Effect of endogenous carbohydrate availability on oral medium-chain triglyceride oxidation during prolonged exercise

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Jeukendrup, Asker E., Wim H. M. Saris, Richard Van Diesen, Fred Brouns, and Anton J. M. Wagenmakers. Effect of endogenous carbohydrate availability on oral medium-chain triglyceride oxidation during prolonged exercise. *J. Appl. Physiol.* 80(3): 949–954, 1996.—The present study examined the medium-chain triglyceride (MCT) oxidation rate of oral carbohydrate (CHO)+MCT supplements after a glycogen-depletion trial [low glycogen (LG)] and in the glycogen-loaded state [normal-to-high glycogen (HG)]. Eight elite athletes cycled four times 90 min at 50% maximal workload (57% maximal O_2 uptake). In two trials, they followed a LG protocol to achieve low-glycogen stores in the leg muscles the evening before the experiment, and in two trials they followed a HG protocol. Subjects received a bolus of 4 ml/kg at the start and 2 ml/kg every 20 min during exercise of either a 15% CHO (long-chain glucose polymer) solution or an equicaloric CHO+MCT suspension. Exogenous MCT oxidation was measured by adding a $[1,1,1-^{13}C]$trioctanoate tracer to the MCT oil and measuring $^{13}CO_2$ production in the breath. The results show that 85% of MCT ingested was oxidized in LG and 69% in HG during the 60- to 90-min period. There was no statistically significant difference in MCT utilization between LG and HG. Peak oxidation rates were 0.15 and 0.13 g/min, respectively. MCT contributed 7.6% (LG) and 6.5% (HG) to total energy expenditure during the 60- to 90-min period. Total fatty acid oxidation was significantly elevated in the LG trial but was not influenced by MCT ingestion. Concomitantly, CHO oxidation was reduced in LG but no effect of MCT was observed. We conclude that *1*) the contribution of MCT to total energy expenditure was small and *2*) strenuous exercise the day before the experiment, followed by a low CHO intake and leading to a low CHO availability, substantially increased total fat oxidation but did not significantly increase MCT oxidation.

trioctanoate; carbohydrate supplementation; substrate utilization; carbon-13 labeling; stable isotopes; exercise; ketone bodies

BECAUSE OF THEIR PHYSICAL characteristics, medium-chain triglycerides (MCT) are frequently used in parenteral and enteral nutrition. It is thought that MCT are a readily available energy source because they have been shown to be rapidly absorbed (2) and, unlike long-chain fatty acids, which are transported in chylomicrons through the lymphatic system, medium-chain fatty acids (MCFA) can directly enter the bloodstream through the portal system. In addition, at the cellular level, MCFA can cross the inner mitochondrial membrane in the liver and muscle independently of the acylcarnitine transferase system (4).

Therefore, it has been suggested that MCT might be a readily available energy source for the working muscle. When ingested orally during exercise, MCT could provide an energy substrate in addition to carbohydrates (CHO). Massicotte et al. (16) suggested that the energetic contribution of exogenous MCT was only slightly lower than that of an equicaloric glucose load during prolonged exercise of moderate intensity. Our laboratory recently reported that 70% of the ingested MCT was oxidized during exercise when coingested with CHOs (12).

As exercise progresses, muscle glycogen levels decline, and this decline is accompanied by a shift in substrate utilization from CHO to fat.

Plasma free fatty acid (FFA) uptake and oxidation increase during exercise (10, 26). Also, plasma glucose turnover and oxidation are increased during exercise at moderate intensities (22, 29). Thus, when intramuscular fuel stores decrease during exercise, there is an increased reliance on plasma fatty acids and plasma glucose for energy provision. We hypothesized that a CHO+MCT supplement can be especially effective under conditions where the reliance on blood substrates is maximal, such as in a glycogen-depleted state. Therefore, the present study examined the metabolic response to CHO+MCT supplementation with low muscle glycogen (LG) and normal-to-high muscle glycogen (HG) stores in a randomized crossover design. To study the oxidation rate of exogenous MCT during exercise, a $[1,1,1-^{13}C]$trioctanoate tracer was incorporated in the drink.

METHODS

Subjects. Eight male highly trained elite triathletes or cyclists [age 28.9 ± 2.5 yr, weight 77.9 ± 3.0 kg, height 184.5 ± 3.1 cm, maximal work rate 434 ± 14 W, and maximal O_2 consumption ($\dot{V}O_{2max}$) 70 ± 2 ml·kg^{-1}·min^{-1}] competing at the international level participated in this study. The nature and the risks of the experimental procedures were explained to the subjects, and their written informed consent was obtained. The study was approved by the local medical ethical committee.

