Aspects of carbohydrate and fat metabolism during exercise

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ASPECTS OF CARBOHYDRATE AND FAT
METABOLISM DURING EXERCISE

Aspects of carbohydrate and fat metabolism during exercise

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit Maastricht,
op gezag van Rector Magnificus, Prof. Mr. M. J. Cohen,
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
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Chapter 1

Introduction

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Historical overview

Man has always been intrigued by the questions of which nutrients are utilized as fuel during exercise and how nutrition can improve exercise performance. The classic Greeks and Romans already were concerned with finding the best food for optimal performance. In as early as 1842, Von Liebig (87) stated that during exercise muscle protein was utilized and he recommended that during periods of heavy physical labor large quantities of meat should be eaten to replenish the protein loss. This view changed when in 1896 Chaveau (10) stated that carbohydrates were the only fuel that could be oxidized by skeletal muscle. In the following years the concept received support from others (44, 65). However, the group of Zuntz (94, 95) did not agree with this view and suggested that both carbohydrates and fat served as a fuel during exercise. In a classical study Krogh and Lindhardt (51) investigated gas exchange in the lungs during exercise. Based on the fact that carbohydrates and fat produce different amounts of CO_2 , and need different amounts of O_2 when they are oxidized, they showed, by measuring VO_2 and VCO_2 in expiratory gases, that fat is an important substrate during endurance exercise. The ratio VCO_2/VO_2 in expired air, the respiratory exchange ratio (R) will be 0.69-0.73 when only fat is oxidized (dependent on the length of the carbon chain of the fatty acid oxidized) and will be 1.0 when only glucose is oxidized. Krogh and Lindhardt (51) demonstrated that after several days of a low-carbohydrate, high-fat diet (they report: very fat bacon, butter, cream, eggs and cabbage) the average R during 2 hours of cycling exercise was reduced to ~0.80 as compared to 0.85-0.90 when a mixed diet was consumed. Conversely, when subjects ate a high-carbohydrate, low-fat diet (potatoes, flour, bread, cake, marmalade, and sugar) R was increased to ~0.95. Very interesting are also the subjective feelings of the subjects reported in that study. The exercise was judged more difficult following the high-fat diet and subjects felt more tired afterwards. Several years later in 1939 Christensen and Hansen (12)

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studied the effect of diet on the relative importance of carbohydrates and fat as a fuel at rest and during exercise. They observed that R was significantly lower after a high-fat diet both at rest and during exercise and time to exhaustion was drastically reduced on this diet. In 1934 Edwards et al (33) studied a runner who ran 6 hours on a treadmill and observed that as exercise duration progressed the relative importance of fat increased and even exceeded 90% of total energy delivery after 6 hours. In 1932 Christensen (11) showed that with increasing exercise intensity the proportion of carbohydrate utilized increased.

Important observations were also made by Levine et al (55) in 1924. They measured blood glucose in some of the participants of the 1923 Boston Marathon and observed that in most runners glucose concentrations were markedly declined after the race. Levine et al (55) suggested that these low blood glucose levels were a cause of fatigue. To test that hypothesis they encouraged several participants of the same marathon one year later to consume carbohydrates during the race (36). This practice, in combination with a high-carbohydrate diet before the race prevented hypoglycemia and significantly improved running performance. The importance of CHO for improving exercise capacity was further demonstrated by Dill et al (29). These investigators let their dogs, Joe and Sally run without feeding them carbohydrates. The dogs became hypoglycemic and fatigued after 4-6 hours. When the test was repeated with the only difference that the dogs were now fed carbohydrates during exercise, Joe and Sally ran for 17-23 hours. These early observations were later verified by the already mentioned studies of Christensen and Hansen (12). They showed that subjects who were exhausted could continue to exercise after they had eaten a large quantity of CHO. These early studies indicated that fat was indeed an important fuel for endurance exercise, but apparently carbohydrates were needed to maintain high exercise intensities and to optimize exercise performance. This work was expanded in the late 1960s with the re-introduction of the muscle biopsy technique (4, 5, 45). These studies indicated the critical role of muscle glycogen as a determinant of endurance exercise performance. These observations lead to the recommendation of a carbohydrate-loading diet. A high (70%) CHO diet, elevated muscle glycogen stores and seemed to enhance endurance performance (4, 70). These observations have been generally accepted and based on these observations athletes are usually recommended to carbohydrate-load (i.e. eat high CHO diets) prior to competition (20, 84). Bergström and Hultman (4) proposed the so-called supercompensation diet as a way to obtain supra-maximal glycogen stores. This regimen consisted of an exhausting exercise trial leading to glycogen depletion followed by three days on a high-fat diet and three days on a high-CHO diet. However this diet has several practical disadvantages such as gastrointestinal problems during the high-fat diet, poor recovery after the exhausting exercise trial etc. Therefore, Sherman et al (85) proposed a more moderate dietary regimen leading to glycogen levels which were only slightly lower than those attained by the classical supercompensation diet.

Carbohydrate ingestion during exercise

Besides the ergogenic effect of carbohydrate-loading, there is also an ergogenic effect of carbohydrate ingestion during prolonged exercise (14, 16, 25, 39, 48, 62, 66, 68, 69, 93). In an elegant series of studies, Coyle et al (23-25) and Coggan and Coyle (14-16) showed that carbohydrate ingestion improved endurance performance by maintaining blood glucose levels and higher rates of CHO oxidation. Without CHO ingestion subjects exhausted when their blood glucose levels dropped below very low levels ($3 \text{ mmol}\cdot\text{l}^{-1}$) but when they ingested CHO, plasma glucose concentrations were maintained, CHO oxidation rates were also maintained and fatigue was postponed. These workers also showed that CHO ingestion during exercise did not affect muscle glycogen breakdown. Since then interest was focused on the formulation of an optimal CHO solution for ingestion during prolonged exercise. A larger contribution of exogenous carbohydrates is believed to be more beneficial to endurance performance since the endogenous substrates are more conserved. In this context it is not surprising that numerous studies tried to measure exogenous CHO oxidation and identify the factors that affect oral CHO oxidation.

Oral CHO oxidation

To investigate the oxidation of ingested CHO, isotopically labeled CHO tracers have to be used. One such isotope is ^{13}C . ^{13}C has a higher mass than ^{12}C , and these isotopes can therefore be distinguished on a mass spectrometer. Both ^{13}C and ^{12}C are naturally abundant in all carbon containing compounds. However, most carbon atoms are ^{12}C (98.9%) whereas only 1.1% of all carbon atoms is ^{13}C . CHO formed by so called C_4 plants, such as corn and sugar cane have a slightly higher ^{13}C -abundance than C_3 plants such as potato, beet sugar and tapioca. The difference is caused by selective isotopic fractionation occurring during the process of photosynthesis in these plants: in the C_4 plants the enzyme preferentially incorporates ^{13}C when CHO are synthesized (38). When CHO derived from C_4 plants are oxidized, the production of $^{13}\text{CO}_2$ increases which, in combination with total CO_2 production, can be used to calculate the oxidation of exogenous CHO. It is also possible to add a certain (small) amount of commercially available ^{13}C -CHO to a drink to artificially elevate the ^{13}C -content of the drink. The first studies looking at exogenous CHO oxidation however, used radioactive isotopes (^{14}C -labeled CHO).

Costill et al (18) were probably the first to investigate the contribution of ingested CHO to energy expenditure during exercise in humans. They reported, however, that only small amounts of a glucose load were oxidized during exercise, leading them to conclude that 'glucose feedings are of limited importance for muscle metabolism. These results were later confirmed by van Handel (86) who also found a very small contribution of ingested glucose to total CHO oxidation. These results, however, are the only reports in the literature that show such a small contribution of exogenous CHO. Pirnay et al (71, 72) were the first to show that

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exogenous CHO could function as a significant energy source during exercise which was later confirmed by many investigations using ^{13}C labeled CHO (8, 27, 40, 42, 49, 50, 52, 54, 57-59, 67, 77, 82, 90, 91). Since it was discovered that ingested CHO could be a major substrate for the working muscle, studies tried to establish the factors that influence exogenous CHO oxidation. The factors studied include the amount and type of carbohydrate ingested (28, 37, 42, 54, 57, 58, 67, 82), the feeding schedule (49, 50, 71), the osmolality of CHO drinks (52) and the exercise intensity (56, 61, 73). (The factors influencing oral CHO oxidation will be discussed in more detail in the *general discussion*). Little or no information is available on the effect of training status and the availability of muscle and liver glycogen on exogenous CHO oxidation rates and these have become two of the questions of this thesis (Chapter 9 and 10).

Maximal exogenous CHO oxidation

An important observation in all the studies that have investigated exogenous CHO oxidation rates during exercise is that there is an upper limit for the oxidation of ingested CHO. This limit is about $1\text{ g}\cdot\text{min}^{-1}$. Independent of feeding schedule, the amount or the type of CHO ingested, the oxidation rate of $1\text{ g}\cdot\text{min}^{-1}$ is not exceeded (see review Hawley et al (41)). To illustrate this, in a study of Rehrer et al. (77) subjects were fed a 4.5% glucose solution (4.5G), a 17% glucose solution (17G) or a 17% maltodextrin solution (17MD) and exogenous CHO oxidation rates were measured. The results of this study show that with the ingestion of the 4.5G a large percentage of the ingested CHO was oxidized whereas only a very small percentage of the 17MD and 17G was oxidized. Oxidation rates did not exceed $1\text{ g}\cdot\text{min}^{-1}$. These results indicated that exogenous CHO oxidation did not increase linearly with the amount ingested and was limited by a yet unknown factor.

The factors that limit exogenous CHO oxidation could theoretically be gastric emptying, absorption, liver glucose metabolism (uptake and release of glucose by the liver), uptake by the muscle or muscle metabolism of glucose. The uptake and oxidation of glucose may be very important since plasma glucose has been shown to be a major substrate for energy metabolism in the muscle, especially late in exercise (80). However, only few attempts have been made to study the fate of plasma glucose or to quantify its contribution to energy expenditure during exercise with or without CHO ingestion. Recently, using tracer methodology, Bosch et al (8) and McConell et al (63) reported that a high rate of appearance of ingested glucose from the gut (R_a gut) reduced the hepatic glucose production and increased glucose uptake during exercise at 70% $\text{VO}_{2\text{max}}$. In the study of McConell et al (63) no plasma glucose oxidation was measured, while Bosch et al (8) found oxidation rates of plasma glucose which exceeded the rate of disappearance of glucose (R_d glucose) by 10-20%. The latter, of course, is theoretically impossible. Coggan et al (17) reported in one subject that 93% of glucose disappearing from the plasma was oxidized with glucose feeding during the second hour of exercise at 70% $\text{VO}_{2\text{max}}$. Thus, although it is often assumed that all glucose taken up by the muscle is oxidized, there is only little evidence to support this hypothesis. Kuipers et al (53)

reported that during low-intensity exercise some of the ingested glucose may be used for glycogen synthesis in the muscle illustrating that not necessarily all glucose disappearing from the plasma is oxidized. Therefore in **Chapter 12** the fate of ingested glucose using different isotopically labeled tracers is investigated.

CHO feedings, high intensity exercise and performance

As discussed above, CHO feedings during exercise maintain euglycemia and high rates of CHO oxidation thereby delaying fatigue and improving exercise capacity (6, 14, 16, 24, 25, 39, 46, 48, 62, 68, 69, 89, 92, 93). Based on these findings recommendations have been made for endurance athletes participating in activities lasting longer than 90 min (9, 32, 41). However, although it is generally accepted that CHO feedings improve performance during exercise at low-to moderate intensities (50-70% $\text{VO}_{2\text{max}}$) there is only little information on the effect of CHO feedings during high intensity exercise ($\geq 80\% \text{VO}_{2\text{max}}$; (1, 3, 74)). So at present it is not clear whether 'sport drinks' (usually CHO-electrolyte solutions) should be recommended to athletes involved in endurance exercise of less than 90 min duration. Therefore, another aim of this thesis was to investigate the effect of CHO-electrolyte solutions on exercise performance in a time trial lasting approximately 1 h (**Chapter 11**). However to be able to detect very small differences in performance a sensitive performance measurement is required. For that reason we investigated the reproducibility of three different 'endurance performance tests' in **Chapter 7**. The most reproducible test was applied to investigate the effect of CHO-electrolyte solutions on performance in **Chapter 11** and the effect of medium chain triglyceride supplementation on performance in **Chapter 8**.

