltodextrin, salt, baking soda, spices and gently fold into the egg mixture.

Add milk, grated carrots, coconut, walnuts and raisins. Stir gently but well.

Pour into greased muffin tins and bake at 190°C for approx 20 – 30 minutes. Leave in tins at least 5 mins prior to turning out.

Chocolate Muffins

<u>Ingredients:</u>	Makes 6	Makes 100
Eggs	1 nos	16 nos
Oil (MCT or LCT)	90 g	1440 g
Pure Honey	60 g	960 g
Maltodextrin	40 g	640 g
Vanilla essence	2.5 ml	30 ml
Flour	130 g	2080 g
Salt	Pinch	Pinch

Baking soda	½ Tspn	8 Tspns
Cocoa powder	25 g	400 g
Milk	50 g	800 g
Blueberries	80 g	1280 g

Method:

Beat egg/s until thick, pale and creamy.

Add oil, vanilla essence, honey. Beat well.

Sift flour, maltodextrin, salt, baking soda, cocoa and gently fold into the egg mixture.

Add milk, blueberries. Stir gently but well.

Pour into greased muffin tins and bake at 200°C for approx 20 – 30 minutes. Leave in tins at least 5 mins prior to turning out.

Chicken Curry

Ingredients: Makes 10 portions

1kg chicken on the bone

250g potato

2@ tomato

100g baba's meat curry powder

50g ground cumin

50g ground coriander

25g ground cinnamon

20g chilli powder

20g turmeric powder

2@onion

3 cloves garlic

15g crushed fresh ginger

1 stalk curry leaf

5g cardamom

5g star anise

5g cloves

150mls MCT or LCT oil combination

Method:

Cut chicken to desired size ,Sauté off onions, curry leaf, cardomon, cloves garlic and ginger until slightly brown in oil. Add all ground ingredients and further sauté for approx.2 minutes followed with the baba's curry powder for a further minute stirring constantly .Add chicken pieces and 1liter of water. Bring to the boil followed with diced potato and diced tomato and simmer until potato is cooked, add salt and pepper to taste .

(Liquid can be alternated between coconut milk and water 1/2 and ½, if a drier version of the curry is desired then omit the liquid totally however the cooking temp. has to be as low as

possible and the dish needs to be stirred constantly to avoid it getting caught to the bottom of the pan)

Dahl Curry

Ingredients: Makes 10 portions

300g yellow split peas
 2 @ onions Diced
 4 cloves of garlic
 30g Ginger
 10g brown mustard seed
 10g Dried Chilli roughly crushed
 10g ground turmeric
 500g potato largely Diced
 1 tin coconut milk 440 ml
 1 stalk curry leaf
 150mls MCT or LCT oil combination

Method:

Soak peas over night, Sauté off 1/2 of the onions, garlic and ginger until lightly coloured in oil. Add peas, turmeric together with 1 litter of water and bring it to the boil. Let it boil for approx 15 min. Add potato and let it simmer further for 15min. Sauté off the remaining onion, garlic, ginger, crushed chilli, mustard seed and curry leaf until golden brown with a little of the oil and add this to the dhal together with salt and pepper to taste.

Muffins and Curries were made by Harvey Bourne, Department of Management and Enterprise Development, Massey University Wellington Campus, PO Box 756, Wellington, NEW ZEALAND.

Chocolate Fudge Bars

<u>Ingredients:</u>	Makes 10
75% Fructose Solution	210 g
MCT or LCT oil	150 g
WP0001	90 g
Skim Milk Powder	60 g
WPC 620	50 g
Supro Soy Isolate	40 g
Rolled Oats	40 g
Glycerine	34 g
Flaked Barley	20 g
Soy Nuggets	20 g

Glucose	20 g
Cocoa	15 g
Vitamins/Minerals	2 g
Potassium Sorbate	0.5 g

Bars were made by George Thorburn, Unit 7 Bar Manufacturing Ltd, Totara Business Park, 22 Matai St, Mt Maunganui, PO Box 5279, Tauranga, NEW ZEALAND.