Pretrials. Subjects' maximal workload (Wmax) was attained on an electronically braked ergometer (Lode Excalibur Sport, Groningen, The Netherlands) during an incremental exhaustive-exercise test (14) 1 wk before the first experimental trial. The results of this initial test were used to determine the 50% Wmax, which was later used in the experimental trials.

Subjects randomly performed two glycogen-depletion trials to achieve LG stores and two glycogen-loading trials (HG). The depletion trial was always performed in the evening

Table 1. *Steady-state $\dot{V}o_2$ and $\dot{V}co_2$ values at different time points during 90 min of exercise in subjects ingesting CHO or a CHO +MCT mixture with normal-to-high glycogen or low glycogen levels*

	Time, min					
	15	30	45	60	75	90
$\dot{V}o_2$, *l/min*						
LG-CHO	3.17 ± 0.09	3.24 ± 0.10	3.25 ± 0.10	3.24 ± 0.08	3.25 ± 0.11	3.36 ± 0.10
LG-CHO + MCT	3.25 ± 0.11	3.24 ± 0.10	3.32 ± 0.10	3.25 ± 0.11	3.28 ± 0.11	3.31 ± 0.11
HG-CHO	3.00 ± 0.08	3.03 ± 0.09	3.01 ± 0.08	3.06 ± 0.11	3.06 ± 0.07	3.04 ± 0.10
HG-CHO + MCT	3.07 ± 0.08	3.15 ± 0.08	3.17 ± 0.11	3.19 ± 0.11	3.23 ± 0.10	3.20 ± 0.12
$\dot{V}co_2$, *l/min*						
LG-CHO	2.61 ± 0.07	2.62 ± 0.08	2.61 ± 0.08	2.59 ± 0.08	2.63 ± 0.10	2.69 ± 0.09
LG-CHO + MCT	2.64 ± 0.08	2.62 ± 0.08	2.59 ± 0.07	2.57 ± 0.07	2.57 ± 0.07	2.63 ± 0.08
HG-CHO	2.67 ± 0.09	2.67 ± 0.09	2.63 ± 0.08	2.65 ± 0.11	2.62 ± 0.09	2.60 ± 0.11
HG-CHO + MCT	2.70 ± 0.08	2.74 ± 0.08	2.71 ± 0.10	2.66 ± 0.08	2.70 ± 0.08	2.74 ± 0.11

Values are means ± SE; n = 8 subjects. $\dot{V}o_2$, O_2 uptake; $\dot{V}co_2$, CO_2 production. LG, low glycogen; HG, normal-to-high glycogen; CHO, carbohydrate; MCT, medium-chain triglycerides.

(8–10 P.M.) preceding the experimental trial. An intermittent exercise protocol was employed, consisting of 2-min bouts at 90% Wmax interspersed with 2 min at 50% Wmax. When the subjects were unable to complete the 2-min 90% Wmax, the high workload was subsequently lowered to 80, 70, and 60% Wmax. The exercise was stopped when the 2-min trial at 60% Wmax could not be completed any more. This protocol has previously been shown to lead to very low muscle glycogen levels (<150 µmol/g dry wt) (13). Subjects were allowed to eat two crackers with cheese (14 g CHO, 4 g fat, 6 g protein) and to drink a cup of coffee or tea in the time between completion of the glycogen-depletion protocol and going to sleep.

The HG trials were preceded by a CHO-rich meal (4,000–5,000 kJ; ±80% CHO, ±10% fat, ±10% protein) at the laboratory, the evening before the experimental test (8–10 P.M.), to ensure a high CHO intake and concomitant HG stores.

Experimental trials. Each subject performed four trials, each separated by at least 7 days. A trial consisted of 90-min cycling at 50% Wmax (~57% $\dot{V}o_{2max}$). O_2 uptake ($\dot{V}o_2$) data are presented in Table 1. Drinks were provided in a randomized order, and both the subjects and the experiment leader were unaware of the content of the drink. Subjects were instructed not to consume any products with a high natural abundance of ^{13}C during the entire experimental period.