Fat supplementation during exercise

Because of the obvious ergogenic effect of CHO, research in the past, has predominantly focused on the role of CHO. Fat metabolism during exercise received relatively little attention and fat supplementation during exercise was generally believed not to support exercise performance or even be detrimental to performance. The absorption of fat in the intestine is a complicated process, involving bile salts, the lymph system and incorporation into chylomicrons which makes fat absorption a relatively slow process. Fat also has a decelerating effect on the gastric emptying of meals. Since gastric emptying and absorption are potentially limiting steps in the oxidation of orally supplied substrates, fat supplementation during exercise is generally regarded as not desirable. With the worldwide introduction of medium chain triglycerides (MCT) in enteral and parenteral nutrition, MCT became also available for sport nutrition. MCT have different physical characteristics compared to 'normal' dietary fat (which contains almost exclusively long chain triglycerides; C16-C18) and it has been reported that MCT are rapidly absorbed and enter the systemic circulation directly, thereby bypassing the slow transport through the lymphatic system. Since exogenous CHO oxidation rates are limited

to 1 g·min⁻¹ it was hypothesized that addition of MCT to CHO drinks may provide an additional exogenous energy source, thereby possibly sparing endogenous energy stores. In vitro studies with rat skeletal muscle have revealed that elevated plasma FFA levels may lead to glycogen sparing during exercise (43, 78, 79) which theoretically could lead to improved endurance performance. Also in human volunteers who were fed triglycerides and later infused with heparin, elevated free fatty acid (FFA) concentrations were found to be associated with a decrease in muscle glycogen utilization during exercise (19, 31, 88). This has led to the suggestion that ingestion of sufficient amounts of medium chain triglycerides (MCT) may elevate plasma FFA levels, spare muscle glycogen and improve performance. Studies with stable isotopes have shown that medium chain fatty acids (MCFA) are rapidly oxidized at rest and during exercise (60, 64). However, pre-exercise MCT feedings did not result in altered rates of fat and CHO oxidation during exercise (26, 47, 83) nor in glycogen sparing (2, 26) or improved exercise capacity (83). However, at present the effects of MCT feedings *during* exercise (in combination with CHO) are not known and these will be investigated in this thesis (Chapter 3, 4, 5, 6 and 8).

The interaction between fat and carbohydrate metabolism

The interaction between fat and carbohydrate metabolism is closely regulated. However, the factors that regulate the interplay between fat and carbohydrate oxidation, especially in skeletal muscle during exercise, are not well understood. The classical concept is that fat metabolism regulates carbohydrate metabolism. The so called glucose-fatty acid cycle first reported by Randle and co-workers (34, 35, 75, 76) proposes that increases in oxidation rates of fatty acids lead to increased acetyl-CoA/CoA ratio's and citrate levels which then inhibit pyruvate dehydrogenase and phosphofructokinase. The subsequent increase in glucose-6-phosphate (G6P) will reduce hexokinase and glucose uptake in rat diaphragm, heart and liver (see Chapter 2). However, there has been little support for the glucose-fatty acid cycle to be operative in exercising skeletal muscle of humans (30, 31, 81). During intense exercise (i.e. cycling at 85% $\dot{V}O_{2\max}$), the elevation of plasma FFA does not increase muscle citrate or G6P and blood glucose uptake is not reduced (30, 31, 81). In exercising people, the major interplay by which plasma FFA alters carbohydrate metabolism appears to be that the lowering of plasma FFA during high intensity exercise deprives the muscle of a plasma substrate and thus increases muscle glycogenolysis as a result of the increased metabolic stress (i.e.; higher AMP levels) (30, 31, 81). Therefore, it generally appears that the ability of fat to regulate carbohydrate metabolism in people during exercise is limited and not in accordance with the classical glucose-fatty acid cycle (34, 35, 75, 76). We therefore tested the converse of the classical concept by hypothesizing that carbohydrate metabolism largely regulates fat oxidation in man during exercise. More specifically, in Chapter 13 we tested the hypothesis that increases in glycolytic flux and carbohydrate oxi-

dation in skeletal muscle reduce fat oxidation by inhibiting the oxidation of long-chain fatty acids, possibly by reducing their transport into mitochondria.

Summary of the contents of this thesis

This thesis describes various aspects of carbohydrate and fat metabolism. A summary of the contents is given below. In contrast to the numerous excellent reviews in the literature describing the role of carbohydrate metabolism during exercise (7, 13, 20-22, 41, 84), there is far less information with respect to fat metabolism during exercise. For that reason **Chapter 2** provides an extensive review of the literature concerning fat metabolism during exercise (In order to provide a complete overview, results of some of the later chapters have been included in **Chapter 2**). The following chapters deal with the questions raised above. **Chapter 3, 4, 5 and 6** will focus on the gastric emptying and the oxidation of ingested medium chain triglycerides, the effect of MCT on carbohydrate metabolism and muscle glycogen stores in particular. We also wanted to investigate the effect of MCT ingestion on endurance exercise performance (**Chapter 8**). However, a review through the literature shows that exercise tests to exhaustion may not always be a reliable and these tests are not a very practical approach to measure exercise performance. Therefore **Chapter 7** will describe the development and validation of an endurance performance test (**Chapter 7**) which was used to measure the effect of MCT ingestion on endurance exercise performance (**Chapter 8**). **Chapter 9 and 10** will extend on the factors that influence exogenous carbohydrate utilization. **Chapter 9** describes the role of endogenous carbohydrate availability and **Chapter 10** the effect of training status on oral glucose oxidation. In **Chapter 11** the effect of CHO feedings on performance during intense exercise of about 1 hour duration will be investigated. **Chapter 12** will describe the fate of the ingested CHO and the effect on endogenous glucose mobilization and oxidation. It will be investigated whether all glucose appearing in the plasma will also be oxidized. Finally in **Chapter 13** we investigated the interaction between fat and carbohydrate metabolism hypothesizing that CHO may regulate fat metabolism in contrast to the classical concept of the Randle cycle that fat regulates CHO metabolism. In **Chapter 14** a general discussion of the results of this thesis is provided.

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Chapter 2

Fat metabolism during exercise: a review

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Fat metabolism during exercise part II: Regulation of metabolism and the effects of training

Fat metabolism during exercise part III: Effects of nutritional interventions

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2.2 Introduction

The two main sources of energy for muscle contraction are carbohydrates and fat. Although branched chain amino acids, as well as other amino acids, can also be oxidized in the muscle, their contribution to total energy expenditure is thought to be negligible during aerobic exercise. The past decennia extensive research has been carried out with regard to the role of carbohydrates during exercise, which has led to a quite complete understanding of carbohydrate metabolism under exercising conditions. Far less information is available on the role of fats during exercise. Hence, our understanding of fat metabolism during exercise is far from complete. The purpose of this review is to give an overview of today's understanding of fat metabolism during exercise. Special attention is given to the factors that limit fat oxidation, the effects of diet on fat metabolism and the effects of training.

2.3 Overview of lipid fuels and nomenclature

In literature different nomenclature is handled with regard to fatty acids. To avoid misunderstandings or misinterpretations the nomenclature in this review article will be explained. Distinction has to be made between fatty acids which are incorporated into triglycerides or other particles, and fatty acids that are not incorporated into triglycerides. The fatty acids that are not esterified to form a mono-, di- or

triglyceride are so called non-esterified fatty acids (NEFAs) or free fatty acids (FFA). The term free fatty acid, might be somewhat ambiguous because, for example in plasma, these fatty acids are bound to albumin and they are not “free”. There is also a very small fraction of fatty acids (less than 0.01% of the plasma FFA pool) that is really “free” and not bound to any other compound (non-protein bound FFA) (79, 189, 190). However, since FFA is the most commonly used term, we will use this term also in this review to designate fatty acids that are not esterified to mono-di, or triglycerides, but might be bound to albumin or fatty acid-binding proteins (FABP). The basic chemical structure of fatty acids is depicted in Figure 2.1.

Figure 2.1 Chemical structure of some common fatty acids. a. stearic acid, 18:0; b. oleic acid, 18:1 (n-7); c. linoleic acid 18:2 (n-6); d. α -linolenic acid 18:3 (n-3).

In humans, the chain length of fatty acids typically varies from C14 to C24, although fatty acids with shorter or longer chain length may occur (Table 2.1). Fatty acids with a chain length of C8 or C10 are called medium chain fatty acids (MCFAs) whereas those with a chain length of C6 or less are called short chain fatty acids (SCFAs) (117). The most abundant fatty acids are the long chain fatty acids (LCFAs) with a chain length of C12 or more. Of the long chain fatty acids palmitic acid (C16) and oleic acid (C18, one double bond) are the most abundant. Fatty acids with no double bonds in their hydrocarbon chain are called saturated fatty acids and those with one or more double bonds unsaturated fatty acids. Fatty acids with one double bond are referred to as monounsaturated fatty acids whereas fatty acids with two or more double bonds are called polyunsaturated fatty acids. Also the position of the double bond is usually indicated. For example C20:4 (n-3) means that this 20 carbon fatty acid with 4 double bonds has the first double bond starting from the 3rd carbon counting from the terminal methyl group (Figure 2.1). Another way of indicating the fatty acid and the position of the double bond is C20:4 ω 3.

Table 2.1 Nomenclature and formulas of fatty acids (FA)

FA	Double Bonds	Common Name	Chemical formula
2:0	–	acetic	CH ₃ COO–
4:0	–	butyric	CH ₃ (CH ₂) ₂ COO–
6:0	–	capronic	CH ₃ (CH ₂) ₄ COO–
8:0	–	caprylic	CH ₃ (CH ₂) ₆ COO–
10:0	–	caprynic	CH ₃ (CH ₂) ₈ COO–
12:0	–	lauric	CH ₃ (CH ₂) ₁₀ COO–
14:0	–	myristic	CH ₃ (CH ₂) ₁₂ COO–
16:0	–	palmitic	CH ₃ (CH ₂) ₁₄ COO–
16:1	n-6	palmitoleic	CH ₃ (CH ₂) ₅ CH=CH(CH ₂) ₇ COO–
18:0	–	stearic	CH ₃ (CH ₂) ₁₆ COO–
18:1	n-9	oleic	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₇ COO–
18:2	n-6	linoleic	CH ₃ (CH ₂) ₄ (CH=CHCH ₂) ₂ (CH ₂) ₆ COO–
18:3	n-6	γ-linolenic	CH ₃ (CH ₂) ₄ (CH=CHCH ₂) ₃ (CH ₂) ₃ COO–
18:3	n-3	α-linolenic	CH ₃ (CH ₂)(CH=CHCH ₂) ₃ (CH ₂) ₆ COO–
20:0	–	arachidonic	CH ₃ (CH ₂) ₁₈ COO–
20:2	n-6	eicosadinoic	CH ₃ (CH ₂) ₄ (CH=CHCH ₂) ₂ (CH ₂) ₈ COO–
20:3	n-6	eicosatrinoic	CH ₃ (CH ₂) ₄ (CH=CHCH ₂) ₃ (CH ₂) ₅ COO–
20:4	n-6	arachidonic	CH ₃ (CH ₂) ₄ (CH=CHCH ₂) ₄ (CH ₂) ₂ COO–
20:5	n-3	eicosapentaenoic (EPA)	CH ₃ (CH ₂)(CH=CHCH ₂) ₅ (CH ₂) ₂ COO–
22:0	–	behenic	CH ₃ (CH ₂) ₂₀ COO–
22:5	n-3	docosapentaenoic	CH ₃ (CH ₂)(CH=CHCH ₂) ₅ (CH ₂) ₄ COO–
22:6	n-3	docosahexaenoic (DHA)	CH ₃ (CH ₂)(CH=CHCH ₂) ₆ (CH ₂)COO–
24:0	–	lignoceric	CH ₃ (CH ₂) ₂₂ COO–

Oxidizable lipid fuels include free fatty acids (FFA), intramuscular triglycerides (IMTG) and circulating plasma triglycerides (TG). These plasma TG are incorporated in lipoproteins like chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), intermediate density lipoproteins (IDL) and high-density lipoproteins (HDL). These lipoproteins differ in their density, TG content, cholesterol content but they also fulfill different functions. VLDL for instance is the main lipoprotein species for transport of triglycerides from the liver to adipose tissue and muscle while HDL is transporting cholesterol from the peripheral tissues to the liver. Therefore it is believed that chylomicrons and VLDL may play a role in energy metabolism during exercise while LDL, IDL and HDL probably do not play a significant role in the energy provision for the muscle. Also fat derived compounds such as ketone bodies (acetoacetate and β-hydroxybutyrate) can serve as a fuel whereas glycerol can be converted into glucose in gluconeogenesis in the liver and subsequently be oxidized. In this review the term “fat” will be used as an overall term for all lipid fuels available.