Diet Drinks

<u>Ingredients:</u>	Per 250 ml serving
Water	193 g
Milk Powder	12.5 g
Skim Milk Powder	5 g
Sodium Casseinate	3.45 g
Dextrose	20 g
MCT or LCT oil	13.75 g
Salt	0.125 g
Carregeenan	0.05 g
Cocoa	1.25 g
Chocolate Flavour	0.5 g
Brown Colour	0.25 g

Method:

All ingredients were thoroughly mixed and homogenised before being stored in sterilised bottles with an air-tight seal.

Diet drinks were made in conjunction with Mike Rockell, Institute of Food Nutrition and Human Health, Massey University, Palmerston North Campus, Private Bag 11 222, Palmerston North, NEW ZEALAND.

Appendix 6

Original and Modified Borg CR10 Perception Scales

Original CR10 Scale

0	Nothing at all
0.3	
0.5	Extremely weak
0.7	
1	Very weak
0.5	
2	Weak
2.5	
3	Moderate
4	
5	Strong
6	
7	Very strong
8	
9	
10	Extremely strong
11	
•	Absolute Maximum

The Borg CR10 Scale (1970, 1985, 1988)

Modified CR10 Scales (this study)

Rating of Perceived Exertion Questions:

- How fatigued do you feel with respect to tiredness?
- How fatigued do you feel with respect to limb soreness?
- How strong do you feel? (ability to sprint)
- How hard do you feel you are exercising?



Rating of Gastrointestinal Distress Questions:

- How much discomfort do you feel in your stomach with respect to fullness/bloatedness?
- How much discomfort do you feel with respect to reflux/burping?
- How much discomfort do you feel with respect to stomach cramps?
- How much discomfort do you feel in with respect to nausea?
- How much discomfort do you feel with respect to an urge to vomit?



Table A1. Mean participant RPE ratings during the 3-h post-treatment and performance tests. Data are presented as mean rating $\bar{x} \pm$ SD.

Test	RPE Scale											
	Tired			Leg Soreness			Strength/Ability to Sprint			Effort		
	LC-MC	MC-MC	LC-CHO	LC-MC	MC-MC	LC-CHO	LC-MC	MC-MC	LC-CHO	LC-MC	MC-MC	LC-CHO
3-h	$\bar{x} \pm 2.18$	$\bar{x} \pm 2.54$	$\bar{x} \pm 1.92$	$\bar{x} \pm 2.28$	$\bar{x} \pm 2.52$	$\bar{x} \pm 1.83$	$\bar{x} \pm 2.04$	$\bar{x} \pm 2.49$	$\bar{x} \pm 1.90$	$\bar{x} \pm 2.21$	$\bar{x} \pm 2.47$	$\bar{x} \pm 1.99$
20	0.220	0.541	0.377	0.284	0.203	0.238	6.272	7.296	6.675	1.140	1.402	0.625
40	0.739	0.829	0.526	0.414	0.732	0.409	5.495	6.428	5.847	1.290	1.238	0.981
60	1.141	1.074	0.770	0.967	0.819	0.585	4.869	5.878	4.801	1.519	1.241	0.925
80	1.497	1.303	1.027	1.012	1.167	0.736	4.619	5.964	5.111	1.928	1.657	1.191
100	1.955	1.753	1.314	1.460	1.694	0.991	4.968	5.296	4.827	1.986	1.566	1.276
120	2.168	1.638	1.487	1.662	1.621	1.349	4.441	5.264	5.187	2.763	1.876	1.330
140	2.538	2.479	1.910	1.958	2.348	1.619	4.079	4.960	4.336	2.297	2.059	1.668
160	4.216	2.148	2.126	2.892	1.931	1.862	3.675	4.244	4.849	2.571	2.083	1.991
180	4.183	3.146	2.577	2.998	2.093	2.300	4.033	4.609	4.620	2.924	2.022	2.435
Sprint	$\bar{x} \pm 1.47$	$\bar{x} \pm 1.46$	$\bar{x} \pm 1.47$	$\bar{x} \pm 2.15$	$\bar{x} \pm 2.38$	$\bar{x} \pm 2.07$	$\bar{x} \pm 1.96$	$\bar{x} \pm 1.85$	$\bar{x} \pm 1.89$	$\bar{x} \pm 1.47$	$\bar{x} \pm 1.47$	$\bar{x} \pm 1.48$
1	4.391	3.604	3.314	2.863	2.549	2.011	3.627	3.498	4.865	7.061	5.495	6.747
4	7.128	5.317	5.188	4.660	4.027	3.357	2.389	2.973	3.691	8.226	6.849	7.860
7	8.522	7.416	6.712	5.984	5.620	4.182	1.894	2.328	2.596	9.254	7.899	7.867
10	11.037	9.153	9.908	6.896	6.840	4.121	1.556	1.734	3.858	11.487	8.525	10.142