Protocol. Subjects reported to the laboratory at 8:00 A.M. after an overnight fast, and a standardized breakfast of two crackers with cheese (14 g CHO, 4 g fat, and 6 g protein) was provided. A Teflon catheter (Baxter Quick Cath, Dupont, Ireland) was inserted into an antecubital vein, and at 8:30 A.M. a resting blood sample was drawn. Resting breath gases were collected for the measurement of $\dot{V}o_2$ (SensorMedics 2900 analyzer, Anaheim, CA), and Vacutainer tubes were filled directly from the mixing chamber in duplicate to determine the ^{13}C/^{12}C ratio in expired CO_2. At 8:50 A.M., a 10-min warm-up began at 100 W. At 9:00 A.M., subjects started cycling at 50% Wmax for 90 min, and in the first minute they drank an initial bolus (4 ml/kg) of either one of the test drinks. Thereafter, every 20 min, a beverage volume of 2 ml/kg was given. Blood samples were drawn at 5, 10, and 15 min and every 15 min thereafter. Expiratory gases were collected every 15 min. Two subjects were tested on the same day, starting the protocol 4 min apart.

Drinks. The drinks consisted of tapioca-derived long-chain glucose polymers of low ^{13}C natural abundance (Sandoz

Nutrition, Bern, Switzerland) or a mixture of CHO and MCT. The MCT contained fatty acids with a chain length of 99% C8 (Estasan GT8–99, Unichema, Barcelona, Spain). To all drinks, 20 mmol/l of NaCl were added.

The composition of the drinks is listed in Table 2. On average, subjects ingested 146.0 g CHO in the CHO trials and 87.1 g CHO plus 26.6 g MCT in the CHO+MCT experiments.

The CHO solution and the CHO+MCT suspension containing 40% (by energy) MCT were equicaloric. Drink temperature was kept constant at 20°C.

Tracer methodology A [1,1,1-^{13}C]trioctanoate tracer (99%), purchased from Cambridge Isotope Laboratories (Woburn, MA), was incorporated in the unlabeled MCT suspension and then mixed with the CHO to form a stable suspension. The ^{13}C enrichment of the MCT was +160.61 8‰ (0.013042 ^{13}C/^{12}C ratio), whereas the ^{13}C enrichment of the CHO was −26.12 8‰ (0.0112372 ^{13}C/^{12}C ratio). The enrichment of the CHO was about the same as the average enrichment of the subjects' resting expired air (−27.21 8‰).

In the present study and in previous studies from our laboratory (12, 20, 24, 27, 28), we have shown that instructing the subjects not to eat any products of high-^{13}C abundance during the experimental period was effective in reducing the background shift (change in [13]CO_2) from endogenous substrate stores (28). We nevertheless decided to correct the background with the change of the ^{13}C enrichment of breath samples observed in the CHO trial (Table 3).

During the initial phases of exercise, some retention of [13]CO_2 in the bicarbonate pool occurs (21) and thus could lead to an underestimation of the calculated exogenous oxidation rates. However, during exercise, the CO_2 production ($\dot{V}co_2$) increases eight- to tenfold, leading to a physiological steady-state situation in which [13]CO_2 in expired air will be in equilibrium with the [13]CO_2/H[13]CO_3 pool. It has been shown

Table 2. *Beverage composition*

Beverage Composition	CHO	CHO + MCT
CHO, energy%	100	60
MCT, energy%	0	40
CHO, g/l	156.2	93.1
MCT, g/l	0	28.5
Energy, kJ/l (kcal/l)	2,620 (627)	2,620 (627)

Subjects ingested 12 ml/kg over 90-min period.

Table 3. *Resting enrichment values and change in enrichment of breath samples at different time points vs. rest sample*

Time, min	LG		HG	
	CHO	CHO+MCT	CHO	CHO+MCT
0	−27.17±0.31	−27.35±0.20	−27.06±0.17	−27.10±0.19
	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
15	0.13±0.09	5.41±0.68	0.10±0.14	3.56±0.69
30	−0.07±0.18	8.42±0.56	0.51±0.13	6.86±0.65
45	0.01±0.15	9.84±1.04	0.55±0.12	8.56±0.69
60	0.24±0.14	11.33±0.83	0.68±0.13	9.74±0.69
75	0.18±0.13	13.04±1.16	0.73±0.13	10.33±0.77
90	0.05±0.25	13.87±0.85	0.70±0.13	10.87±0.90

Values are means ± SE, expressed in δ‰ vs. PDB.

that the dilution of $^{13}CO_2$ becomes negligible and recovery of $^{13}CO_2$ approaches 100% after 60 min of exercise (18). Therefore, in the present study, data of exogenous MCT oxidation are presented for the 60- to 90-min period unless stated otherwise.