2.4 Fatty acids versus carbohydrates

Fatty acids have several biochemical and physical properties which distinguish them from carbohydrates and, in many cases, make them the substrate of choice. One of these properties is that fat contains more than twice as much energy per gram than do carbohydrates; $38 \text{ kJ}\cdot\text{g}^{-1}$ ($9 \text{ kcal}\cdot\text{g}^{-1}$) for fats versus $18 \text{ kJ}\cdot\text{g}^{-1}$ ($4 \text{ kcal}\cdot\text{g}^{-1}$) for carbohydrates). Furthermore, carbohydrates are stored in the presence of water, whereas fat is stored almost anhydrous (1 gram of glycogen binds about 2 grams of water (110)). This makes fat a far more efficient fuel per unit of weight. If all the fat in our body would be replaced by an equicaloric amount of carbohydrates our weight would double. Fat appears to be the ideal fuel for long lasting exercise in situations in which the provision of food is limited. A good example to illustrate this are certain animal species, like migratory birds, who fly for days without eating. These birds who rely heavily on their endogenous energy stores, store almost exclusively fat as a fuel (78).

In humans, both fat and carbohydrate is stored. Carbohydrates are stored as glycogen in muscle and liver. Muscle glycogen can be used directly to fuel contractile processes while glucose from liver glycogen first has to be transported by the blood, and taken up by the muscle before it can be oxidized. Gluconeogenic substrates such as lactate, glycerol and amino acids can be converted into glucose in the liver and can indirectly serve as an energy substrate. In addition, exogenous carbohydrate sources can also provide glucose for oxidative processes in the muscle after they are absorbed in the gut and have entered the systemic circulation. The carbohydrate stores are small. The total amount of muscle glycogen of an 80 kg man is about 400 grams (Table 2.2) although trained individuals may have larger glycogen stores. Liver glycogen represents about 80-100 grams. The total amount of plasma substrates (glucose and lactate) is about 20 grams. Expressed in terms of energy, the body carbohydrate stores represent approximately 8000 kJ (2000 kcal). In comparison with this, fat stores are very large and theoretically the fat store could provide energy for days whereas the glycogen stores can become depleted within 60-90 min. Calculating the amount of energy stored as fat for an 80 kg man and 60 kg woman (average body composition) this would provide respectively 450,000 kJ (110,000 kcal) and 550,000 kJ (135,000 kcal). Energy stored in the form of carbohydrates can vary in a range from 6000 kJ (1500 kcal) to 12,000 kJ (3000 kcal). In other words, if only fat or only carbohydrates could be utilized as a fuel, the carbohydrate stores would deliver energy for no more than 90 min of marathon running while energy derived from fat stores would be satisfactory for 119 hours of continuous marathon running (167).

Of course adipose tissue contains the largest quantity of fat, and most of the fat in man is stored in subcutaneous and deep visceral adipose tissue. The storage of fat is dynamic, which means that in case of a negative energy balance, the size of the individual fat cells will decrease whereas with a positive balance, the excess of fatty acids will be converted into triglycerides and hypertrophy of fat cells will result.

Although adipose tissue is by far the most important site of storage, fat is also stored within the muscle. The size of this fat pool is difficult to determine, but has been estimated to vary from 7 to 40 mol·kg ww⁻¹ (23, 62, 121, 202). The total amount of fat stored in all muscle cells has been estimated to be approximately 300 grams (17). The amount of fatty acids stored varies substantially between tissues but also within tissues. In muscle tissue for instance, type I fibers have been shown to have a higher TG content than type II fibers (62, 71). Besides these TG stores in adipose tissue and muscle there is a small fraction of fatty acids in the blood, transported by lipoproteins or as a "free" fatty acid (FFA), bound to albumin.

Table 2.2 The energy stores of an 80 kg man

substrate		weight (kg)	energy (kJ)
carbohydrates	plasma glucose	0.02	320
	liver glycogen	0.1	1600
	muscle glycogen	0.4	6400
	total (approx.)	0.52	8000
fat	plasma FFA	0.0004	14
	plasma triglycerides	0.004	140
	adipose tissue	12	450000
	intramuscular triglycerides	0.3	11000
	total (approx.)	12.3	460000

The values given are estimates for a 'normal' man of 80 kg. The amount of protein in the body is not mentioned but this would be about 10 kg (160,000 kJ) mainly located in the muscle.

Fatty acids provide more ATP per molecule than glucose does. A glucose molecule produces 38 ATP whereas a molecule of stearic acid produces 147 ATP. However, to produce the same amount of ATP, oxidation of fatty acids requires more oxygen than the oxidation of carbohydrates (159). The oxidation of one molecule glucose requires 6 molecules of oxygen while, for example, the complete oxidation of stearic acid requires 26 molecules of oxygen.

Furthermore, per unit of time more ATP can be derived from carbohydrates (glucose) than from the oxidation of fatty acids (159). When blood-borne fatty acids are oxidized the maximum rate of high energy phosphate (HEP) formation is ~0.40 mol HEP·min⁻¹ while the aerobic or anaerobic breakdown of endogenous glycogen can generate ~1.0-2.4 mol HEP·min⁻¹ (236). Because metabolic pathways beyond the formation of acetyl-CoA are identical for carbohydrate and fatty acid oxidation, the rate-limiting step in overall fat utilization must be proximal to the tri-carboxylic acid (TCA)-cycle (possibly β -oxidation, activation of the fatty acid, carnitine mediated transport across the mitochondria or transport of fatty acids from

the blood to the intracellular site of activation). At present the question as to which part of overall fatty acid utilization is rate limiting is yet unsolved. The possible limiting steps will be discussed below.

2.5 Fat as a fuel during exercise

In the early years, Zuntz (261, 262), Krogh and Lindhardt (143) and later Christensen and Hansen (33) showed that a mixture of carbohydrates and fat are used as a fuel at rest and during exercise. Based on the fact that carbohydrates produce different amounts of CO_2 , and require different amounts of O_2 when they are oxidized, they used VO_2 and VCO_2 measurements in expiratory gases to obtain information about substrate utilization. The ratio VCO_2/VO_2 in expired air, the respiratory exchange ratio (R) will be 0.69-0.73 when only fat is oxidized (depending on the length of the carbon chain of the fatty acid oxidized) and will be 1.0 when only glucose is oxidized. These early studies showed not only that both carbohydrate and fat were used during exercise but also that their relative contribution changed dependent on the exercise intensity, the exercise duration, and the diet prior to exercise (33, 56, 143). In general over 50% of the energy requirements in rest is derived from fatty acid oxidation (86). Fatty acids remain a very important substrate during exercise, as long as the exercise intensity is below 80-90% $\text{VO}_{2\text{max}}$ (82, 86, 195). Above this exercise intensity carbohydrates are the predominant substrate (1, 82, 86, 195).

While early studies were mainly based on respiratory gas exchange measurements, after World War II radioactive and stable isotope tracers became available and with tracer dilution techniques it was possible to investigate the kinetics of substrate mobilization and utilization (175). Very important was also the reintroduction of the muscle biopsy technique by Bergström and Hultman (15, 16, 118) in the late sixties. These techniques made it possible to quantify substrate fluxes and measure tissue substrate concentrations.

2.6 Processes that potentially limit fat oxidation

Even though the fat stores are relatively large, the capacity to oxidize fatty acids is limited and in many cases carbohydrates are the dominant substrate. The reason of this limitation in the use of fat stores is still not fully elucidated. The limitations in fat oxidation might be located at different levels:

1. Mobilization of FFA from adipose tissue
2. The transport of fatty acids to the muscle
3. The uptake of fatty acids by the muscle cell
 - a. The uptake of plasma FFA
 - b. The uptake of FFA from plasma lipoprotein-TG

4. Fatty acid mobilization from intramuscular triglyceride and cytoplasmic transport
5. The transport of fatty acids into the mitochondria
6. The oxidation of FFA in the mitochondria

2.6.1 Mobilization of fatty acids

Most tissues (i.e. muscle, liver, intestine, brain) contain only small amounts of fat and the ability of these tissues for *de novo* synthesis of fats is limited. Therefore most tissues depend on a continuous supply of fatty acids from the diet and from adipose tissue. This also holds for muscle. The rate of mobilization of FFA from adipose tissue is dependent on 1. the rate of lipolysis, 2. the rate of reesterification of FFA within the adipocyte and 3. the rate of export of FFA from adipose tissue via the blood.

2.6.1.1 Lipolysis in adipose tissue

In adipose tissue, fatty acids are constantly mobilized by lipolysis (hydrolysis of triglycerides), a process that can be initiated by the sympathetic nervous system (SNS). A first step in the process of lipid mobilization from adipocytes is the transfer of triglycerides from the main lipid droplet to the site of enzymatic cleavage in the cytoplasm of the adipocyte (Figure 2.2). Although there is only little information about this process, it is believed not to be rate-limiting (18). A second step in the mobilization of fatty acids is the enzymatic cleavage of the triglyceride in the cytoplasm. The fatty acids in the outer or β -position of the triglycerides are hydrolyzed by the action of hormone sensitive lipase (HSL). This enzyme is subjected to hormonal regulation. The remaining monoglyceride (glycerol with a fatty acid in the α -(inner) position) is then hydrolyzed by the more active enzyme monoglyceride lipase.

Glycerol cannot be reutilized by the adipocyte to form new triglycerides since the enzyme glycerokinase, is only present in very low concentrations or even absent in adipose tissue (and muscle) (194). Glycerol is a small water soluble molecule which can easily diffuse through the cell membrane into the blood. Therefore all the glycerol produced by lipolysis in the adipocyte, is released into the circulation. For this reason glycerol appearance in the blood is often used as a measurement of (whole-body) lipolysis. However, it must be noted that in certain conditions such as ischaemia, there may also be other sources of glycerol production (i.e. hydrolysis of glycerol-3-phosphate into glycerol) as shown by de Groot et al (48) for heart muscle. Furthermore, Elia et al (58) raised the possibility that glycerol released from IMTG might be directly oxidized, which may result in an underestimation of tracer determined whole-body lipolysis. However Romijn et al (195) argued that although Elia et al (58) showed a significant isotope exchange of glycerol across the human forearm, it was not clear whether this was due to slow equilibration of enriched glycerol with the muscle pool of glycerol or if it was related to concomitant muscle glycerol oxidation. Romijn et al (195) use their fat oxidation data to provide*

Figure 2.2 Mobilization of fatty acids from adipose tissue. Triglycerides are transported from the lipid droplet to the cytoplasm of the adipocyte. This triglyceride is subjected to cleavage by the enzyme hormone-sensitive lipase (HSL); a monoglyceride is formed and two fatty acids which can diffuse into the circulation. The remaining monoglyceride is split by the enzyme monoglyceride lipase (MGL) into glycerol and another fatty which can also diffuse into the circulation. The rate limiting step in this mobilization of fatty acids from the lipid droplet is the HSL. This enzyme is present in an active and an inactive form and can be activated by adrenergic stimulation, which will trigger a cascade of events leading to phosphorylation of the inactive hormone sensitive lipase. Insulin will stimulate phosphodiesterase leading to decreased cAMP levels and decreased activation of HSL. (PK = protein kinase, HSL = hormone sensitive lipase, TG = triglyceride, FFA = free fatty acids).

support for the contention that Ra glycerol is an accurate reflection of whole body lipolysis.

Unlike glycerol, fatty acids can be reesterified to form new triglycerides, a process called triglyceride-fatty acid cycling. So, some of the fatty acids and all of the glycerol formed by lipolysis in the cytoplasm will diffuse out of the adipocyte into the circulation. The rate of lipolysis is largely dependent on the activation of hormone sensitive lipase. The regulation of the activity of this enzyme is of primary importance for the mobilization of fatty acids from adipose tissue. The activity of the enzyme is dependent on several inhibitory and stimulatory factors. The SNS and circulating epinephrine concentrations appear to be the major stimulatory factors and insulin is probably the main counterregulatory hormone. Studies in isolated human adipocytes have revealed that at physiological concentrations, catecholamines, glucocorticoids, thyroid stimulating hormone and growth hormone are good stimulators of lipolysis (42, 99). On the contrary, lactate (95) ketone bodies (42) and especially insulin (99) have an inhibitory effect on the enzyme. The relative importance of these stimulating and inhibiting factors on lipolysis is not fully elucidated.

The transfer of SNS activity to the cellular level occurs through adrenergic receptors. In human adipose tissue, catecholamines have both α -adrenergic inhibitory and β -adrenergic stimulatory effects on the rate of lipolysis (42, 65). Through the adrenergic receptors a cascade of events is triggered: the adenylate cyclase system and the protein kinase system are activated. The protein kinase will activate the lipase by phosphorylation (Figure 2.2). Insulin acts mainly by stimulating phosphodiesterases which break cAMP down to AMP, thereby preventing the stimulation of HSL. On the other hand caffeine, for instance, is a known stimulator of the adenylate cyclase system, and therefore also a potent stimulator of lipolysis (see section 2.10.2.1 Caffeine).