Test	GI Distress Scale											
	Fullness/Bloatedness				Reflux				Stomach Cramp			
	LC-MC	MC-MC	LC-CHO	Baseline	LC-MC	MC-MC	LC-CHO	Baseline	LC-MC	MC-MC	LC-CHO	Baseline
3-h	x/÷ 4.77	x/÷ 3.52	x/÷ 2.88	x/÷ 4.22	x/÷ 4.80	x/÷ 3.40	x/÷ 3.12	x/÷ 4.13	x/÷ 3.04	x/÷ 2.67	x/÷ 3.00	x/÷ 2.92
20	0.446	0.560	0.442	0.892	0.367	0.476	0.353	0.669	0.199	0.222	0.138	0.211
40	0.512	0.559	0.324	0.606	0.501	0.520	0.350	0.857	0.161	0.370	0.176	0.191
60	0.793	0.729	0.596	0.913	0.694	0.827	0.425	0.886	0.414	0.409	0.363	0.464
80	1.143	1.191	0.606	0.666	1.170	0.974	0.667	0.839	0.532	0.430	0.457	0.654
100	1.302	1.829	0.765	1.740	1.761	1.543	0.741	1.766	0.761	0.610	0.295	0.797
120	1.689	1.690	0.734	1.358	1.920	1.977	1.027	1.348	0.686	0.607	0.353	0.559
140	2.111	2.172	1.148	1.479	1.791	2.116	1.052	1.410	1.145	0.732	0.366	0.669
160	2.163	2.229	0.900	2.019	1.845	2.165	0.923	1.961	0.904	0.624	0.336	1.027
180	2.124	2.495	0.675	2.708	2.366	1.982	0.791	2.490	0.821	0.737	0.359	1.444
Sprint	x/÷ 2.07	x/÷ 2.66	x/÷ 2.16	x/÷ 2.35	x/÷ 2.10	x/÷ 2.43	x/÷ 2.10	x/÷ 2.20	x/÷ 2.84	x/÷ 3.49	x/÷ 2.85	x/÷ 3.16
1	1.863	1.571	0.406	1.009	1.767	1.745	0.496	1.295	1.141	0.838	0.632	0.795
4	3.551	2.068	1.243	2.915	4.121	1.987	1.796	3.308	1.203	1.100	0.604	1.163
7	4.082	2.937	2.112	3.276	4.404	2.532	2.669	3.615	1.729	1.443	1.317	1.846
10	4.680	3.485	1.989	3.121	4.119	2.788	2.377	3.203	1.675	1.382	1.400	1.663