Analysis. Blood (10 ml) was collected into EDTA tubes and centrifuged for 4 min. Aliquots of plasma were frozen immediately in liquid nitrogen and stored at −40°C until analysis of glucose (Roche, Uni Kit III, 0710970), lactate (9), β-hydroxybutyrate (17), FFAs (Wako NEFA-C test kit, Wako Chemicals, Neuss, Germany), and glycerol (Sigma Chemical, GPO-trinder 337) on a COBAS BIO analyzer. From breath samples ($\dot{V}CO_2$, $\dot{V}O_2$) and stable-isotope measurements (IRMS, Finnigan MAT 252), total energy expenditure and oxidation rates of total fat, total CHO, and exogenous MCT were calculated. Breath samples were collected in 20-ml Vacutainer tubes (Becton Dickinson, Meylan Cedex, France) and stored at room temperature until analysis.

Calculations. From $\dot{V}CO_2$ and $\dot{V}O_2$, CHO and fat oxidation rates were calculated by using stoichiometric equations (19)

$$\text{glucose oxidation} = 4.585 \ \dot{V}CO_2 - 3.226 \ \dot{V}O_2$$

$$\text{fat oxidation} = 1.695 \ \dot{V}O_2 - 1.701 \ \dot{V}CO_2$$

The isotopic enrichment was expressed as the δ‰ difference between the $^{13}C/^{12}C$ ratio of the sample and a known laboratory reference standard according to the formula

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

The $\delta^{13}C$ was then related to an international standard, Pee Dee Belemnitella (PDB) limestone (PDB1 standard = 1.12372 $^{13}C/^{12}C$ ratio).

The amount of exogenous MCT oxidized was calculated according to the formula

exogenous MCT oxidation

$$= \dot{V}CO_2 \cdot (\delta_{bkg} - \delta_{exp})/(\delta_{bkg} - \delta_{ing}) - 1/k$$

where δ_{bkg} is the ^{13}C enrichment of expired air during the CHO trial (background), δ_{exp} is the ^{13}C enrichment of expired air during exercise at different time points, δ_{ing} is the ^{13}C enrichment of the MCT in the ingested CHO+MCT suspension, and k is the amount of CO_2 (in liters) produced by the oxidation of 1 g trioctanoate ($k = 1.2369$ liters CO_2/g MCT).

Statistics. Analysis of variance for repeated measures was used to compare differences in substrate utilization and in
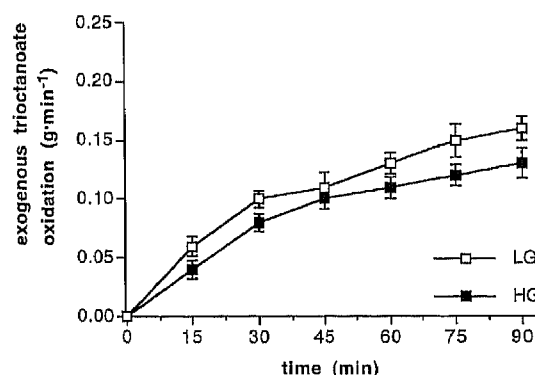

Fig. 1. Exogenous trioctanoate oxidation [medium-chain triglycerides (MCT)] from carbohydrate (CHO)+MCT suspensions during exercise in high-glycogen (HG) and low-glycogen (LG) trials. No significant differences between LG and HG were found.

blood-related parameters among the four endurance rides. A Scheffé's post hoc test was used in the event of a significant ($P < 0.05$) F-ratio.

RESULTS

$\dot{V}O_2$ was relatively constant throughout the experiments, and there were no differences among the four trials (Table 1). Average background ^{13}C enrichment measured from the resting breath samples was −27.21 ± 0.61 δ‰ (Table 3). Changes in isotopic composition of expired CO_2 in response to exercise are presented in Table 3. With ingestion of CHO (of low ^{13}C natural abundance) there was a slight, but statistically not significant, increase of ^{13}C in the expired air. In the CHO+MCT trials, the rise in ^{13}C was highly significant, reaching a δ‰ difference of ≥10–13 toward the end of 90-min exercise (compared with CHO experiment breath samples). There was no difference in the rates of $^{13}CO_2$ appearance in expired air between the HG and LG trials. Exogenous MCT oxidation showed a gradual increase over time both in the LG and in the HG states (Fig. 1). Peak oxidation rates were reached at the end of exercise (90 min) and were 0.15 g/min (LG) and 0.13 g/min (HG). No differences were observed between the LG and HG trials.