Studies using microdialysis have shown that in man, α -adrenergic inhibitory mechanisms modulate lipolysis at rest, whereas β -adrenergic stimulatory effects are predominant during exercise (5).

During exercise the plasma insulin concentration will decrease, mainly due to an increased concentration of norepinephrine. Because insulin is a strong inhibitor of lipolysis, the net effect will be an increased lipolysis. At the same time the sensitivity of β -adrenergic receptors for catecholamines will increase in adipose tissue (240). The combination of these effects results in an increased lipolysis in adipose tissue during exercise.

2.6.1.2 Export rate of FFA from adipose tissue

Although the major regulatory factors controlling adipose tissue FFA mobilization are through adrenergic stimulation and insulin, the rate at which FFA are removed from the adipose tissue appears to be another factor. The removal rate of FFA from the adipose tissue is dependent on the plasma albumin concentration, the arterial FFA/albumin ratio and the blood flow through adipose tissue (25). The plasma albumin concentration in plasma is fairly constant (about $0.6 \text{ mmol} \cdot \text{l}^{-1}$) while in normal conditions the fatty acid concentration varies between 0.2 – $1.0 \text{ mmol} \cdot \text{l}^{-1}$. During moderate intensity exercise arterial plasma FFA concentrations may increase up to twentyfold. This leads to changes in the FFA/albumin ratio from a resting value of 0.2 to values of 3 – 4 during exercise. Since albumin binds FFA with decreasing affinity when more binding sites are occupied (210), increases in the FFA/albumin ratio are accompanied by increased concentrations of the non-protein bound FFA fraction in plasma. This in turn favors reesterification within the adipocyte since the FFA/albumin ratio in plasma is increased even more. It has also been shown that adipose tissue blood flow markedly increases the removal rate from adipose tissue and thus the mobilization of FFA (25, 28). Madsen et al (150) showed that in perfused adipose tissue increases in the FFA/albumin ratio as well as decreases in the adipose tissue blood flow decreased FFA mobilization. They suggested that the decreased net FFA output was due to increased rates of reesterification or a decreased transport capacity of the blood. During exercise blood flow through adipose tissue may increase up to threefold which may partly compensate for the decreased transport capacity caused by the increased plasma FFA/albumin ratio (24, 26, 27).

2.6.1.3 Triglyceride-fatty acid cycling

As mentioned before, glycerol which is liberated after lipolysis, cannot be incorporated into triglycerides since the enzyme glycerol kinase is virtually absent in adipose tissue and muscle (194). In adipose tissue this enzyme is needed to convert glycerol to glycerol-3-phosphate (G-3-P), which is the backbone of a triglyceride molecule. Therefore the rate of appearance (Ra) of glycerol is often used as a direct index of lipolysis (253). The G-3-P required for reesterification is indirectly derived from glucose in the adipose tissue. Formation of G-3-P involves the reduction of dihydroxyacetone phosphate, a glycolytic intermediate. The newly formed G-3-P can then be bound to acyl-CoA to synthesize triglycerides. With low blood glucose levels, less G-3-P will be formed and consequently the rate of fatty acid reesterification will decrease (254, 255). The excess FFA will be released into the circulation. This TG hydrolysis and subsequent reesterification, was the first documented substrate cycle and is called the triglyceride-fatty acid cycle (Figure 2.3). The functioning of this cycle allows the adipocyte to adjust the release of FFA rapidly in response to alterations in metabolic demands. For instance, when there is less glucose available, reesterification will be decreased and more FFA will be mobilized from the adipose tissue (254). Reesterification may occur within the adipocyte (intracellular reesterification) or fatty acids may be released and reesterified in some other tissue (extracellular reesterification).

Figure 2.3 The triglyceride-fatty acid cycle. Fatty acids (FFA) mobilized after hydrolysis of triglycerides (TG) can either be released into the circulation or reesterified with a molecule of glycerol-3-phosphate (G-3-P). This TG hydrolysis and subsequent reesterification is called the triglyceride-fatty acid cycle.

The release of 1 molecule glycerol should be accompanied by the release of 3 molecules FFA if no reesterification occurs after hydrolysis of triglycerides. However, the release ratio between glycerol and FFA is usually 1-2. Stimulation of lipolysis takes place very rapidly after the onset of exercise (5). However, the rate of lipolysis is far in excess of the need for fatty acids in oxidative processes. Therefore it was concluded that fatty acid mobilization was regulated by reesterification (254). During the first 30 min of exercise at 40% $\text{VO}_{2\text{max}}$, the reesterification of FFA was markedly decreased. Whereas at rest approximately 70% of all released FFAs were reesterified, during exercise only 25% was reesterified (254). Similar findings were obtained by Hodgetts et al (109) during exercise at 50-70% $\text{VO}_{2\text{max}}$. During the first 30 min of exercise, reesterification is suppressed and at the same time lipolysis is increased and adipose tissue blood flow is increased (24). This results in a massive increase in rate of appearance and availability of FFA in the plasma. The rate of reesterification is also dependent on the ability of plasma to carry away released FFA (i.e. the number of free albumin binding sites for FFA and the adipose tissue blood flow), and the availability of glucose to produce G-3-P. In addition, lactate has been shown to increase the rate of FFA reesterification (125, 176, 204). Shaw et al. (204) stated that a lactate concentration of 2 mmol·l⁻¹ reduces FFA output by 35-40% in the whole animal.

2.6.2 Transport of lipids by the blood

When fatty acids have passed the cell membrane of the adipocyte either passively or mediated by a membrane associated protein such as fatty acid translocase (FAT) or fatty acid-transport protein (FATP) (101, 203, 236), they will move through the interstitium bound to albumin, pass the vascular wall of the capillaries and again bind to the circulating albumin. Since albumin has (at least) three high-affinity fatty acid binding sites, under physiological circumstances a minority of binding sites is occupied. The largest fraction of FFA in the plasma (more than 99.9%) is carried bound to albumin (189, 190). Prior to extraction by skeletal muscle, FFA have to be released from albumin since the permeability of endothelial cells, lining the wall of muscle capillaries, is very low for the albumin-FFA complex (12). Because solubility of FFA in water is poor, a large fraction of fatty acids will be esterified into triglycerides in the liver, and incorporated into lipoproteins (very low density lipoproteins VLDL). Also other lipoproteins function as carriers for fatty acids through the blood (chylomicrons, low density-lipoprotein LDL, high-density lipoprotein HDL).

2.6.3 Uptake of fatty acids

Havel et al (102) showed in 1967 that during exercise plasma FFA concentrations increased along with the FFA uptake although the fractional extraction rate decreased. Studies in men and dogs have suggested that a linear relationship would exist between FFA concentration in the blood and the utilization of FFA, suggesting that the uptake of FFA by the muscle is a passive process that occurs by passive diffusion (4, 122). However, this traditional view has recently been challenged (12,

Figure 2.4 Transport of FFA from the vascular space to the mitochondria of the skeletal muscle cell. FFA in plasma are bound to albumin and after transport into the interstitial space again bound to albumin. FFA cross the sarcolemma either passively or by a plasma membrane bound fatty acid-binding protein (FABP_{pm}) which may function as a true translocator or as an acceptor for fatty acids, thereby creating a steep transmembrane gradient. In the sarcoplasm FFA will be bound to a cytoplasmic fatty acid-binding protein (FABP_c). Subsequently FFA will be activated by acetyl-CoA synthase to form an acyl-CoA which can be transported via carnitine acyl transferase I (CAT I), a translocase and carnitine acyl transferase II (CAT II) into the mitochondrial matrix. This transport into the mitochondria is carnitine dependent. Acyl-CoA units inside the mitochondrial matrix may be subjected to enzymatic cleavage in the β -oxidation.

235). Evidence is accumulating indicating the existence of a "carrier system" for FFA across the cell membrane. Although the details of the transport of FFA into the muscle cell are largely unknown, it is generally accepted that one or more membrane-associated proteins are involved (29, 181, 230). Recently carrier proteins such as FAT and FATP with fatty acid-transport stimulating properties have been identified in adipocytes (101). FABPpm (29), FAT (2, 80), and FATP (2, 80) have also been identified in skeletal muscle.

Besides protein mediated transport, part of the fatty acids will passively pass the cellular membranes because of the lipophilic nature of the fatty acids (80, 236).

The initial step in the uptake of FFA from the plasma would be the translocation through the luminal membrane of the endothelial cell, the cytoplasmic compartment of the endothelial cell and subsequently through the abluminal membrane of the endothelial cell (Figure 2.4). Next, the FFA have to be transported through the interstitial space most probably bound to albumin (235). Then the FFA will be transported through the sarcolemma, either by passive diffusion or facilitated by plasma membrane bound FABP (FABPpm) (235) or as recently suggested by a protein in the membrane named FAT or FATP (80, 230). In the cytoplasm, FFA are bound to cytoplasmic FABP (FABPc) and transport of the FFA within the cytoplasm occurs bound to this protein (80, 81, 230). Vork et al (233) reported a significant positive correlation between the percentage of muscle FABP content and the percentage of oxidative fibers in skeletal muscle, suggesting that FABP plays an important role in the rate of FFA oxidation.

Although the mechanism of FFA transport into the muscle cell is largely unknown, the existence of a carrier mediated transport might explain the findings of Turcotte et al (226) and Kiens et al (138). In isolated perfused rat skeletal muscle (226) and in man (138), it was observed that the uptake of non-protein bound FFA by the muscle followed saturation kinetics and did not increase linearly with the increasing FFA concentration in the blood as was generally believed. Above a certain FFA concentration, saturation of the transport process occurred, which could indicate a limitation in the transport over the cell membrane although limitations distal from the FFA uptake cannot be excluded (i.e. oxidation). The data implying saturation kinetics of FFA uptake are derived from plots of FFA uptake against the delivered non-protein bound FFA fraction (90, 138, 209, 215, 226). Although the largest fraction of FFA in plasma (99.9%) are carried bound to albumin, FFA uptake is suggested to be dependent on the small non-protein bound FFA fraction in the plasma (<0.1% of the total FFA pool in plasma) (138, 226).

2.6.4 Availability of FFA

Because fat is stored mainly outside the muscle cell in adipose tissue, availability of FFA as a substrate is dependent on lipolysis in adipose tissue, transport through the blood and FFA uptake by the muscle. During the first minutes of exercise plasma FFA concentration decreases as a result of a delay between the increased uptake

of FFA directly after the onset of exercise and the stimulation of lipolysis (31). Once lipolysis is fully activated and reesterification is suppressed, the plasma FFA concentration will rise (254). Plasma FFA concentration increases progressively with increasing submaximal exercise intensity (83, 84, 167). At high exercise intensities ($>80\% \text{VO}_{2\text{max}}$), however, FFA availability may be limited as a result of decreased FFA release from adipose tissue (Ra FFA) (195).

At cessation of exercise the utilization of FFA is drastically reduced whereas the lipolytic activity will be maintained because of the metabolic activation. As a result FFA levels in the blood will increase in the post-exercise period (167). After 10-15 min a peak FFA concentration is reached, and concentrations will decline again to resting levels ($0.2\text{--}0.5 \text{ mmol}\cdot\text{l}^{-1}$). After exercise, and sometimes even during exercise, FFA concentrations may increase up to $2 \text{ mmol}\cdot\text{l}^{-1}$. It is generally assumed that FFA concentrations above $2 \text{ mmol}\cdot\text{l}^{-1}$ are toxic in humans (128, 167). High post-exercise FFA concentrations can also be regarded in a positive way, since they replenish muscle TG stores (241). Hagenfeldt et al (98) showed that FFA oxidation is in large part dependent on the plasma FFA concentration. However, the rate of FFA oxidation in the working muscle is not directly determined by the plasma FFA concentration but the FFA concentration in the cytoplasm, the number and the size of mitochondria and probably also by the presence of other substrates such as pyruvate (112). There are indications that FFA oxidation is dependent on the availability of pyruvate. In the absence of carbohydrates, the FFA oxidation is inhibited in isolated perfused hindlimb from the rat (192, 225). Apparently a minimal level of glycolysis is required to provide the TCA-cycle via anaplerotic reactions with carbon skeletons. TCA cycle intermediates may be formed from glucose in muscle by carboxylation of pyruvate and reversal of the phosphoenolpyruvate carboxykinase reaction (228, 238, 239). Decreased FFA oxidation in the absence of carbohydrates (192, 225) may therefore be in agreement with the theory that glycogen depletion leads to fatigue because the number of TCA-cycle intermediates decreases below a critical level (200, 237).