Test	GI Distress Scale							
	Nausea				Urge to Vomit			
	LC-MC	MC-MC	LC-CHO	Baseline	LC-MC	MC-MC	LC-CHO	Baseline
3-h	x/÷ 2.92	x/÷ 2.92	x/÷ 2.92	x/÷ 2.92	x/÷ 7.15	x/÷ 3.47	x/÷ 3.47	x/÷ 4.25
20	0.425	0.267	0.120	0.351	0.156	0.310	0.185	0.200
40	0.329	0.553	0.151	0.342	0.190	0.449	0.150	0.163
60	0.509	0.531	0.337	0.575	0.338	0.452	0.313	0.259
80	0.699	0.857	0.396	1.146	0.413	0.562	0.320	0.656
100	1.199	1.164	0.221	1.603	0.630	0.850	0.187	1.253
120	1.609	1.138	0.457	1.204	0.792	0.855	0.263	1.144
140	2.185	1.981	0.438	1.303	0.976	1.447	0.327	0.890
160	1.994	1.788	0.505	1.730	0.938	1.478	0.299	0.927
180	1.677	1.862	0.358	2.310	1.049	1.632	0.371	1.363
Sprint	x/÷ 3.21	x/÷ 3.25	x/÷ 3.14	x/÷ 3.44	x/÷ 3.81	x/÷ 3.24	x/÷ 3.23	x/÷ 3.48
1	1.561	1.659	0.400	1.301	1.088	1.324	0.297	0.887
4	3.326	2.504	0.869	2.690	3.124	1.622	1.085	1.964
7	3.786	2.381	0.877	4.049	2.949	1.755	1.028	1.937
10	3.140	2.033	1.219	1.836	3.216	1.959	1.434	1.664

Table A2. Mean GI distress ratings during the 3-h post-treatment and performance tests. Data are presented as mean rating x/÷ SD.

Appendix 7

Gas Combustion Isotope Radio Mass Spectrometry Methodology (GC-IRMS)

Gas Combustion IRMS combines gas chromatography with mass spectrometry, allowing components of gaseous mixtures to be detected and analysed sequentially. Gaseous molecules are first ionised and separated using gas chromatography. The mass spectrometer then disperses the ions according to their mass-to-charge ratios, which separates ^{12}C and ^{13}C . The relative peaks of ^{12}C and ^{13}C are displayed on a histogram, which can then be used to determine the quantity of each substance present in the sample in $\delta\text{-mil}^{-1}$ vs. PDB.

Gas Combustion Isotope Radio Mass Spectrometry of expired air was carried out by Dr. Asker Jeukenrup, School of Sport and Exercise Sciences, University of Birmingham, Edgbaston, England, B15 2TT.

Elemental Analyser Isotope Radio Mass Spectrometry Methodology (EA-IRMS)

Analysis

Samples and reference materials were weighed into tin capsules, sealed, and then loaded into an automatic sampler on a Europa Scientific ANCA-GSL elemental analyser. From here they were dropped in sequence into a furnace held at $1000\text{ }^{\circ}\text{C}$ and combusted in the presence of oxygen. The tin capsules flash combusted, raising the temperature in the region of the sample to $\sim 1700\text{ }^{\circ}\text{C}$. The combusted gases were swept in a helium stream over combustion catalyst (Cr_2O_3), copper oxide wires (to oxidize hydrocarbons), and silver wool to remove sulphur and halides. The resultant gases, N_2 , NO_x , H_2O , O_2 , and CO_2 were swept through a reduction stage of pure copper wires held at 600°C . This removed any oxygen and converted NO_x species to N_2 . A magnesium perchlorate chemical trap was used to remove water. Nitrogen and carbon dioxide were separated using a packed column gas chromatograph held at an isothermal temperature of 110°C . The resultant carbon dioxide peak entered the ion source of the Europa Scientific GEO 20-20 IRMS where it was ionised and accelerated. Gas species of different mass were separated in a magnetic field then simultaneously measured using a Faraday cup collector array to measure the isotopomers of CO_2 at m/z 44, 45, and 46.

Both references and samples were converted to CO₂ and analysed using this method. The analysis proceeded in a batch process by which a reference is analysed followed by a number of samples and then another reference.

Reference Standards

The reference material used for analysis was Iso-Analytical Ltd. Working reference standard IA-R024 olive oil with a $\delta^{13}\text{C}$ value of -29.27‰ vs. V-PDB. IA-R024 is traceable to NBS-22 (Mineral Oil), distributed by the IAEA, with an accepted $\delta^{13}\text{C}$ value of -29.81‰ vs. V-PDB. IA-R024 and IA-R002 (Iso-Analytical mineral oil standard, $\delta^{13}\text{C}$ value of -28.06‰ V-PDB, traceable to NBS-22) were used as quality control check samples within the batch analysis.