In Table 4, the amount of CHO and exogenous and endogenous fat oxidation during the exercise period is presented. Over the 60- to 90-min period, 4.24 ± 0.27 g of 5 g exogenous MCT were oxidized in the LG trial and

Table 4. *Total CHO oxidation, endogenous fat, and exogenous MCT oxidation during 60- to 90-min period in LG and HG trials*

	LG		HG	
	CHO	CHO+MCT	CHO	CHO+MCT
CHO total, g	45.5±3.5*	38.7±3.1*	69.2±5.1	60.5±5.4
Fat total, g	32.0±1.3*	35.1±2.3*	19.9±1.3	25.7±2.4
Fat exo (MCT), g	0.0±0.0	4.2±0.3	0.0±0.0	3.5±0.3
Fat endo, g	32.0±1.3*	30.9±2.0*	19.9±1.3	22.2±2.0

Exo, exogenous; endo, endogenous. *Significant difference between LG and HG ($P < 0.05$).

3.45 ± 0.26 g in the HG trial. This represented 85 and 69% of the total amount of MCT ingested, respectively. Exogenous MCT contributed 7.6% to total energy expenditure in the LG trial and 6.5% in the HG trial (during the 60- to 90-min period). These differences, however, were not statistically significant.

No differences in energy expenditure between the four trials were observed (Fig. 2). There were large differences in substrate utilization between the HG and LG trials, but differences between the CHO and CHO+MCT trials were not statistically significant. Total CHO utilization over 90 min was significantly higher in the HG trials [63 (CHO) and 53% (CHO+MCT) of total energy expenditure compared with the LG trials; 37 (CHO) and 33% (CHO+MCT) of total energy expenditure, respectively].

Resting plasma FFA concentrations were significantly higher in the LG trials (Fig. 3). Plasma FFA concentrations rose during exercise in all trials, except for the glycogen-loaded trial with CHO supplementation. FFA concentrations were elevated in the CHO+MCT trials compared with the CHO trials and in the LG trials compared with the HG trials. Glycerol concentrations were not significantly different in the resting situation and increased during exercise in all trials (Fig. 3). However, the increase was significantly greater in the LG trials from 15 min on. No difference was observed between CHO and CHO+MCT. Plasma

β-hydroxybutyrate was significantly elevated after 30 min until the end of exercise for the MCT-containing drinks (Fig. 3). There was no significant difference between HG and LG. There were no large changes in plasma glucose concentrations (Fig. 3). At 15 and 30 min, however, a slight but significant higher glucose concentration was observed in the HG-CHO trial compared with the LG trials.

DISCUSSION

Exogenous MCT oxidation. The amount of MCT oxidized was ~69–85% of the amount ingested during the final 30 min of exercise, representing 6.5–7.6% of total energy expenditure. When calculated over the entire 90 min of exercise, and neglecting a possible delay in the $^{13}CO_2$ appearance in the expired air due to entrapment in the bicarbonate pool, about one-third of the ingested amount was oxidized, covering 5.2–5.9% of energy expenditure, which is in accordance with previous findings of others (7, 16) and ourselves (12). Massicotte et al. (16) showed that 54% of 25 g MCT was oxidized during 120 min of exercise at 65% $\dot{V}o_{2max}$. The MCT, provided in a preexercise meal, contributed 7% to total energy expenditure. Décombaz et al. (7) reported that 30% of a preexercise MCT meal (25 g) was oxidized during 120 min of exercise at a comparable exercise intensity (60% $\dot{V}o_{2max}$). MCT contributed 11% to the energy yield.

We recently reported that the rate of MCT oxidation was maximally 70% of the rate of ingestion of MCT provided as MCT or in a CHO+MCT suspension during 180 min of exercise (12). MCT contributed 3–7% to total energy expenditure. In this study, peak MCT oxidation rates as well as the percentage of ingested MCT that was oxidized (i.e., 69–85%) were somewhat higher. The high oxidation rates of MCT suggest that the MCFA are very rapidly oxidized once they are in the systemic circulation.

