Minor amounts of plasma medium-chain fatty acids and no improved time trial performance after consuming lipids

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Abstract

Vistisen, Bodil, Lars Nybo, Xuebing Xu, Carl-Erik Høy, and Bente Kiens. Minor amounts of plasma medium-chain fatty acids and no improved time trial performance after consuming lipids. J Appl Physiol 95: 2434–2443, 2003.—Medium-chain triacylglycerols (MCT) have a potential glycogen-saving effect during exercise due to rapid hydrolysis and oxidation. However, studies comparing intake of carbohydrates (CHO) plus 80–90 g MCT with intake of CHO alone have revealed different results. The present study tested performance after consumption of specific structured triacylglycerol, consisting of a mixture of medium-chain fatty acids and long-chain fatty acids, to prevent the adverse effects observed by MCT (pure medium-chain fatty acids) regarding gastrointestinal distress. Seven well-trained subjects cycled 3 h at 55% of maximum O2 uptake during which they ingested CHO or CHO plus specific structured triacylglycerols. Immediately after the constant-load cycling, the subjects performed a time trial of ~50-min duration. Breath and blood samples were obtained regularly during the experiment. Fatty acid composition of plasma triacylglycerols, fatty acids, and phospholipids was determined. Performance was similar after administration of CHO plus specific structured triacylglycerol [medium-, long-, and medium-chain fatty acid (MLM)] compared with CHO (50.0 ± 3.6 min, respectively). No plasma 8:0 was detected in the plasma lipid classes, but the amount of phospholipid fatty acids was significantly higher after CHO+MML compared with CHO intake. The lacking time trial improvement after intake of medium-chain fatty acids might be due to no available 8:0 in the systemic circulation. A higher level of plasma phospholipid fatty acids in the CHO+MML compared with the CHO group was probably due to endogenous phospholipid release into chylomicrons.

Specific structured triacylglycerol; well-trained cyclists; fatty acid composition; plasma lipid classes

It has been proposed that medium-chain triacylglycerol (MCT) may have a glycogen-saving effect due to its absorption and distribution properties. Increasing plasma medium-chain fatty acids may lower carbohydrate (CHO) oxidation and enable saving of glycerogen, which is connected with delay of fatigue (5, 36).

The theoretical potential of MCT as an energy source during exercise is based on the fast hydrolysis and absorption of MCT compared with long-chain triacylglycerols (10, 16). The hydrophilic nature of medium-chain fatty acids enables absorption in the enterocyte without incorporation into micelles of bile salts and phospholipids, and the majority of medium-chain fatty acids pass the enterocyte and enter the portal vein, which flows to the liver (19, 33). The rapid metabolism of MCT was demonstrated by Jeukendrup et al. (26), who found that ~70% of the ingested MCT was oxidized during 3 h of exercise.

Several studies have compared endurance performance in cyclists consuming CHO or CHO+MCT before and during exercise (12, 21–24). In all of these studies, relatively small amounts of MCT (20–30 g) were ingested, and no glycogen-sparing effects or improved endurance performance with CHO+MCT compared with CHO intake were revealed.

With administration of an increased amount of MCT (80–90 g) + CHO, Van Zyl et al. (39) demonstrated an improved cycling performance compared with that with CHO ingestion. Contrary to this, Jeukendrup et al. (27) and Angus et al. (1) observed no differences in performance after CHO, CHO+MCT (~85 g), or placebo solution intake. Several studies observed, however, gastrointestinal distress during and after exercise when MCT was ingested.

Medium-chain fatty acids and long-chain fatty acids can be chemically or enzymatically esterified to the same glycerol backbone, forming so-called structured triacylglycerols. Parenteral feeding with structured triacylglycerols (up to 100 g/day) of postoperative patients or patients in critical conditions was advantageous compared with long-chain triacylglycerol or MCT infusion (higher fat oxidation and improved nitrogen balance) (30, 35, 37). The structured triacylglycerol combines the positive effects of long-chain triacylglycerol and MCT. Medium-chain fatty acid is an energy source due to rapid hydrolysis and oxidation, whereas the long-chain fatty acid ensures adequate intake of essential fatty acids.

The specific structured triacylglycerols are native triacylglycerols, which are lipidase interesterified with donor fatty acids. The lipase only hydrolyzes fatty acids of position sn-1 and sn-3 in the glycerol backbone.

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When long-chain fatty acids in these positions are replaced with medium-chain fatty acids, the resulting specific structured triacylglycerol is MLM (M = medium-chain fatty acid, L = long-chain fatty acid) (41). It contains two-thirds of the medium-chain fatty acids in MCT, and the long-chain fatty acid may prevent gastrointestinal implications. In healthy subjects consuming 70 g of MLM, no adverse effects were observed (4). Therefore, a specific structured triacylglycerol may be of interest as an energy solution for endurance exercise.

The aim of the present study was thus to examine whether the consumption of MLM in combination with CHO, in contrast to CHO alone, during prolonged submaximal exercise would have a beneficial effect on exercise performance in a subsequent time trial. Furthermore, the different circulating fatty acid species were measured, as this has not been done in similar studies.