2.6.5 Uptake of fatty acids from circulating lipoproteins

Another potential source of fatty acids is triglycerides bound to lipoproteins (VLDL and chylomicrons) (102). Muscular endothelium is virtually impermeable for circulating lipoproteins. Before FFA can be taken up by the muscle, they have to be released from the triglycerides forming the core of the lipoproteins (VLDL and chylomicrons). Only after hydrolysis of the lipoprotein-TG by lipoprotein lipase (LPL), FFA can be transported into the muscle cell. LPL is located on the luminal surface of the vascular wall (21) and will hydrolyze some of the TG in the lipoproteins passing through the capillary bed. LPL activity is found in most, if not all, tissues. The highest activities have been reported in cardiac and red skeletal muscle and adipose tissue. The response of LPL activity to certain stimuli (like fasting or exercise) appears to be tissue specific (144). For example during fasting or exercise the LPL activity is increased in heart and skeletal muscle whereas at the same time the LPL activity in adipose tissue is reduced (144).

Attempts have been made, using arteriovenous balances and radioactive labeled TG, to quantify the oxidation of fatty acids derived from plasma TG. From most of these studies, contribution of plasma TG to the total fat metabolism during exercise appears to be very small (100, 102, 172, 173). Attempts to quantify VLDL-TG utilization during exercise have revealed that the fatty acid uptake from plasma lipoprotein triglycerides occurs slowly (258) and accounts for less than 5% of the fatty-acid derived CO_2 during prolonged exercise (102, 123). Therefore it is often believed that plasma TG contribute only minimally to energy production during exercise (102, 123, 258). However, there are some interesting observations that need further investigation. For instance LPL activity is significantly increased after training (140) and after a high-fat diet (139), both situations in which fat oxidation is markedly increased. In addition, acute exercise also stimulates muscle LPL activity (220). Furthermore, studies in exercising dogs showed that the contribution of VLDL-TG is small (221). These findings together suggest that LPL may play an important role although attempts to measure the contribution of plasma TG directly report that plasma TG are not a major fuel during exercise. Therefore the contribution of VLDL-TG (and chylomicron-TG) to energy expenditure remains uncertain.

2.6.6 Muscle triglycerides

Muscle triglycerides are stored in depots, mostly adjacent to the mitochondria (115). Skeletal muscle contains on average about $12 \text{ mmol} \cdot \text{kg}^{-1} \text{ ww TG}$ (23, 62, 91, 121, 202) but this can vary markedly because of other factors such as fiber type, nutrition and physical exercise. Histological studies revealed that Type I fibers contain more TG than Type IIa fibers in humans. Type IIb fibers displayed the lowest concentration of intramuscular TG (62). Glycolytic fibers rely less on TG as a source of energy and as a consequence TG pools can be smaller in these fibers. As in adipocytes, lipolysis in muscle is stimulated by SNS activity and circulating epinephrine concentrations. In isolated perfused heart it was shown that with epinephrine the glycerol release was increased (245). Furthermore by perfusion with dibutyryl adenosine 3',5'-cyclic monophosphate (cAMP), TG decreased by 50% in perfused rat hearts (77). This may indicate that, as in adipose tissue, cardiac lipolysis is mediated through the classical cAMP cascade. Cleroux et al. (35) showed that muscle triglyceride lipolysis is mediated by β_2 -adrenoceptors. In subjects performing prolonged exercise till exhaustion, muscle triglyceride utilization was completely blocked by nadolol, a non-selective β -blocker, whereas it was unchanged with atenolol, a selective β_1 -adrenoceptor blocker. Indirect support for these observations in skeletal muscle comes from studies showing that muscle triglyceride lipolysis occurs mainly in slow-twitch fibers (211, 214) which also have a higher density of β_2 -adrenoceptors. Because results indicated that extramuscular lipolysis decreased to the same extent with β_2 -adrenoceptor- or $\beta_1 + \beta_2$ -adrenoceptor-blockade it was concluded that adipose tissue lipolysis is only partially controlled by the adrenergic system and mainly through β_1 -adrenoceptors, whereas skeletal muscle triglyceride breakdown appears to be controlled by the adrenergic system

through $\beta 2$ -adrenoceptors (35). However, at present it is still not completely clear to what extent $\beta 1 + \beta 2$ and $\beta 3$ -adrenoceptors are involved in skeletal muscle lipolysis.

Early studies using ^{14}C -labeled fatty acids to quantify fatty acid turnover indicated that muscle TG were used during exercise (97, 102). These studies revealed that during submaximal exercise (60-120 min), plasma FFA contributed only 50% to total fat oxidation, implicating that the residue must have come from other fat sources, most likely intramuscular triglycerides. With electron microscopy, fat vacuoles in the muscle were studied before and after exercise (170). The size of the vacuoles was smaller after exercise indicating that indeed fatty acids were mobilized from these vacuoles and subsequently oxidized (170). Studies using muscle biopsies indicated that muscle TG content decreases during exercise (23, 63, 71, 186). However, other studies were not able to find differences between pre and post-exercise TG content in muscle biopsies (83, 130). Part of the inconsistent results can be explained by the muscle biopsy technique applied. The muscle biopsy, usually 50-150 mg wet weight, represents only a small part of the whole muscle and might not be representative for the whole muscle TG stores. TG are not stored homogeneously over the muscle compartments and as noted above, different fiber types have different TG contents (202). Wendling et al. (243) recently showed that TG measured in repeated muscle biopsies displayed a large variation resulting in coefficients of variation between 20-26%. They suggest that only changes in IMTG larger than 24% may be detectable. In addition, to accurately quantify intramuscular triglyceride utilization, it is of utmost importance that all surrounding fat is carefully dissected out from the muscle fibers before the triglyceride content is analyzed. If this dissection is not performed, the measured triglyceride content will be the sum of intramuscular triglycerides and adipocytes located between muscle fibers.

It was estimated that the contribution of muscle TG to total energy expenditure during exercise (65% $\text{VO}_{2\text{max}}$) was 15-35% (121, 130). It must be noted however, that this contribution was calculated by subtracting plasma FFA uptake from total fat oxidation and the difference theoretically reflects TG in the muscle, TG between muscle fibers and TG in VLDL. In this study it was assumed that the contribution of VLDL-TG was negligible. Romijn et al (195) investigated the energetic contribution of different substrates during exercise at three different exercise intensities. The contribution of intramuscular TG was 7%, 26% and 8% during exercise at 25%, 65% and 85% of $\text{VO}_{2\text{max}}$ respectively (Figure 2.5). This suggests that there is an optimum in intramuscular TG utilization somewhere between 25% and 85% $\text{VO}_{2\text{max}}$. Endurance trained athletes rely more on their IMTG. Martin et al (153) showed that training increased the contribution of IMTG utilization to total energy expenditure. This will be discussed in more detail in section 2.9.5 Effect of training on IMTG utilization.

In general it can be concluded that muscle TG are utilized during submaximal exercise, although methodological problems make exact quantification of intramuscular triglyceride utilization very difficult. Attempts to quantify muscle TG utilization,

Figure 2.5 Substrate utilization (in $\text{J}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) at different exercise intensities (25% VO_2max , 65% VO_2max and 80% VO_2max). Data adapted from Romijn et al. (195).

included measurements of arterio-venous concentration differences of FFA of specific muscle groups, muscle biopsies and more recently also by stable isotope techniques. The latter is determined by measuring plasma FFA oxidation (by use of a palmitate or oleate tracer) and subtracting this from the total fat oxidation obtained from indirect calorimetry; total fat oxidation minus plasma FFA oxidation is believed to be the (minimal) contribution of IMTG.

2.6.7 Transport of FFA across the mitochondrial membrane

Once FFA enter the cytoplasm of the muscle cell they can either be esterified and stored into intracellular TG (or incorporated in phospholipids) (46) or the FFA can be bound to the FABP for transport to the site of oxidation and activated to a fatty acyl-CoA by the enzyme acyl-CoA synthase. The activation of the FFA is an extramitochondrial process. The mitochondrial inner membrane is impermeable for acyl-CoA (or FFA) so that a carrier is needed for translocation of the activated fatty acid over the mitochondrial inner membrane. First the acyl-CoA ester is converted into acyl-carnitine by carnitine-acyl-CoA transferase (CAT I) located at the outer face of the inner mitochondrial membrane and reconverted into fatty acyl-CoA at the matrix side of the inner mitochondrial membrane by the enzyme CAT II (Figure 2.4). Acyl carnitine crosses the inner membrane in a 1:1 exchange with a molecule free carnitine, a transport step which is controlled by the protein acyl carnitine translocase (169, 236). It is generally believed that medium- and short chain fatty acids can more freely diffuse into the mitochondrial matrix, where they are converted to their respective CoA esters. However, at least part of these fatty acids are transported by specific carrier proteins short- or medium chain acyl-CoA transferases (96, 198). Because malonyl-CoA, an intermediate of biosynthesis of fatty acids, inhibits CAT I this is believed to be involved in the regulation of overall fat utilization (45, 156-158, 198, 247, 248, 250). The possible role of malonyl-CoA

in the regulation of fatty acid oxidation will be discussed in section 2.7.4 Regulation through malonyl-CoA.

The importance of the carnitine-acyl-transport system across the inner mitochondrial membrane becomes obvious when regarding the serious complications observed in carnitine-deficient patients (216). These patients are unable to use fat as a fuel. Consequently, they rely heavily on their glycogen stores, which will become depleted earlier during exercise. Early fatigue and decreased performance will result.

There is preliminary indirect evidence suggesting that the carnitine dependent transport of long chain fatty acids into the mitochondria may be a rate limiting step in the process of fatty acid oxidation. When intravenous infusions of long chain fatty acids are given (which are dependent on the carnitine transport system to cross the inner mitochondrial membrane), lower oxidation rates are found compared to medium chain fatty acids which are less dependent on this carnitine transport system (160). However, at present it is uncertain to what extent the carnitine dependent transport of FFA into the mitochondria is rate limiting.

2.6.8 Oxidation of FFA

In the β -oxidation, the fatty acyl-CoA is stepwise degraded to acetyl-CoA and an acyl-CoA residue, shortened by two carbons. The acetyl-CoA units can enter the tricarboxylic acid cycle (TCA-cycle) and follow the exact same pathway as acetyl-CoA units from pyruvate. The shortened fatty acyl-CoA acts again as a substrate for the β -oxidative pathway until it is completely oxidized. The rate at which fatty acids are oxidized depends on the type of fatty acids. Fatty acyl moieties that are poorly oxidized by the mitochondria (i.e. very long chain fatty acids) may be oxidized in peroxisomes (236). Both the number of carbon atoms and the degree of saturation have been shown to influence the oxidation rate. It is known that medium chain fatty acids (MCFA) are more rapidly and more completely oxidized than long chain fatty acids (LCFA) (6).

Jones et al (137) studied whole body oxidation rates of fatty acids with a similar chain length (C18) but different degrees of saturation. Remarkable differences in the oxidation rates were observed: oleic acid (C18:1 n-9) was more rapidly oxidized than linoleic acid (C18:2 n-6) which in turn was more rapidly oxidized than stearic acid (C18:0). In vivo studies in rats (147) showed that the oxidation rates of fatty acids decreased with increasing chain length (C12:0 > C14:0 > C16:0 > C18:0). There were considerable differences in the oxidation rates of different unsaturated fatty acids (18:3 n-3 > 18:1 n-3 > 18:3 n-6 > 22:6 n-3 > 20:4 n-6).

Oleic acid (18:1) was oxidized at a remarkable high rate, almost as fast as lauric acid (12:0). Of the n-6 fatty acids linoleic acid (18:2 n-6) was oxidized at a faster rate than any of its metabolites, with arachidonic acid (20:4 n-6) being oxidized at the lowest rate. The rate of oxidation of α -linolenic acid (18:3 n-3) was almost as fast as lauric (C12:0) and oleic acid (C18:1 n-9). In incubated hepatocyte cultures, rates of fatty acid uptake and ketone body formation were of the order 16:1 > 16:0 > 18:2 > 18:1 > 18:0 (60). Also Hagenfeldt and Wahren (97) observed a slight prefe-

rential extraction of linoleic (18:2 n-6) and oleic acid (18:1) compared to palmitic acid (C16:0) across the forearm. From these studies it appears that saturated and unsaturated fatty acids are oxidized at different rates.