The time course of $^{13}CO_2$ appearance in the expired air is similar to that of glucose: a plateau in enrichment is reached after ~60 min. The time required to reach maximal oxidation rates is dependent on several factors, including dilution in the bicarbonate pool, gastric emptying, and absorption.

Recently, we examined the gastric emptying rate of CHO+MCT emulsions (3). Four equicaloric CHO+MCT suspensions were studied with varying MCT contents. These suspensions varied from no MCT to maximally 30% MCT. It appeared that the suspension that emptied most rapidly from the stomach was the suspension with the highest concentration of MCT, whereas the CHO solution with no MCT was the slowest. Therefore, it was concluded that MCT did not reduce gastric emptying of CHO+MCT suspensions and that CHO content may be a major factor determining the gastric emptying rate.

In vitro studies (8) as well as in vivo studies (8, 15) have shown that the rate of absorption is fast compared
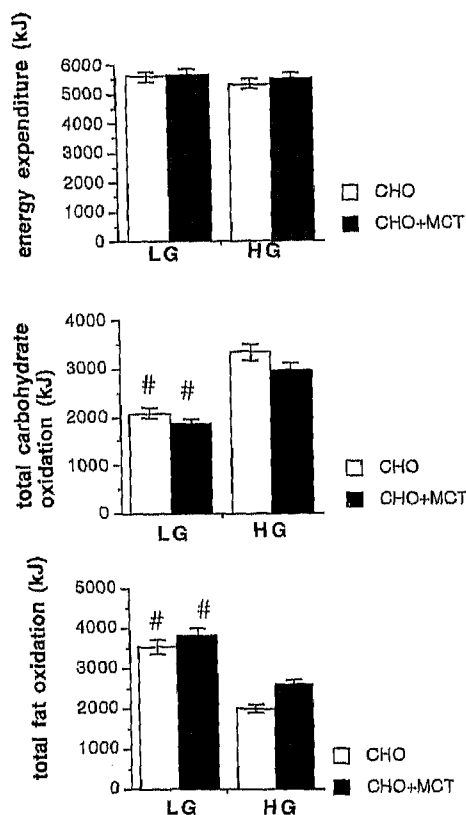

Fig. 2. Total energy expenditure, CHO oxidation, and fat oxidation over 90 min of exercise with ingestion of CHOs or a CHO+MCT suspension in HG and LG trials. #Significant difference ($P < 0.001$) between LG and HG.
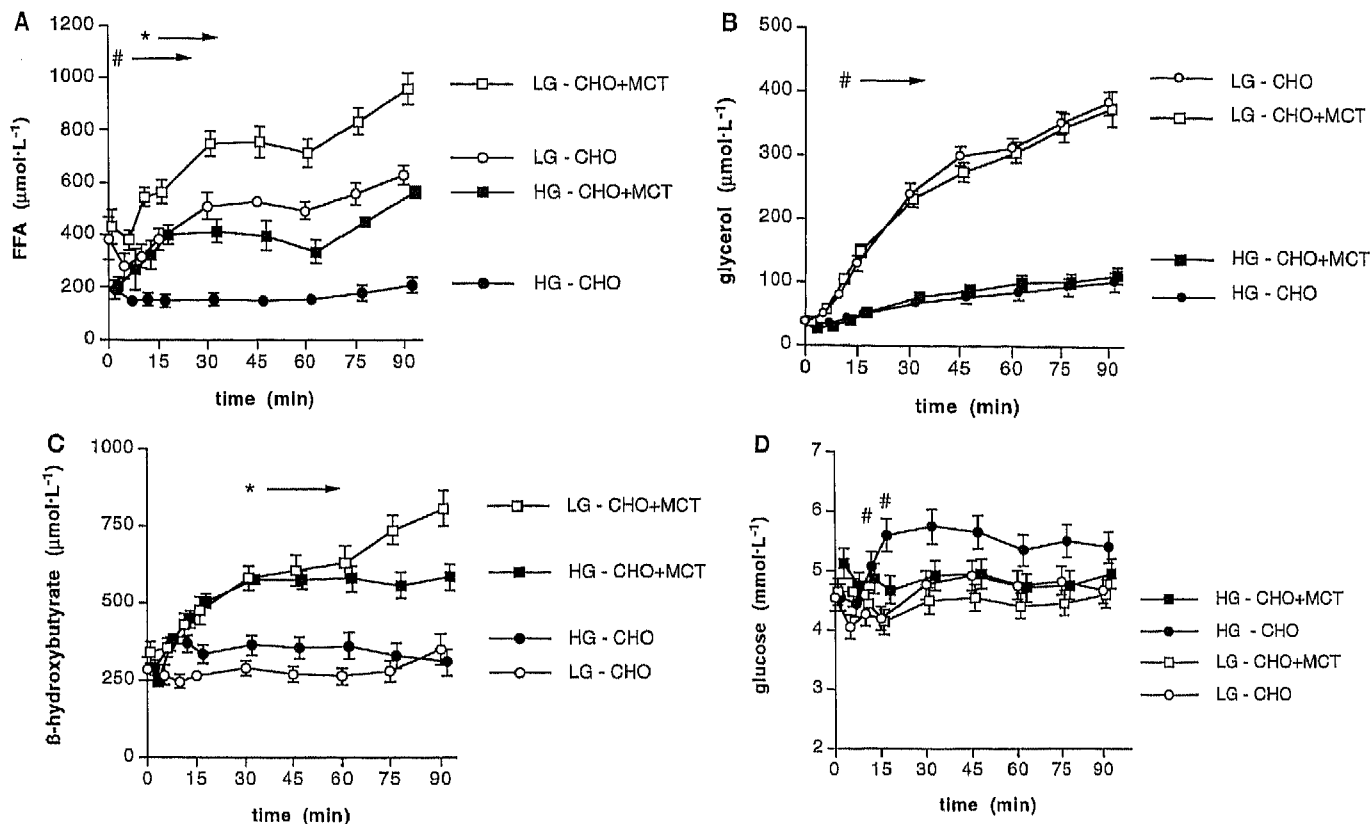
Fig. 3. Plasma free fatty acids (FFA; A), glycerol (B), β-hydroxybutyrate (C), and glucose (D) concentrations during 90 min of exercise in LG and HG trials with CHO or CHO+MCT ingestion. *Significant difference ($P < 0.05$) between CHO and MCT until end of exercise; #significant difference ($P < 0.05$) between LG and HG until end of exercise.