**METHODS**

**Experimental Procedures**

**Subjects.** Seven well-trained male cyclists participated in the study. Their maximum O₂ uptake (\(V_{\text{O}_2 \text{max}}\)) averaged 4.85 ± 0.22 l/min, and mean body weight (BW) was 74.1 ± 2.9 kg. They were 29 ± 1.5 yr old and all nonsmokers. Subjects received written information about the experiment and signed a consent form. The experiment protocol was approved by the Copenhagen and Frederiksborg Ethics Committee (project no. KP 01-241/01).

**Experimental Design**

On 2 different days, the subjects performed an exercise test on cycle ergometers, which consisted of a submaximal test lasting 3 h followed by a time trial. During the 3-h test, the subjects consumed either a solution of CHO or CHO plus specific structured triacylglycerol (CHO + MLM). One to two weeks before the experimental days, the subjects completed a test on a bicycle to determine \(V_{\text{O}_2 \text{max}}\).

On the day of the experimental trial, subjects arrived at the laboratory at 9 AM by bus or car. After 1 h of rest, a catheter was inserted into the forearm vein for blood sampling. The catheter was regularly flushed with sterile saline to avoid coagulation. Before exercise, oxygen uptake (\(V_{\text{O}_2}\)) was measured, and a blood sample was collected. Then the subjects consumed ~400 ml of either CHO or CHO + MLM solution and filled in a visual analog scale (VAS) score (see below) concerning the impression of the solutions. Subjects cycled 3 h at 55 ± 1% of \(V_{\text{O}_2 \text{max}}\). Every 15 min they consumed ~200 ml of energy solution, and rate of gastrointestinal distress (RGI) and rate of perceived exertion (RPE) were registered (see below). Breath and blood samples were collected frequently during the exercise. After 5 min of resting, the subjects commenced a self-paced time trial equal to 800 kJ.

During the first 5 min of the time trial, the subjects exercised at 266 ± 29 W, corresponding to ~70% of \(V_{\text{O}_2 \text{max}}\). Thereafter, with the purpose to accomplish 800 kJ as fast as possible, the subjects themselves adjusted the work rate by asking the leader of the experiment to raise or lower power output in steps of 5 or 10 W. The subjects were not informed about the actual workload or elapsed time, but they continuously received information about the total distance (accumulated work) covered. Breath and blood samples were obtained two times, and RGI and RPE values were regularly estimated. The same person encouraged the subjects during the time trial on both experimental days without knowing the energy drink composition. During cycling, the subjects had free access to water and were cooled with an electric fan. After the experiment, subjects filled in a VAS score again.

The experimental days were randomized, and 4–7 days elapsed between the two trials.

**Methods.** All cycling experiments were performed on Monark 839E bicycles (Monark Exercise, Vansbro, Sweden). \(V_{\text{O}_2 \text{max}}\) was determined by measuring O₂ and CO₂ of breath samples at two submaximal intensities and subsequently increasing working load 25 W every minute (starting from 200 W). The subjects cycled to exhaustion, breath samples were collected, and \(V_{\text{O}_2 \text{max}}\) was determined. The submaximal \(V_{\text{O}_2}\) values were used to define the working load during the 3-h cycling at 55% of \(V_{\text{O}_2 \text{max}}\).

To increase the reproducibility of the performance test, the participants practiced the time trial ~1 wk before the first experimental day (25, 38).

Two days before the experimental day, subjects only performed light exercise. The day before the experimental day, they sustained from exercise, drank no coffee or alcohol, and consumed a standardized diet (64% CHO, 20% fat, and 16% protein, 212 kJ/kg BW). In the morning of the experimental day, subjects had a standardized breakfast at 7 AM (67% CHO, 18% fat, 15% protein, 44 kJ/kg BW), which was 3 h before the commencement of the exercise bout.

**RGI scale, RPE scale, and VAS score.** Subjects rated their perceived exertion (RPE) using the Borg scale (6) and their gastrointestinal conditions (“how full is your stomach?”) using the RGI scale shown in Table 1. Subjects filled in VAS scores (13) to describe their impression of the energy solution. The subjects marked smell, taste, aftertaste, and total impression on a 10-cm line (e.g., with headline “smell” and in each end of the line “good” and “bad”). Ratings were evaluated by measuring the distance (in cm) to the positive expression (13). The higher value, the worse the impression of smell, taste, the higher the amount of aftertaste, and the worse the total impression.

**Preparation of energy solutions.** The energy solutions consisted of 2.4 g/kg BW CHO (neutral Maxim, maltodextrines, Maxim Europe, Helmond, Holland) alone or 2.4 g/kg BW CHO and 1.5 g/kg BW MLM (emulsified-specific structured triacylglycerol, M = caprylic acid, 8:0, located in position sn-1,3 of the glycerol backbone and L = safflower oil fatty acids located in position sn-2).

Subjects received in total 93–128 g MLM. The 8:0 content of the beverages was ~30 g. The fatty acid compositions of MLM and of the sn-2 position of MLM are presented in Table 2. MLM was emulsified with stabilizer (0.2% guar gum, 2% light bloated 3 4 bloated 5 6 very bloated 7 8 light nausea 9 10 nausea 11

**Table 1. Rate of gastrointestinal distress: “How full is your stomach?”**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>light bloated</td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>bloated</td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>very bloated</td>
</tr>
<tr>
<td>7</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>light nausea</td>
</tr>
<tr>
<td>9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>nausea</td>
</tr>
<tr>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>
sodium alginate; Danisco Cultor) and emulsifier (0.2% sodium stearoyl-2-lactate; Danisco Cultor) in water (1:1). Preparation of the emulsion increased the solubility of the lipid in the energy solution and disguised the "oily" taste of the fluid. Strawberry flavor (Danisco Ingredients) was added to the CHO solution too, to obtain a comparable taste of the two beverages.