2.6.9 Other fat metabolites

Other fat related compounds such as glycerol and ketone bodies can also serve as a fuel during exercise. Glycerol is released after hydrolysis of TG and will be transported to the vascular space. Via the blood glycerol will be transported to the liver where it can serve as a gluconeogenic precursor for glucose. However, studies with glycerol administration showed that the conversion rate of glycerol to glucose was inadequate to contribute significantly to the energy expenditure during exercise (161, 165).

The ketone bodies (acetoacetate and β -hydroxybutyrate) are products of incomplete fatty acid oxidation. Under normal circumstances, the liver is the only organ with the ability to produce ketone bodies. On the other hand ketone bodies can be oxidized by most tissues including skeletal muscle. The concentration of ketone bodies in the plasma is usually very low (50-150 $\mu\text{mol}\cdot\text{l}^{-1}$) but may increase markedly after fasting or during prolonged exercise (23, 51, 52). During fasting, the production of ketone bodies is extremely important since they serve as an alternative fuel for glucose in the brain. Glucose and ketone bodies are the only fuels that can be utilized by the brain. Normally there is ample glucose to provide the brain with energy substrates. However, during fasting the availability of glucose is markedly reduced and ketone bodies can serve as an alternative fuel in muscle cells. With respect to energy provision during exercise, the contribution of ketone bodies is generally believed to be negligible (68, 167).

2.7 Regulation of substrate utilization

2.7.1 Hormonal regulation

Changes in gluconeogenesis, lipolysis and ketogenesis during exercise can at least partly be explained by changes in hormone concentrations. The catabolic hormone profile as observed during intense endurance exercise will be promoted after a low-carbohydrate diet or a negative energy balance (74, 142, 149). Hormones may primarily affect the mobilization of FFA. The insulin concentration decreases while glucagon, epinephrine, norepinephrine but also growth hormone (GH) and cortisol levels increase. GH has a facilitating effect on the catecholamines and thus also on the release of FFA into the bloodstream, while insulin is a strong inhibitor of lipolysis (87). See also section 2.6.1.1 Lipolysis in adipose tissue. Galbo (74) concluded that the changes in concentration of these hormones were primarily caused by changes in plasma glucose concentrations. However, a sympathetic stimulation of hormone release could not be excluded. The role of hormones during exercise and their influence on substrate utilization has been extensively reviewed in several recent publications (66, 72, 73, 191, 232).

2.7.2 The glucose-fatty acid cycle at rest

The interaction between carbohydrate and fat metabolism could partly be explained by the existence of the so called glucose-fatty acid cycle proposed by Randle in 1963 (183) (Figure 2.6). An increased plasma FFA concentration would lead to an increased fat oxidation. The accelerated flux through the β -oxidation pathway would result in accumulation of acetyl-CoA and NADH, which in turn would inhibit the activity of the enzyme pyruvate dehydrogenase (PDH) and thus inhibit pyruvate oxidation. Inhibition of pyruvate dehydrogenase would lead to a sparing of carbohydrates since, once pyruvate has been converted into acetyl-CoA it is committed to oxidation. In addition an increased acetyl-CoA concentration and in fact an increase in the acetyl-CoA/CoA ratio, would result in an increased citrate concentration leading to an inhibition of the enzyme phosphofructokinase (PFK), a key (rate-determining) enzyme in the glycolytic pathway. These effects would result in a decreased rate of glycolysis and glycogenolysis. Lower FFA concentration would of course result in the opposite adaptations.

Figure 2.6 Glucose-fatty acid cycle (Randle cycle). This cycle describes the mechanisms by which FFA oxidation may inhibit glucose oxidation. (PDH = pyruvate dehydrogenase; PFK = phosphofructokinase; P = phosphate).

Although evidence in favor of the existence of this glucose-fatty acid cycle has been obtained in rat cardiac muscle (75, 76, 183, 184) and in rat diaphragm (75, 76, 183, 184) initial studies in rat skeletal muscle suggested that availability of FFA did not inhibit glucose disposal or oxidation (14, 89, 193). However results were rather conflicting since other studies reported decreased glucose utilization after adding FFA to the perfusion medium in isolated rat skeletal muscle (176, 187, 188). Rennie et al. (188) found that increasing the FFA availability decreased the rate of glycogen degradation in stimulated rat skeletal muscle. Citrate concentrations increased in slow twitch (ST; soleus) and fast twitch oxidative (FTO; deep portion of vastus lateralis) but not in fast twitch glycolytic muscle (FTG; superficial portion of vastus lateralis). So the differences in the above mentioned studies may be in part explained by the different muscle types studied. Another difference between the studies, which could explain part of the conflicting results, may be the time point of the measurement. Zorzano et al (260) concluded that the glucose-fatty acid cycle does not operate in the resting state in the soleus muscle of a starved rat, but it does operate in the post-exercise period.

Although, most (9, 67, 69, 223, 242, 257), but not all (89, 260) studies provide data that support the existence of the Randle cycle in resting skeletal muscle, this issue still remains a matter of continuous debate. Ferrannini et al. (69) showed that elevated plasma FFA levels inhibited glucose uptake, a finding which was later confirmed by Walker et al (242). Studies in which glucose tolerance was investigated with high and low plasma FFA levels suggest a direct inhibiting effect of FFA on glucose transport (67). Wolfe et al (252) on the other hand, observed that elevated FFA levels did not affect plasma glucose oxidation when glucose uptake was kept constant. Instead, elevated FFA levels suppressed the oxidation of glycogen. Baron et al. (11) administered Intralipid® and heparin and observed that insulin-mediated whole body glucose utilization at rest was inhibited.

Most studies in resting human subjects support the concept that an increased availability of fatty acids affects the utilization of intracellular or extracellular glucose or both. However, the underlying mechanisms are largely unknown and although there are indications that the Randle-cycle is operative in resting human skeletal muscle, there is little support for the cycle in exercising conditions.

2.7.3 The glucose-fatty acid cycle during exercise

In one of the first studies looking at the glucose-fatty acid cycle in man during exercise, it was observed that glucose uptake during exercise was inhibited by an increase in plasma FFA concentrations (43). In a classical study Costill et al. (43) fed their subjects a high-fat meal and gave them a heparin infusion to elevate plasma FFA. Subjects exercised 30 min on a treadmill at 70% $\text{VO}_{2\text{max}}$. The elevation of plasma FFA ($\sim 1 \text{ mmol}\cdot\text{l}^{-1}$) decreased the rate of muscle glycogen breakdown by 40% as compared to a control trial ($\sim 0.2 \text{ mmol}\cdot\text{l}^{-1}$). Vukovich et al (234) observed similar glycogen sparing effects with fat feeding and Intralipid® infusion in combination with heparin infusion. These findings were further confirmed by Dyck et al. (55) who infused Intralipid® and heparin to elevate plasma FFA and observed sig-

nificant glycogen sparing after 15 min of cycling at 85% VO_2max . However, the latter did not observe any differences in muscle citrate, acetyl-CoA and PDH between the Intralipid® and the control trials, suggesting that another mechanism than the glucose-fatty acid cycle must be responsible for the regulation of fuel utilization. The authors suggest regulation at the level of glycogen phosphorylase (55). Hargreaves et al. (100) elevated plasma FFA concentration by infusion of Intralipid® and heparin. During exercise (knee extensions), arteriovenous differences of different substrates were measured and muscle biopsies were taken. The uptake of glucose was inhibited, while no differences could be found in the muscle respiratory quotient and glucose-6-phosphate. Elevated FFA concentration did not result in a decreased glycogen breakdown. The authors suggested that FFA have a direct effect on the uptake of glucose from the vascular space. These results were obtained during low intensity exercise with no changes in hormone concentrations (100). However, Romijn et al. (196) showed that during intense exercise, glucose uptake was not inhibited by increasing plasma FFA concentrations. To further illustrate the contradicting findings, Ravussin et al. (185) could not find a change in the relative contribution of fat and carbohydrates to total oxidation during exercise at 44% VO_2max , although plasma FFA were significantly elevated by providing a pre-exercise medium-chain triglyceride (MCT) containing meal.

The results of several studies are far from consistent, especially during exercise (36, 43, 108, 185, 187, 188). However, some of the differences in the results can be explained by the experimental setup. Some studies have been performed in situations with very low rates of fatty acid oxidation (148). These low rates of fatty acid oxidation can be found for instance during either very low or very high exercise intensities. Some studies administered only small amounts of exogenous fat, which might have been insufficient to significantly elevate fat oxidation (38, 148). Also other factors may have caused the non-uniformity in the results. In addition, the glucose-fatty acid is probably subjected to influences of several hormones which makes comparison of different investigations difficult. In the literature, the different findings of studies, some of which observed glycogen sparing, and others did not, is generally explained by the extent to which plasma FFA levels were elevated. In other words, in those studies that could not observe glycogen sparing, plasma FFA were not elevated enough above resting levels to result in a decreased muscle glycogen breakdown.

Another way of looking at the seemingly conflicting results is that the glycogen sparing effect seen in some of the studies is not a result of a reduction in glycogen breakdown but rather an accelerated glycogen breakdown in the control trials. Studies in which glycogen sparing was observed with elevated FFA levels (43, 55, 234), had very low plasma FFA levels in their control groups (below $\sim 0.2 \text{ mmol}\cdot\text{l}^{-1}$) which may have deprived the muscle of a plasma substrate. On the other hand, the FFA levels in those studies not able to demonstrate this effect, were somewhat higher (above $\sim 0.4 \text{ mmol}\cdot\text{l}^{-1}$) (185). This is analogous to the finding that nicotinic acid,

a strong inhibitor of FFA mobilization which decreases FFA availability usually below $\sim 0.2 \text{ mmol}\cdot\text{l}^{-1}$ increased muscle glycogenolysis. This view is further supported by observations by Dyck et al. (55) who showed a poor energy status of the muscle (low AMP, PCr) in the control trials in which FFA availability was low compared to the Intralipid® trials with very high plasma FFA concentrations.

In conclusion, there is no evidence that the glucose-fatty acid cycle as originally proposed is operative in exercising human skeletal muscle. Although several studies showed decreased glucose utilization when FFA are elevated, regulation may not be through the classical glucose fatty acid cycle. The question remains: if the glucose-fatty acid cycle is not the regulating mechanism during exercise then what determines carbohydrate and fat utilization?

2.7.4 Regulation through Malonyl-CoA

Another possible mechanism is regulation through Malonyl-CoA. Carnitine acyl transferase I, the rate limiting step for the transport of LCFA-CoA into the mitochondria has been suggested to be an important regulating site of FFA oxidation (Figure 2.7). CAT I-activity in turn is regulated by malonyl-CoA, which is formed by acetyl-CoA carboxylase (ACC) in the rate limiting step of fatty acid synthesis. CAT I is very sensitive to changes in the malonyl-CoA concentration (156). In starved and diabetic rats, hepatic malonyl-CoA levels are decreased and fatty acid oxidation as well as ketosis is increased (158). In situations of glucose paucity such as fasting, AAC is inactivated, and the result is a decreased malonyl-CoA concentration and an increased rate of β -oxidation. After refeeding, the previously starved rats have increased malonyl-CoA levels and decreased fatty acid oxidation (158).

These observations suggest that malonyl CoA may play an important role in the regulation of fatty acid oxidation (157). Although these effects have been shown in animal studies of liver (155, 157), heart (197), there is also evidence for the regulation through malonyl-CoA in skeletal muscle (57, 157, 198, 199, 247, 248). Evidence collected in isolated and perfused skeletal muscle suggests that the availability of carbohydrates may be an important factor determining the utilization of FFA (199). In liver, heart, and also in muscle, increases in glucose or insulin and especially the two in combination will lead to an increased activity of ACC and an increased formation of malonyl-CoA (53, 199). In addition, exercise (247, 248) and contractions induced by electrical stimulation (53, 199) have been shown to decrease acutely the concentration of malonyl-CoA in rat skeletal muscle. However, all these findings are in animal models. In addition no direct measures were made of fatty acid oxidation or any aspect of fat metabolism. Odland et al. (171) were the first to measure malonyl-CoA in human skeletal muscle. They reported surprisingly low levels at rest and a 20% decline during exercise that failed to be statistically significant (171). In a follow-up study, Spriet et al. (213) measured malonyl-CoA levels in muscle biopsies during exercise at different exercise intensities (35% VO_2max , 65% VO_2max and 90% VO_2max). Although there were considerable changes in fat oxidation no differences were observed in muscle malonyl-CoA levels (213). So,

Figure 2.7 Mechanism by which malonyl-CoA may regulate fatty acid flux into the mitochondria. Glucose and insulin may stimulate acetyl-CoA carboxylase (ACC) leading to an accumulation of malonyl-CoA which in turn may inhibit carnitine dependent long chain fatty acid (LCFA) transport across the inner mitochondrial membrane. Medium chain fatty acid transport across the inner mitochondrial membrane which is less sensitive for changes in malonyl-CoA concentration may be inhibited to a lesser extent.

the first data in humans are not very conclusive and further research is needed to elucidate the role of malonyl-CoA in the regulation of fat oxidation. However animal models indicate that when the muscle is supplied with a surplus of fuels other than fatty acids, or it does not use available fuels, malonyl-CoA levels increase (53, 199, 247, 248). Conversely, malonyl-CoA levels decrease when the muscle is fuel deprived or energy use is increased by contraction (53, 199, 247, 248).