Elemental Analyser Isotope Radio Mass Spectrometry of the ^{13}C -enriched randomised oil was carried out by IsoAnalytical Ltd., Millbuck Way, Springvale Industrial Estate, Sandbach, Cheshire, CW11 3HT.

Table A3. Effect of test order on sprint and recovery mean power, RPE and GI ratings. Test 1 refers to Baseline, and tests 2-4 refer to LC-MC, MC-MC and LC-CHO conditions. Data are presented as means x/ SD.

Variable	Test Comparison		
	2-1	3-2	4-3
Sprint Mean Power		0.996 (x/÷ 1.067, 0.91)	0.986 (x/÷ 1.067, 0.67)
Recovery Mean Power		0.993 (x/÷ 1.022, 0.55)	1.015 (x/÷ 1.022, 0.20)
RPE 3-h post-treatment			
Tired	0.471 (x/÷ 1.194, <0.0001)	0.660 (x/÷ 1.194, <0.0001)	0.670 (x/÷ 1.194, <0.0001)
Leg Soreness	0.452 (x/÷ 1.217, <0.0001)	0.595 (x/÷ 1.206, <0.0001)	0.725 (x/÷ 1.206, 0.0008)
Strong/Ability to Sprint	1.219 (x/÷ 1.177, 0.02)	0.996 (x/÷ 1.171, 0.96)	1.142 (x/÷ 1.171, 0.10)
Effort	0.818 (x/÷ 1.167, 0.01)	0.709 (x/÷ 1.166, <0.0001)	0.883 (x/÷ 1.167, 0.11)
RPE performance			
Tired	0.660 (x/÷ 1.168, <0.0001)	0.800 (x/÷ 1.168, 0.005)	0.770 (x/÷ 1.168, 0.001)
Leg Soreness	0.586 (x/÷ 1.239, <0.0001)	0.822 (x/÷ 1.240, 0.07)	0.856 (x/÷ 1.239, 0.15)
Strong/Ability to Sprint	1.367 (x/÷ 1.306, 0.02)	1.294 (x/÷ 1.306, 0.06)	1.209 (x/÷ 1.305, 0.16)
Effort	0.817 (x/÷ 1.107, 0.0002)	0.907 (x/÷ 1.107, 0.06)	0.847 (x/÷ 1.107, 0.002)
GI Distress 3-h post-treatment			
Fullness/Bloatedness	0.640 (x/÷ 1.375, 0.004)	0.677 (x/÷ 1.327, 0.007)	0.761 (x/÷ 1.318, 0.05)
Reflux	0.599 (x/÷ 1.350, 0.0009)	0.596 (x/÷ 1.323, 0.0003)	0.894 (x/÷ 1.320, 0.43)
Stomach Cramp	0.448 (x/÷ 1.381, <0.0001)	0.889 (x/÷ 1.324, 0.41)	0.789 (x/÷ 1.308, 0.08)
Nausea	0.418 (x/÷ 1.445, <0.0001)	0.678 (x/÷ 1.392, 0.02)	0.726 (x/÷ 1.390, 0.06)
Urge to Vomit	0.430 (x/÷ 1.494, <0.0001)	0.769 (x/÷ 1.427, 0.15)	0.699 (x/÷ 1.411, 0.04)
GI Distress performance			
Fullness/Bloatedness	0.462 (x/÷ 1.422, <0.0001)	0.758 (x/÷ 1.418, 0.12)	0.958 (x/÷ 1.419, 0.81)
Reflux	0.489 (x/÷ 1.384, <0.0001)	0.794 (x/÷ 1.368, 0.15)	1.057 (x/÷ 1.368, 0.73)
Stomach Cramp	0.807 (x/÷ 1.491, 0.29)	0.628 (x/÷ 1.442, 0.01)	1.043 (x/÷ 1.443, 0.82)
Nausea	0.513 (x/÷ 1.614, 0.007)	0.901 (x/÷ 1.594, 0.66)	1.193 (x/÷ 1.594, 0.45)
Urge to Vomit	0.493 (x/÷ 1.564, 0.0004)	1.014 (x/÷ 1.540, 0.95)	0.896 (x/÷ 1.534, 0.61)