MLM was produced by pilot scale lipase interesterification of 8:0 and safflower oil (41). The lipase used in the interesterification was sn-1,3 specific.

**Analysis of blood samples, plasma lipids, and the MLM of the energy solution.** Glucose, lactate, Na\(^+\), and hemoglobin contents of the blood samples were immediately measured on a blood-gas oximetry, electrolyte, and metabolite system (ABL, 615/610, Radiometer, Copenhagen, Denmark). Blood samples for fatty acid determination were centrifuged, and plasma was frozen in liquid nitrogen and stored at \(-80^\circ\text{C}\) until further analysis.

Internal standards (triacylglycerol (TG-15:0), phospholipid (PC-15:0), and fatty acid (15:0); Sigma Aldrich, Steinheim, Germany) were added to plasma samples before extraction (14). The extract was separated in lipid classes by thin-layer chromatography (Silica gel 60, Merck, Darmstadt, Germany) in a system of heptane-isopropanol-acetic acid (95:5:1 vol/vol/vol). The different bands were identified by 2,7-dichlorofluorescein under ultraviolet light by use of standards. Triacylglycerol, phospholipids, and fatty acid spots were scraped off and transmethylated with BF\(_3\)-catalyzed methylation (2, 18).

MLM was transmethylated by KOH in methanol (9) for determination of fatty acid composition. The structure (the fatty acid composition of the sn-2 position) was determined by Grignard degradation (3) followed by thin-layer chromatography, separation, recovery of the sn-2 monoacylglycerol, and KOH methylation.

The fatty acid methyl esters were analyzed by a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard, Waldbronn, Germany) on a fused silica capillary column (SP-2380, 60 m, ID 0.25 mm, 0.20-μm film; Supelco, Bellafonte, PA). The injector was 260°C, and it was used in the split mode with a split ratio of 1:20. The fatty acids were separated by an initial temperature of 70°C, which was kept for 0.5 min, then raised to 160°C at 15°C/min, at 1.5°C/min to 200°C, which was held for 15 min, and finally raised to 225°C at 30°C/min. The last temperature was held for 10 min. The temperature of the detector (flame ionization) was 300°C. A standard sample (Nu-Chek Prep, Elysian, MN) with known composition and quantities of fatty acid methyl esters was run daily. This standard contained fatty acid methyl esters with chain length of C\(_6\) to C\(_{22}\), which ensured detection of all plasma fatty acid methyl esters within this range. Fatty acids were identified by retention times of the standard fatty acid methyl esters.

**Table 2. Fatty acid composition of the triacylglycerol and of the sn-2 monoacylglycerol of MLM**

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>TG</th>
<th>2-MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caprylic acid (8:0)</td>
<td>38.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Palmitic acid (16:0)</td>
<td>2.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Stearic acid (18:0)</td>
<td>1.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Oleic acid [18:1(n-9)]</td>
<td>14.0</td>
<td>20.3</td>
</tr>
<tr>
<td>Linoleic acid [18:2(n-6)]</td>
<td>42.1</td>
<td>77.0</td>
</tr>
<tr>
<td>Linolenic acid [18:3(n-3)]</td>
<td>0.3</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Data represent averages of 3 determinations in mol%. TG, triacylglycerol; 2-MG, sn-2 monoacylglycerol; MLM, medium-, long-, and medium-chain fatty acid.

**Statistics**

Data are given as means ± SE.

Time- and treatment-related differences in plasma fatty acids concentrations, respiratory exchange ratio (RER), VO\(_2\), and blood glucose values during the 3-h ride and the time trial were tested with one-way ANOVA for repeated measurement, and differences were evaluated with the Tukey test. Differences in RPE and RGI during the time trial were tested with two-way ANOVA of repeated measurements. The level of significance was \(p < 0.05\). All statistical calculations were performed with SigmaStat (version 2.03, Jandel, Erkrath, Germany).

**RESULTS**

Time trial performance after 3-h cycling at a constant workload and consuming either CHO or CHO+MLM revealed no difference between treatments (Fig. 1). The time trial averaged 50.8 ± 3.6 min after ingestion of CHO and 50.0 ± 1.8 min after ingestion of CHO+MLM.

An interesting finding was that, even though subjects consumed 30 g of caprylic acid (8:0), no 8:0 and only traces of capric acid (10:0) were detected in plasma triacylglycerol, phospholipid, and fatty acid.

**Results of the 3 h of Cycling at 55% of VO\(_2\)\(_{max}\)**

**Plasma triacylglycerol fatty acids.** The sum of plasma triacylglycerol fatty acids revealed an insignificant decline during the 3-h ride, when CHO was consumed, whereas no time-related effect was observed after intake of CHO+MLM (Table 3). Thus the sum of plasma triacylglycerol fatty acids in CHO compared with CHO+MLM was similar during the 3-h ride (Fig. 2A).