2.8 The effect of exercise intensity on fat metabolism

The intensity of exercise is the main factor determining the degree of fat or carbohydrate oxidation during exercise. Relatively, fatty acids will be more important during low-intensity exercise. During exercise at $25\% \text{VO}_2\text{max}$ almost all of the energy expenditure was derived from fat (195). During exercise at $65\% \text{VO}_2\text{max}$ fat oxidation accounted for 50% of the energy expenditure but since the energy turnover was so much higher, the absolute fat oxidation rates were greater. The absolute rates of energy provision from fat seem to have an optimum at exercise levels

between 50 and 70% VO_2max . At high exercise intensities (70% VO_2max) both the absolute and the relative contribution of fat was reduced. Romijn et al. (195) also distinguished between plasma FFA uptake and IMTG utilization using stable isotopes. They find that during low intensity exercise (25% VO_2max) IMTG contribute minimally to energy provision. Plasma FFA and glucose appeared to be the most important substrates at this intensity where fat is by far the predominant fuel. At a moderate exercise intensity (65% VO_2max) substrates in the muscle (IMTG and glycogen) became more important (Figure 2.5). IMTG were oxidized at high rates at this exercise intensity while plasma FFA were used at a slightly lower rate compared to low intensity exercise (Figure 2.5). When the exercise intensity was further increased to 85% VO_2max , the contribution of plasma FFA became even smaller, while also IMTG oxidation decreased. The decreasing contribution of plasma FFA as observed in this study may be caused by a decreased availability of FFA which in turn may be caused by lower rates of appearance of FFA into the plasma (RaFFA) (136). This decreased RaFFA (136) despite a similar rate of lipolysis (195) may indicate that FFA are trapped within the adipocyte (109). However, although FFA availability is one of the factors which may be partially responsible for the decreased FFA oxidation during exercise at high intensities, this may not be the only factor. When plasma FFA levels were elevated to high levels during exercise at 85% VO_2max by Intralipid® and heparin infusion, fat oxidation rates were somewhat increased (196) but still lower compared to exercise at 65% VO_2max (195). This indicates that other (intramuscular) factors may reduce fatty acid oxidation during high intensity exercise. A possible mechanism may be that high rates of glycolysis at those exercise intensities inhibit long chain fatty acid transport into the mitochondria at the level of CAT I by increasing malonyl-CoA concentrations (45). However, at present there is no direct evidence available to support this hypothesis.

In addition, during intense exercise, more fast-twitch (FT) fibers will be recruited and less slow-twitch (ST) fibers (202). Since the FT-fibers have a smaller capacity to oxidize fatty acids, fat oxidation will decrease and concomitantly carbohydrate oxidation will increase when more of these fibers will be recruited (202).

The contribution of fatty acids can increase markedly when glycogen stores in the muscle become depleted (3). This implies, however, that the "high" exercise intensity can not be sustained and that exercise has to be continued at a lower level (177) because the rate of ATP production will decrease.

2.9 Training and fatty acid oxidation

Endurance training affects both substrate utilization and the exercise capacity. A large number of studies, both in animal and man, have established a marked adaptive increase in oxidative potential in response to an increased physical activity (111, 112). A notable consequence and probably contributing factor to the enhanced exercise capacity after endurance training is the shift of metabolism to a greater use

of fat and concomitant sparing of the glycogen stores (10, 37, 39, 85, 86, 111-113, 121, 201, 202). The increased performance and the shift to fat metabolism during submaximal exercise are more pronounced than the change in total-body maximal oxygen uptake ($\text{VO}_{2\text{max}}$) (82) and it seems unlikely that other training adaptations such as an increased maximal cardiac output or pulmonary function, will be a major factor in explaining the shift from carbohydrate to fat metabolism in the trained skeletal muscle (86). The contribution of fat to total energy expenditure increases after training at both the same relative and absolute exercise intensity (43, 107, 121, 130). This is of utmost importance during prolonged exercise of moderate to high intensity (50-90% $\text{VO}_{2\text{max}}$) since carbohydrates are required to maintain those levels of exercise (i.e. high rates of ATP production). As soon as glycogen stores become depleted and carbohydrate oxidation falls below a critical level, the exercise intensity has to be reduced since ATP can not be generated at a sufficient rate (166). Although the advantages of increased fat oxidation during exercise are obvious, the cellular and molecular mechanisms underlying this beneficial effect of training are incompletely understood. Several adaptations may contribute to a stimulation of fat oxidation in trained subjects: 1) An increase in the number of oxidative enzymes, 2) Increased mitochondrial content in trained muscle, 3) Increased muscle triglyceride oxidation, 4) Increased FFA uptake by the muscle and 5) Alterations in mobilization of FFA from adipose tissue.

Not only the cause of the increased fatty acid oxidation after training is uncertain, also the source of these fatty acids is still not completely known.

2.9.1 An increase in the number of oxidative enzymes

Studies on whole muscle homogenates have shown that rat skeletal muscle undergoes adaptive increases in the capacities to oxidize fatty acids (10, 162) and ketones (249). Based on enzyme kinetics Gollnick and Saltin (85) calculated that enhanced activity of enzymes involved in fatty acid oxidation may be the prime cause of the increased fat oxidation after training. Increased levels of enzymes involved in the activation, transport and β -oxidation of long chain fatty acids have been frequently reported (10, 32, 44, 111, 162). Increased levels of FABP (22) and of 3-hydroxyacyl CoA dehydrogenase have been found in endurance trained rats (49) and in man (121, 130). Other fatty acid handling enzymes of which the activity or content is increased after training include: carnitine palmitoyl transferase I (162), carnitine acyl transferase (162) and fatty acyl-CoA synthase (162). However, not only the enzymes involved in fatty acid activation, transport and oxidation are increased in trained muscle, but also the enzymes involved in the TCA-cycle and respiratory chain (32, 44, 107). Since endurance training increases both the capacities to oxidize fatty acids and pyruvate, the question remains: why are proportionally more fatty acids and less carbohydrates oxidized?

2.9.2 Increased mitochondrial content in trained muscle

Endurance training increases both the size and the number of the mitochondria (112). This increases the surface area where exchange of substrates and ADP can

take place and possibly also the number of transport proteins. Gollnick and Saltin (85) proposed that the increased total mitochondrial volume as seen after endurance training, increases the capacity to transport ADP formed by the contractile cycle into the mitochondria. Consequently, free ADP (ADP_f) levels are lower in muscles of exercise trained compared to untrained muscles at the same contractile activity (41). The ADP_f concentration, ATP/ADP_f ratio and the ATP/(ADP_f × Pi) ratio in the cytosol and in the mitochondria have been shown to be key regulatory factors of metabolism (8, 54, 217). Besides these factors also extramitochondrial Pi and the supply of hydrogen have been proposed as important regulatory factors of mitochondrial respiration (217). According to the model of Gollnick and Saltin (85) by maintaining lower levels of ADP_f or increases in the ATP/ADP_f ratio, a greater entry of acetyl-CoA from fatty acids into the oxidative pathways would be favored because ADP_f and ATP/ADP_f have a stimulating influence on glycolysis (167). Compared to untrained, trained subjects have a higher mitochondrial content (i.e. higher oxidative capacity) and during exercise at a certain workload less ADP_f will be formed (along with higher ATP/ADP_f and ATP/(ADP_f × Pi) ratios). These changes will directly control energy metabolism by stimulating glycogen phosphorylase, PFK and PDH resulting in an increased glycolytic flux. Although the enzyme activity and mitochondrial density are increased after training, it remains to be determined whether the enzyme activity is the main limitation in lipid oxidation in the exercising muscle cell. An increase in the oxidative capacity should be accompanied by an quantitatively similar increase in the supply of fatty acids to the mitochondria (236). Evidence to support this view is the observation that runners with similar rates of fat oxidation during a 60 min run at 70% VO₂max, displayed considerable differences in carnitine palmitoyl transferase I and succinyl dehydrogenase activity (44).

2.9.3 Malonyl-CoA and fat utilization after training

Malonyl-CoA may be an important regulator of fatty acid oxidation in skeletal muscle during exercise (247, 248). Decreased malonyl-CoA levels during exercise may allow more long chain fatty acids to be transported into the mitochondrial matrix, since CAT I is less inhibited. The decreased muscle malonyl-CoA concentrations during exercise parallel a decreased carbohydrate flux. Recently evidence was provided that carbohydrate flux directly regulated fatty acid oxidation during exercise at 50% VO₂max (45, 205). It is therefore tempting to speculate that training may result in a greater fall in muscle malonyl-CoA concentration during exercise, thereby relieving inhibition of CAT I, and enhancing fatty acid oxidation. This is an attractive hypothesis since it has been shown that the activity of acetyl CoA carboxylase (ACC), the enzyme that synthesizes malonyl-CoA, is inhibited by epinephrine (250). Since the epinephrine response to exercise is blunted after training, this could lead to decreased malonyl-CoA levels and consequently increased fatty acid oxidation. In addition lower ADP_f concentrations during exercise after training may lead to less activation of glycolysis and less malonyl-CoA formation. This in turn could explain increased fatty acid oxidation in trained

because CAT dependent transport of FFA into the mitochondria is less inhibited. However, these mechanisms are rather speculative and additional research is required to test these challenging hypotheses.

2.9.4 Effect of training on plasma FFA utilization

With FFA uptake and oxidation being dependent on their vascular concentration and from the increased oxidative potential of endurance-trained skeletal muscle (4, 102, 122, 138, 226), it might be expected that an enhanced rate of lipolysis would accompany training. This could be envisaged as a mechanism to provide more FFA to the working muscle to support the increased potential to oxidize FFA. However, lower plasma FFA concentrations after training (47, 121, 153, 251) suggest that the effect of training on plasma fatty acid oxidation during exercise is either a diminished mobilization from adipose tissue or an increased extraction of FFA by the muscle.

A study of Martin et al. (153) using isotopic labeling of palmitate shows that both the rate of appearance (R_a) and the rate of disappearance (R_d) of FFA are diminished as a result of training. Henriksson (107) found similar rates of FFA uptake between a trained and an untrained leg during 50 min moderate-intensity two-legged exercise and Jansson and Kaijser (130) found no effect of training on leg extraction of plasma FFA. So, although fat oxidation is markedly increased it is unlikely that plasma FFA are the main source of fatty acids that explain this increased fat oxidation. The above mentioned studies (107, 121, 130, 153) support the concept that training does not increase extraction of plasma FFA by the muscle.

However, Turcotte et al. (227) reported that, above certain plasma FFA levels, thigh uptake of plasma FFA was limited in untrained compared to trained during the third hour of single-leg knee extension exercise at 60% $\dot{V}O_{2\max}$. Whereas plasma FFA uptake rose linearly with increasing (non-protein bound) plasma FFA concentrations in trained subjects, the uptake followed saturation kinetics (i.e. leveled off) in untrained subjects above plasma concentrations of $\sim 700 \mu\text{mol}\cdot\text{l}^{-1}$. The observation that the uptake of FFA is a saturable process was previously shown in a rat model (226). Training may provoke changes in the transport across the membrane (227). It has been suggested that FABP, FAT and FATP may play an important role the transport across the membrane and inside the cytosol (81, 236). In rats, training increased FABP content of heart muscle, but not in the EDL and soleus (22). However, as discussed by Coggan (39) the saturation kinetics displayed in studies using knee extension exercise as applied by Turcotte et al. (227) cannot be easily extrapolated to whole body exercise since with this knee extension exercise hormonal changes are much smaller compared to whole body exercise. Since the extraction of FFA does not seem to be increased after training during whole body exercise (130, 153), the reduction of plasma FFA oxidation must be due to reduced lipolysis. The most likely explanation for an effect of training on adipose tissue lipolysis is probably a decrease in the sympathoadrenal response to exercise of the same absolute intensity. The level of sympathoadrenal activity, which is a major determinant of adipose tissue lipolysis in humans, is markedly blunted even after a

few weeks of training (251). Winder et al. (251) observed a 55% reduction in the plasma catecholamine concentrations during prolonged exercise (decreased heart rate, may be an indicator of decreased sympathoadrenal activity after training). Endurance training also attenuates the exercise-induced increase in plasma concentrations of other lipolytic hormones such as glucagon and growth hormone (251). In addition, Lavoie et al. (146) showed that after endurance training the inhibiting effect of insulin on lipolysis is increased, which leads to reduced release (Ra) of FFA (146, 153) and reduced plasma FFA concentrations (37, 146, 153, 251). However, although the reduction of the release of FFA after training may in large part be explained by the decreased rate of lipolysis, it cannot be excluded that trained subjects have higher rates of triglyceride-fatty acid cycling.