with long-chain triglycerides and can occur even in the absence of lipase (6, 8). Therefore, it seems that both the rate of gastric emptying and the rate of absorption of MCT are comparable to those of glucose, which is reflected in a similar time course of $^{13}CO_2$ appearance in expired gases.

Although total fat utilization was significantly higher, MCT oxidation was not elevated in the LG trials. After a glycogen-depletion protocol as applied in the present study, muscle glycogen (13) and muscle triglycerides (5) are drastically reduced. Several studies have shown that late in exercise the rate of disappearance of glucose and FFA is increased (22, 23), providing evidence for the increased reliance on plasma glucose and FFA. Therefore, we hypothesized that plasma FFA, and thus also plasma MCFA oxidation, would even be higher in the LG trials. The glycogen-depletion trial, however, had no effect on exogenous MCT oxidation in the present study. This makes it more likely that the main limiting factor for oxidation of MCT is the entrance of MCFA in the systemic circulation, as suggested previously (12).

Ingestion of MCT did not significantly influence total CHO utilization. This is in agreement with previous studies (7, 16, 25) that also found no changes in endogenous CHO oxidation or muscle glycogen utilization with MCT ingestion.

Substrate / metabolite concentrations. Glycerol concentrations did not change as a result of MCT ingestion. Probably MCT are hydrolyzed in the lumen, and both MCFA and glycerol enter the liver directly via the portal vein. The data suggest that glycerol is converted into glucose by gluconeogenesis in the first pass through the liver. In the glycogen-depleted state, however, lipolysis in adipose tissue is stimulated during exercise, and large amounts of glycerol enter the main circulation. The increase in plasma β-hydroxybutyrate concentration seems to suggest that part of the MCFA are oxidized in the liver. Ketone bodies are formed in the liver when the production of acetyl-CoA exceeds the energy needs of the hepatic tissue. It is known that MCTs are highly ketogenic (2). In this study, MCT supplementation elevated β-hydroxybutyrate concentrations markedly, which is in accordance with previous studies giving MCT as a preexercise feeding (1, 7, 11, 16, 25).

Summary. We conclude that lowering of muscle glycogen on the previous day substantially increases total fat oxidation during 90 min of exercise in comparison with CHO loading the previous day. However, no effect was seen on the oxidation of MCTs that were coingested with CHOs during 90 min of exercise.

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