In the CHO ride, the concentrations of plasma triacylglycerol fatty acid species remained unchanged after 1 h of submaximal exercise compared with resting...
levels (Table 3). After 2 h of exercise, a tendency to decrease was seen in the concentrations of 14:0, 16:0, 18:0, 18:1(n-9), 18:2(n-6), and 18:3(n-3). After 3 h of cycling, a decline ($P < 0.05$) was observed in 18.0 and 18.3(n-3) compared with the values obtained after 1 h of exercise. The other plasma triacylglycerol fatty acids tended to decrease after 3 h of exercise, except 20:4(n-6) and 22:6(n-3), which remained unchanged during the ride. The decline averaged 40% (range: 30–50%) (Table 3).

In the CHO+MLM group, a tendency toward an increase in plasma triacylglycerol fatty acids from resting values to values after 1 h of exercise appeared. After the 3-h ride, a significant decrease was observed in the plasma triacylglycerols 12:0, 14:0, 18:3(n-3), and MCFA compared with the values after 1-h cycling, despite a frequent intake of MLM (Table 3). The decline averaged 39% (range: 28–60%).

A tendency to obtain higher amounts of 14:0 was observed after 1 and 2 h of cycling, when comparing CHO+MLM with CHO treatment (Table 3). Similarly, a tendency to higher 18:2(n-6) concentration after 2- and 3-h cycling ($P = 0.08–0.11$) was observed when comparing CHO+MLM with CHO consumption (Table 3).

**Plasma fatty acids.** After consumption of both energy solutions, the sum of plasma fatty acids was significantly higher after 3 h of cycling compared with the resting values and the values after 1 h of cycling. No differences between the sum of plasma fatty acids appeared after CHO compared with CHO+MLM consumption (Fig. 2B).

Generally, the pattern of plasma fatty acids revealed a decline from the resting values to the concentration after 1-h cycling and then an increase during the rest of the 3-h ride. With CHO consumption, significant increases from resting values to the concentration after the 3-h ride were observed in plasma 16:0 and 18:1(n-9). The concentrations of these fatty acids, together with 14:0 and 18:0, were also significantly increased from 1 to 3 h of exercise (Table 4).

In the CHO+MLM group, the plasma 14:0, 16:0, 18:1(n-9), and 18:2(n-6) concentrations after 3 h of exercise were significantly higher than at rest. A significant increment was also observed for 16:0, 18:1(n-9), and 18:2(n-6) from 1 to 3 h of cycling (Table 4).

The plasma fatty acid concentrations during the 3-h ride were similar when comparing CHO and CHO+MLM consumption (Table 4).

**Plasma phospholipid fatty acids.** The sum of phospholipid fatty acids remained unchanged during the 3-h ride in the CHO group. During the CHO+MLM treatment, the sum of plasma phospholipid fatty acids tended to be higher after 1 h of cycling compared with the resting value ($P = 0.119$) (Table 5). The sum of the plasma phospholipid fatty acids revealed significantly higher values for CHO+MLM compared with CHO after 1 h and tended to be higher after 2 h of cycling ($P = 0.075$) (Fig. 2C).

In the CHO group, no significant differences were observed in individual plasma phospholipid fatty acids during the 3-h ride. In the CHO+MLM group, a significant increase from resting values to 1 h of cycling was found in plasma phospholipid 18:2(n-6), whereas
After 1 h of exercise, RER was significantly higher in the CHO group (0.91) compared with the CHO+MLM group (0.87) (Table 6). At the end of the 3-h ride, a decrease in RER was observed in both treatments. RER values remained insignificantly higher after CHO compared with CHO+MLM intake during the 2nd and 3rd h of the ride.

RPE increased significantly during the 3 h, from 10.6 to 12.7–13.5 (P < 0.001), with no difference between CHO and CHO+MLM consumption. RGI raised significantly from 1.6 ± 0.4 to 3.9 ± 0.8 and from 1.9 ± 0.4 to 2.7 ± 0.5 after intake of CHO and CHO+MLM, respectively.

Glucose, lactate, hemoglobin, and Na+. In the CHO+MLM group, blood glucose was significantly higher after 1 h of cycling compared with 2 h, but no difference was observed between treatment groups, even though CHO ingestion resulted in lower average values in all blood samples (Table 6).

In the CHO and the CHO+MLM groups, resting blood lactate was 1.24 ± 0.08 and 1.06 ± 0.11 mmol/l, respectively, and, during the 3-h ride, it was 0.9–1.07 mmol/l in both experimental groups.

Hemoglobin did not differ between treatment groups, but increased from 8.7–8.8 mmol/l at rest to 9.3–9.4 mmol/l after 3 h of exercise (Table 6). Blood Na+ content was 139–141 mmol/l throughout both treatments (Table 6).

Results of the Time Trial

Plasma triacylglycerol, fatty acids, and phospholipids. The sum of plasma triacylglycerol and phospholipid fatty acids was similar during the time trial (Tables 3 and 5). However, the sum of plasma fatty acids was significantly higher at the end of the time trial compared with 400 kJ in the CHO group (Table 4). There was a significantly higher sum of triacylglycerol fatty acids after CHO+MLM compared with CHO intake at 400 and 800 kJ (Table 3). Similarly, a significantly higher sum of phospholipid fatty acids was observed after 400 kJ in the CHO+MLM compared with CHO treatment (Table 5).

No time-related effects were observed during the time trial in plasma triacylglycerol and phospholipid fatty acid species (Tables 3 and 5). However, significant increases in plasma 18:1(n-9) and 18:2(n-6) after CHO treatment were observed during the time trial (Table 4).

Significant higher concentrations of plasma triacylglycerol 18:2(n-6) after 400 and 800 kJ were observed in CHO+MLM compared with CHO (Table 3).

Plasma phospholipid 18:0 and 18:2(n-6) were significantly higher after 400 and 800 kJ following CHO+MLM compared with CHO treatment (Table 5).

Breath samples, RPE, and RGI. During the time trial, subjects exercised at 78.6 ± 10.2% VO2 max (range: 68.8–91.6%) with an oxygen consumption of 3.9 ± 0.3 l/min at 400 kJ and 3.7 ± 0.3 l/min at 800 kJ after CHO intake. The corresponding values after CHO+MLM intake were 3.8 ± 0.2 and 3.7 ± 0.2 l/min, respectively.
There were no differences between treatments and no time-related effect concerning oxygen consumption. A tendency of lower RER values after administration of CHO+MLM compared with CHO was observed (Table 6). No time-related effect was observed.

RPE increased significantly during the time trial from ~16.5 to ~18.5. There were no differences in rating after CHO or CHO+MLM consumption. Gastro-intestinal distress was rated to 2.5–4.5 during the time trial, with significant lower ratings after 400 compared

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### Table 4. Fatty acid composition of plasma fatty acid during 3 h at 55% of VO\(_{2\text{max}}\) and subsequent time trial of 800 kJ

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Energy Solution</th>
<th>Resting</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>400 kJ</th>
<th>800 kJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:0</td>
<td>CHO</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>CHO + MLM</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>12:0</td>
<td>CHO</td>
<td>1.4 ± 0.9</td>
<td>0.8 ± 0.6</td>
<td>0.9 ± 0.6</td>
<td>1.2 ± 0.6</td>
<td>1.5 ± 0.5</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>CHO + MLM</td>
<td>1.4 ± 0.8</td>
<td>0.8 ± 0.5</td>
<td>0.9 ± 0.6</td>
<td>0.4 ± 0.4</td>
<td>0.9 ± 0.6</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>14:0</td>
<td>CHO</td>
<td>3.6 ± 1.2</td>
<td>3.1 ± 0.7†</td>
<td>4.6 ± 0.6</td>
<td>6.9 ± 0.8</td>
<td>6.6 ± 0.8</td>
<td>9.1 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>CHO + MLM</td>
<td>3.1 ± 0.9†</td>
<td>3.9 ± 0.3</td>
<td>5.2 ± 0.6</td>
<td>6.5 ± 0.9</td>
<td>6.1 ± 1.5</td>
<td>8.1 ± 2.1</td>
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<tr>
<td>16:0</td>
<td>CHO</td>
<td>38.7 ± 7.3</td>
<td>32.4 ± 4.0†</td>
<td>49.7 ± 7.2</td>
<td>81.3 ± 11.4</td>
<td>67.2 ± 10.2</td>
<td>113.4 ± 22.1</td>
</tr>
<tr>
<td></td>
<td>CHO + MLM</td>
<td>37.4 ± 3.3‡</td>
<td>41.6 ± 4.3‡</td>
<td>62.5 ± 11.4</td>
<td>89.6 ± 19.9</td>
<td>76.5 ± 17.5</td>
<td>103.6 ± 30.7</td>
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<tr>
<td>18:0</td>
<td>CHO</td>
<td>18.9 ± 3.0</td>
<td>11.7 ± 1.3†</td>
<td>16.1 ± 2.3</td>
<td>23.0 ± 2.5</td>
<td>22.9 ± 1.7</td>
<td>38.4 ± 9.4</td>
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<tr>
<td></td>
<td>CHO + MLM</td>
<td>18.6 ± 1.8</td>
<td>14.7 ± 1.5</td>
<td>18.1 ± 2.1</td>
<td>22.7 ± 3.1</td>
<td>21.1 ± 3.1</td>
<td>27.5 ± 5.9</td>
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<tr>
<td>18:1(n-9)</td>
<td>CHO</td>
<td>32.9 ± 8.8†</td>
<td>27.9 ± 3.9†</td>
<td>53.8 ± 10.4</td>
<td>103.3 ± 20.6</td>
<td>76.0 ± 20.3‡</td>
<td>142.3 ± 31.4</td>
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<tr>
<td></td>
<td>CHO + MLM</td>
<td>33.0 ± 4.8†</td>
<td>35.8 ± 10.1†</td>
<td>67.2 ± 20.9</td>
<td>101.4 ± 27.5</td>
<td>84.5 ± 26.3</td>
<td>123.2 ± 46.5</td>
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<td>18:2(n-6)</td>
<td>CHO</td>
<td>13.3 ± 3.2</td>
<td>13.1 ± 2.7</td>
<td>21.8 ± 4.4</td>
<td>37.4 ± 7.0</td>
<td>26.7 ± 7.6‡</td>
<td>49.3 ± 11.1</td>
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<td>CHO + MLM</td>
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<td>18.4 ± 6.0†</td>
<td>35.6 ± 8.5</td>
<td>55.8 ± 10.9</td>
<td>45.2 ± 11.4</td>
<td>61.4 ± 17.9</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>CHO</td>
<td>1.6 ± 0.9</td>
<td>1.7 ± 0.8</td>
<td>1.9 ± 1.0</td>
<td>2.0 ± 0.7</td>
<td>2.1 ± 0.9</td>
<td>2.5 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>CHO + MLM</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>20:0(n-6)</td>
<td>CHO</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.0 ± 0.5</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>CHO + MLM</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.4 ± 0.3</td>
<td>ND</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>CHO</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.5 ± 0.3</td>
<td>2.6 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>CHO + MLM</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.5 ± 0.3</td>
<td>2.6 ± 1.8</td>
</tr>
<tr>
<td>MCFA</td>
<td>CHO</td>
<td>5.7 ± 2.2</td>
<td>4.4 ± 1.4</td>
<td>9.5 ± 1.2</td>
<td>8.5 ± 1.5</td>
<td>8.5 ± 1.0</td>
<td>11.4 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>CHO + MLM</td>
<td>4.5 ± 1.8</td>
<td>4.7 ± 0.8</td>
<td>6.1 ± 0.9</td>
<td>6.9 ± 0.9</td>
<td>7.0 ± 1.8</td>
<td>8.7 ± 2.6</td>
</tr>
<tr>
<td>Sum</td>
<td>CHO</td>
<td>106.9 ± 23.0*</td>
<td>89.8 ± 10.6†</td>
<td>148.3 ± 24.3</td>
<td>257.1 ± 41.8</td>
<td>203.9 ± 39.6‡</td>
<td>362.6 ± 71.8</td>
</tr>
<tr>
<td></td>
<td>CHO + MLM</td>
<td>109.3 ± 13.6*</td>
<td>116.6 ± 20.1†</td>
<td>192.4 ± 41.2</td>
<td>278.6 ± 59.6</td>
<td>237.9 ± 59.3</td>
<td>330.2 ± 104.3</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/l; \( n = 7 \). * \( P < 0.05 \) between resting and 3-h values in same row. † \( P < 0.05 \) between 1- and 3-h values in same row. ‡ \( P < 0.05 \) between 400- and 800-kJ values in same row.
with 800 kJ (Fig. 3). There was no difference in gastrointestinal discomfort between energy solutions.

Glucose, lactate, hemoglobin, and Na⁺. Blood glucose during the time trial was ~4 mmol/l after CHO consumption, whereas the value tended to increase from 3.9 ± 0.3 to 4.4 ± 0.3 mmol/l after CHO + MLM intake (Table 6). Blood lactate during the time trial was ~4–5 mmol/l after both treatments. Blood Na⁺ and hemoglobin during the time trial were ~140–142 and 9.4–9.6 mmol/l, respectively (Table 6). No time- or treatment-related differences in blood glucose, lactate, Na⁺, and hemoglobin were observed during the time trial.

Impression of Energy Solution

The two solutions resulted in smell, taste, aftertaste, and total impression at the same level (Table 7). A tendency to obtain higher values at the end of exercise compared with the beginning was observed in both treatment groups.

DISCUSSION

The present study demonstrated no time trial performance improvement after ingestion of CHO in combination with specific structured triacylglycerol (MLM) compared with ingestion of CHO during a prior 3-h ride.

To our knowledge, we present for the first time a gas chromatographic measurement of the plasma contents of short- and medium-chain fatty acids (6:0, 8:0, 10:0, and 12:0) after consumption of specific structured triacylglycerols containing medium-chain fatty acids during exercise. We demonstrated that 8:0 was not detected in plasma triacylglycerols, fatty acids, or phospholipids after consumption of a large amount of 8:0. The advantages of medium-chain fatty acids compared with long-chain fatty acids as substrate during exercise are due to faster hydrolysis during digestion (10, 16), portal vein transport as fatty acids (29), and more efficient uptake and oxidation in the muscle cell, e.g., carnitine palmitoyl transferase-independent transport into mitochondria (8, 15, 17). Furthermore, medium-chain fatty acid oxidation in contrast to long-chain fatty acid oxidation is not inhibited by simultaneous CHO intake (11). However, if no 8:0 reaches the systemic circulation after passing the liver, it excludes utilization of these potential beneficial properties of medium-chain fatty acids in the muscles.

That we were unable to detect 8:0 in the circulation, despite the intake of a large amount of 8:0, could be due to the fact that tissue oxidation (in muscle tissue and liver) removed 8:0 from the general circulation so fast that detection was impaired. Several studies have demonstrated a complete oxidation of MCT (TG-8:0) in rat liver (19, 43). However, it has been demonstrated that a minor amount of medium-chain fatty acids was observed in plasma after overfeeding of MCT in humans (20). This finding indicates that, if 8:0 were available in the systemic circulation of cyclists in the present study, it would have been detectable by our analysis.

The question is then: What happened with the large amount of 8:0, which the subjects received with CHO + MLM during exercise? Jeukendrup et al. (26) demonstrated that the oxidation rate of MCT was ~70% of the ingestion rate. This whole body estimate,

### Table 6. RER, blood glucose, hemoglobin, and Na⁺ during 3-h ride and subsequent time trial

<table>
<thead>
<tr>
<th>Energy Solution</th>
<th>Resting</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>400 kJ</th>
<th>800 kJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>RER</td>
<td>CHO</td>
<td>0.84±0.03</td>
<td>0.91±0.01</td>
<td>0.89±0.02</td>
<td>0.87±0.01</td>
<td>0.91±0.02</td>
</tr>
<tr>
<td></td>
<td>CHO+MLM</td>
<td>0.83±0.02</td>
<td>0.87±0.01</td>
<td>0.88±0.02</td>
<td>0.84±0.01</td>
<td>0.89±0.01</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>CHO</td>
<td>3.43±0.17</td>
<td>5.07±0.12</td>
<td>4.83±0.14</td>
<td>4.94±0.16</td>
<td>4.17±0.36</td>
</tr>
<tr>
<td></td>
<td>CHO+MLM</td>
<td>4.69±0.09</td>
<td>5.39±0.14</td>
<td>4.97±0.10</td>
<td>5.04±0.19</td>
<td>3.91±0.26</td>
</tr>
<tr>
<td>Hemoglobin, mmol/l</td>
<td>CHO</td>
<td>8.81±0.18</td>
<td>9.33±0.16</td>
<td>9.37±0.20</td>
<td>9.43±0.24</td>
<td>9.60±0.21</td>
</tr>
<tr>
<td></td>
<td>CHO+MLM</td>
<td>8.73±0.12</td>
<td>9.36±0.18</td>
<td>9.36±0.17</td>
<td>9.27±0.14</td>
<td>9.60±0.16</td>
</tr>
<tr>
<td>Na⁺, mmol/l</td>
<td>CHO</td>
<td>139.29±0.42</td>
<td>140.00±0.69</td>
<td>140.14±0.99</td>
<td>140.00±1.07</td>
<td>140.57±1.17</td>
</tr>
<tr>
<td></td>
<td>CHO+MLM</td>
<td>139.14±0.50</td>
<td>141.50±0.78</td>
<td>140.14±0.59</td>
<td>139.43±1.09</td>
<td>142.29±1.43</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7. RER, respiratory exchange ratio.

### Table 7. Impression of beverages (CHO or CHO + MLM) after initial intake and after experiment

<table>
<thead>
<tr>
<th></th>
<th>CHO (I)</th>
<th>CHO + MLM (I)</th>
<th>CHO (II)</th>
<th>CHO + MLM (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smell</td>
<td>4.9±0.3</td>
<td>4.7±0.7</td>
<td>5.8±0.8</td>
<td>5.5±0.8</td>
</tr>
<tr>
<td>Taste</td>
<td>6.0±1.0</td>
<td>5.6±0.9</td>
<td>6.8±0.9</td>
<td>6.8±0.8</td>
</tr>
<tr>
<td>Aftertaste</td>
<td>5.7±0.9</td>
<td>5.8±1.0</td>
<td>5.6±0.9</td>
<td>6.3±1.2</td>
</tr>
<tr>
<td>Total impression</td>
<td>5.6±0.8</td>
<td>5.9±1.0</td>
<td>6.9±0.8</td>
<td>6.9±0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7. The lower value, the better the smell, taste, aftertaste, and total impression. I, after initial intake; II, after experiment.
however, does not indicate the site of oxidation. Possibly 8:0 was also elongated and recovered as 14:0 and other long-chain fatty acids in the circulation. Support for this is the tendency to retain higher 14:0 values in the CHO+MLM compared with the CHO group. When 8:0 is absorbed in the intestine, it is preferably transported to the liver (33), where elongation may take place (32). An elongation of 8:0 to longer chain fatty acids might also eventually happen in the enterocyte (7, 42) before a (quantitatively) minor lymphatic transport.

Oxidation of 8:0 in the liver results in the formation of acetyl-CoA, which can be metabolized further into ketone bodies. Van Zyl et al. (39) found a significantly higher β-hydroxybutyrate concentration after administration of CHO+MCT compared with CHO. Furthermore, administration of structured triacylglycerol (not sn-1,3 specific but consisting of medium-chain and long-chain fatty acids) to sedentary subjects resulted in a significant increase in plasma ketone bodies compared with administration of long-chain triacylglycerols (31). Ketone bodies are potential energy sources of the peripheral tissue. However, higher serum β-hydroxybutyrate plasma concentration observed in subjects ingesting MCT alone, compared with CHO+MCT, did not improve performance (27, 39).

Like the present study, Jeukendrup et al. (27) and Angus et al. (1) did not demonstrate differences in cycling performance after administration of CHO+MCT [8:0 (27) or 8:0 mixed with 10:0 (1)] compared with CHO alone. The lack of improvement in these studies could be ascribed to the study design, with time trials lasting ~15 min (27), which might have been too short to reveal a benefit of the lipid solution, or 3 h (1). It could also be because some of the subjects experienced gastrointestinal distress after intake of MCT, or it could simply be due to the fact that 8:0 or 10:0 was not available in the circulation during exercise, as demonstrated in the present study.

In contrast, Van Zyl et al. (39) observed an improved performance in a time trial lasting ~1 h after 2 h of prolonged exercise, while CHO+MCT was consumed, compared with CHO.

Van Zyl et al. (39) observed a large amount of medium-chain fatty acids in the blood samples of cyclists after 2-h cycling at submaximal intensity. They defined, however, the serum medium-chain fatty acid content as the difference between measurements of total serum fatty acids and long-chain fatty acids (measured by two different methods). Long-chain fatty acids were defined as C16–C20, and medium-chain fatty acids as 8:0 and 10:0, which left 12:0 and 14:0 to none of the groups. Thus, by indirect measurement, Van Zyl et al. found a contribution of ~0.20 mmol/l of medium-chain fatty acid after CHO+MCT ingestion. In the present study, in which direct measurement of medium-chain fatty acids was performed, this amounted to 0.009 mmol/l (if 12:0 and 14:0 were included in the group) (Table 4).

The time trial of the present study was similar to that in the study of Van Zyl et al. (39): designed to last ~1 h after a prolonged 3-h ride, which was expected to involve lipids as the major energy source. Furthermore, the medium-chain fatty acids in the present study were administered as specific structured triacylglycerol, which might minimize the gastrointestinal distress, as our laboratory (4) has previously observed no side effects of administration of ~70 g of the same specific structured triacylglycerol to healthy resting subjects. Despite these initiatives, no performance improvement was observed in the time trial in the present study after MLM+CHO intake.

In the present study, we observed a significantly lower RER after a 1-h ride and a tendency to lower values of RER during the rest of the 3-h ride after CHO+MLM compared with CHO consumption. This indicates a larger lipid oxidation during exercise when CHO+MLM was ingested compared with CHO.

In the circulation, the sum of plasma fatty acids increased during the 3-h ride to a similar extent after CHO and CHO+MLM treatment, and this increase included a broad range of plasma fatty acids [14:0, 16:0, 18:0, 18:1(n-9), and 18:2(n-6)] (Table 4).

The sum of plasma triacylglycerol fatty acids tended to decrease during the 3-h ride with CHO consumption. A decrease was not observed after CHO+MLM treatment due to the constant fat loading. Nevertheless, no differences in the sum of triacylglycerol fatty acid were observed between treatments after 3-h cycling.

An interesting finding in the present study was that the plasma phospholipid fatty acid concentrations, with CHO+MLM treatment, were increased after 1 h of exercise, with increments and tendencies to increments in several fatty acid species [16:0, 18:0, 18:2(n-6), and 20:4(n-6)]. Plasma phospholipids are parts of the chylomicrons and other lipoprotein particles of the blood (34). Due to the difference between treatments, it is most likely that the surplus phospholipids were incorporated into chylomicrons. The phospholipids of the chylomicrons originate, to a large extent, from endogenous sources (40), which also explains the broad range of the increased fatty acids. Probably, a small fraction of the exogenous 18:2(n-6) in MLM was incorporated into resynthesized phospholipid, but mainly the plasma phospholipids were of endogenous origin and increased due to a stimulation of the lymphatic transport by the MLM ingestion (28). Then the intestinal absorption and chylomicron assembly had proceeded very fast, due to the significant increment, already 1 h after the first intake of energy solution.

The sum of plasma phospholipid fatty acids was higher in the CHO+MLM group compared with the CHO group, which, in contrast to plasma fatty acids and triacylglycerols, indicated a different effect of the two beverages with influence on the energetic basis before the time trial. However, these differences in the circulating profile before the time trial did not influence physical performance in the time trial.

In the present study, no significant differences between RPE values were observed after administration of CHO+MLM compared with CHO. Similarly, the impression of the energy solutions was the same in
both experimental groups. During cycling, the Na\(^+\) and hemoglobin content of the blood samples did not differ in relation to treatment or time. This indicated that the subjects were not dehydrated during the tests. These results enhanced the probability of detecting differences between the energy solutions.

The RGI values did not differ after administration of CHO+MLM or CHO, except for a tendency to higher RGI values in the last part of the time trial after CHO+MLM ingestion. However, the day after the experiment, subjects were questioned about eventual reactions in the hours after the experiment (when the subjects went home). Several of the subjects experienced side effects after the experiment with CHO+MLM, from eructation to diarrhea and vomiting, whereas no reactions were experienced after the CHO treatment. This revealed that, during hard and enduring exercise, no difference regarding gastrointestinal distress after MLM compared with MCT intake could be demonstrated, even though structured triacylglycerols under other circumstances caused no gastrointestinal adverse effects (35, 37). Therefore, if MLM distresses the gastrointestinal system less than MCT, it was concealed in an experimental design like the present study.

In summary, the present study reveals no performance improvement after consumption of specific structured triacylglycerol [MLM: M = 8:0, L = 18:2(n-6)] during endurance exercise before the time trial. The 8:0 was not recovered in plasma samples obtained during exercise, and this excludes the proposed glyco-GEN-saving effect (due to muscle oxidation of circulating medium-chain fatty acids).

Plasma fatty acid and triacylglycerol fatty acid concentrations were similar after the 3-h ride at submaximal cycling with both treatments, but the plasma phospholipid fatty acid concentration was enlarged after consumption of CHO+MLM compared with CHO. The plasma glucose levels were similar in both groups after the 3-h ride. This indicates only minor differences in the metabolism of energy substrates during cycling.

In the present study, consumption of MLM during endurance exercise did not enhance performance. This might be due to a lack of medium-chain fatty acid appearing in the systemic circulation.

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DISCLOSURES

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REFERENCES