2.9.5 Effect of training on IMTG utilization

Reduced plasma FFA oxidation despite higher rates of total fat oxidation after training suggest that the additional fat oxidized during exercise has to be derived from other sources than adipose tissue triglycerides, possibly muscle triglycerides (102, 121, 123, 153). Trained skeletal muscles may have increased intramuscular triglyceride concentrations (138) which can serve as a significant energy source during exercise (30, 62, 63, 71, 121, 186, 211). Because lipid droplets are located in close proximity to the mitochondria (115) the transit time of FFA from the TG in an intramuscular lipid droplet to the outer mitochondrial membrane will be very short. So, also from a teleological point of view, the increased intramuscular triglyceride stores would have a practical advantage. The observation that training increases the number of lipid droplets (116) is in line with the increased intramuscular triglyceride oxidation after training (102, 121, 123, 153).

2.9.6 Effect of training on plasma VLDL-TG utilization

It is tempting to believe that increased activity of LPL (7, 140, 168) and a greater capillary endothelial surface area after training (140) will be responsible for increased hydrolysis of plasma triglycerides after training. Increases in LPL activity have been shown to be linearly related to increases in capillarization of trained muscle (140). Although extraction rates of circulating TG were 8% in untrained and 15% in trained thigh muscles under resting conditions, differences in VLDL-TG utilization during exercise were relatively small and inconsistent (140). Kiens and Lithell (140) therefore suggested that VLDL-triglyceride degradation is probably more important for its potential long-term influence on blood lipid profiles than in contributing to a higher rate of fat oxidation during exercise after training. Moreover, training seems to decrease the availability of circulating VLDL rather than increase it (92) by reducing the production of VLDL by the liver.

In conclusion, the increased mitochondrial density after training and increased oxidative enzymes may partly explain the increased fatty acid oxidation during exercise after training. However, also supply of FFA to the mitochondria may be important. The available evidence suggests that the additional fatty acids oxidized

after training are primarily derived from intramuscular triglycerides and not from adipose tissue derived FFA or circulating triglycerides.

2.10 Nutrition and substrate utilization during exercise

2.10.1 Fat supplementation

Fat supplementation during exercise is usually regarded as undesirable since endogenous fat stores are very large and there may not be a need to supplement additional fat. In addition there is the risk that ingestion of long chain triglycerides leads to an increase of the adipose tissue compartment and increased body weight. An argument which has been suggested in favor of fat supplementation is a possible glycogen sparing effect. Fat supplements can be ingested as either long chain triglycerides or medium chain triglycerides.

2.10.1.1 Ingestion of long chain triglycerides during exercise

Nutritional fats include triglycerides, phospholipids and cholesterol of which triglycerides and possibly phospholipids can contribute to any extent to energy provision during exercise. In contrast to carbohydrates, nutritional fats reach the circulation only slowly since they are potent inhibitors of gastric emptying (120). Furthermore, the digestion in the gut and absorption of fat are also rather slow processes compared to the digestion and absorption of carbohydrates. Bile salts, produced by the liver and lipase secreted by the pancreas are needed to split the long chain triglycerides (LCT) into glycerol and three long chain fatty acids (LCFA) or monoacylglycerol and two fatty acids. The fatty acids move into the intestinal mucosa cells and are reesterified in the cytoplasm to form long chain triglycerides (LCT) (6). These LCTs will be encaptured by a coat of proteins (chylomicrons) to make them water soluble (167). These chylomicrons are then released through the interstitial compartment into the lymphatic system which ultimately drains in the systemic circulation. Exogenous fat enters the systemic circulation much slower than carbohydrates which are absorbed as glucose (or to minor extents as fructose or galactose) and directly enter the main circulation through the portal vein. Long chain dietary fatty acids typically enter the blood 3-4 hours after ingestion (59, 88). Also important is that these long chain fatty acids enter the circulation esterified in chylomicrons and it is generally believed that the rate of breakdown of chylomicron-bound triglycerides by muscle during exercise is relatively low and contributes only minimally to energy expenditure (140). It has been suggested that one of the roles of these triglycerides in chylomicrons is the replenishment of intramuscular triglyceride stores after exercise (173).

In summary, long chain triglyceride ingestion during exercise is not desirable because of the following reasons: 1. It slows gastric emptying 2. The LCT only slowly appear in the systemic circulation and 3. LCT enter the systemic circulation in chylomicrons which are believed to be no major fuel during exercise but may serve to replenish intramuscular TG stores after exercise.

2.10.1.2 Ingestion of medium chain triglycerides

Although fat in general is slowly hydrolyzed and absorbed this also depends on the chain length of the fatty acids. In contrast to the long chain triglycerides, short- and medium-chain triglycerides (MCT) are more rapidly absorbed and they are not transported by chylomicrons but directly enter the systemic circulation through the portal vein. Consequently they will enter the systemic circulation more rapidly. With the introduction of medium chain fatty acids (MCFA) in enteral and parenteral nutrition, their possible role in sports nutrition became subject of investigation. MCFA contain 8 or 10 carbons, whereas LCFA contain 12 or more carbons. Unlike most LCT, MCT are liquid at room temperature. This is in part the result of the small molecular size of MCT. MCT are more polar and therefore better soluble in water. This greater water solubility and smaller molecular size has consequences at all levels of metabolism. MCT are more rapidly digested and absorbed in the intestine than LCT. Furthermore MCFA follow the portal venous system and enter the liver directly while LCFA follow the slow lymphatic system (6, 114, 126). It has been suggested that MCT may be a valuable exogenous energy source during exercise in addition to carbohydrates (132). Also, it has been suggested that MCT ingestion may improve exercise performance by elevating plasma FFA levels and sparing muscle glycogen (231) since it has been observed that increased availability of plasma FFA reduced the rate of muscle glycogen breakdown and delayed the onset of exhaustion (43, 108, 124, 188, 234).

MCT added to CHO drinks did not inhibit gastric emptying (13). In fact the drinks with MCT emptied faster from the stomach than an isocaloric CHO drink. In a subsequent study the oxidation rates of orally ingested MCT were investigated (132). Eight well-trained athletes cycled 4 times 180 min at 50% $\dot{V}O_{2\max}$ (57% $\dot{V}O_{2\max}$). Subjects ingested either CHO, CHO+MCT, or MCT. During the second hour (60 - 120 min period) the amount of MCT oxidized was 72% of the amount ingested with CHO+MCT whereas during the MCT trial only 33% was oxidized. It was concluded that more MCT is oxidized when ingested in combination with CHO. Data confirmed the hypothesis that oral MCT might serve as an energy source in addition to glucose during exercise since the metabolic availability of MCT was high during the last hour of exercise. However, the maximal amount of oral MCT that could be tolerated in the gastrointestinal tract is small (about 30 g) and this limited the contribution of oral MCT to total energy expenditure to values between 3 and 7%. We also investigated MCT oxidation in CHO+MCT supplements under conditions where the reliance on blood substrates is maximal such as in a glycogen depleted state (133). Subjects exercised to exhaustion the evening before the experiment in which MCT oxidation was determined during exercise at 57% $\dot{V}O_{2\max}$. Although total fat oxidation was markedly increased, MCT oxidation increased only marginally. The contribution of MCT to total energy expenditure was still small (about 6-8%) (133).

It has also been suggested that elevating plasma FFA levels by infusing Intralipid and Heparin will spare muscle glycogen and improve exercise performance (43, 234). In theory, MCT ingestion could also be a way to elevate plasma FFA levels.

However, well-trained athletes who ingested CHO+MCT or CHO displayed no significant differences in glycogen breakdown between the trials nor in the respiratory exchange ratio during exercise (135). This may be attributed to the relative small amount of MCT (about 30 grams) that can be tolerated in the gastro-intestinal tract. Van Zeyl et al. (231) suggested that the amount of MCT ingested was too small to exert positive effects on performance. Therefore they gave subjects 86 g of MCT during 2 hours of exercise followed by a 40 km time trial. They observed decreased glycogen breakdown and increased performance when a CHO+MCT drink was ingested. However, recently we performed a similar experiment in which subjects received 85 g of MCT in a MCT or CHO+MCT drink during 2 hours of cycling exercise at 60% VO_2max (134). CHO+MCT did not improve subsequent (14 min) time trial performance compared to CHO or placebo ingestion. However, when only MCT was ingested, performance measured with a validated time trial protocol (131) decreased which was related to gastro-intestinal complaints reported by the subjects (134). It is not known why subjects in the study of van Zeyl (231) did not experience gastro-intestinal complaints after MCT feeding and in our study subjects did (134).

In conclusion: 1. MCT is rapidly emptied from the stomach, absorbed and oxidized, and 2. the oxidation of exogenous MCT was enhanced when co-ingested with CHO. 3. Ingestion of 30g MCT did not affect muscle glycogen breakdown and 4. contributed only 7% to energy expenditure. 5. Ingestion of larger amounts of MCT resulted in gastrointestinal distress. Therefore MCT does not appear to have the positive effects on performance that are often claimed.

2.10.2 Substances and conditions that are claimed to promote fat oxidation

2.10.2.1 Caffeine

Caffeine has been shown to stimulate the mobilization of FFA. Costill et al. (43, 64, 127) showed that caffeine ingested one hour prior to the start of an exercise bout increased plasma FFA concentrations and improved performance. The improvement in performance was explained by the increased availability of FFA, which would lead to a suppression of carbohydrate metabolism and, consequently, to a decreased glycogen utilization. However, the results of studies investigating the ergogenic effects of caffeine and the possible role of FFA are contradictory. Several studies showed increased plasma FFA concentrations but did not see any effect on fat oxidation (141, 219) or performance (61, 182, 219). Other studies did not observe any effect on either fat metabolism or performance (19, 222, 246). Recent studies showed large improvements in performance with high doses of caffeine (93, 94, 174, 212) and these improvements were not always accompanied by changes in plasma FFA or fatty acid oxidation. For example Pasman et al. (174) studied subjects who exercised at 80% VO_2max until exhaustion with caffeine doses ranging from 3 to 9 $\text{mg}\cdot\text{kg}^{-1}$. Time to exhaustion increased by 10-20%, independent of the dose and this effect could not be explained from the increased availability of FFA. It is concluded that, although caffeine stimulates FFA mobilization, the ergogenic

effect of caffeine cannot be explained by the increased availability of FFA (64, 94). For details on caffeine metabolism and the ergogenic effects of caffeine we refer to several recent reviews (34, 50, 207, 218, 229, 244).

2.10.2.2 Carnitine

The importance of carnitine in energy metabolism has led to the belief that L-carnitine supplements may increase fat oxidation, thereby reducing the reliance on endogenous carbohydrate stores which theoretically could improve endurance performance. L-carnitine supplements were introduced as ergogenic aids. L-carnitine came into play when rumors were spread that the Italian soccer team, that became World Champion in 1982 had used L-carnitine supplements. Although few investigations reported effects on heart rate, maximal oxygen uptake and performance (151), the majority of the studies could not find any effect of either orally or intravenously administered L-carnitine on substrate utilization or performance (20, 104, 208, 224, 256). (For review see (237) and (103)).

2.10.3 Fasting

Fasting has been proposed as a way to increase fat utilization, spare muscle glycogen and favorably influence exercise performance. Plasma FFA concentrations are elevated at rest and during exercise after fasting (51, 52, 142, 149, 259). Loy et al. (149) reported decreased time to fatigue by 20-25% following a 24 hour fast which was associated with lower muscle glycogen and blood glucose levels at the onset of exercise.

In summary, fasting increases the availability of lipid substrates resulting in increased oxidation of fatty acids at rest and during exercise. However since the glycogen stores are not maintained, fatigue resistance and exercise performance are impaired.

2.10.4 Effect of a short-term high-fat diet

A change in the carbohydrate-fat content of nutrition can cause a shift in substrate utilization. The ratio of energy providing substrates can be expressed as a food quotient (FQ) in analogy to RQ. A high-fat diet (low FQ), and concomitant low-carbohydrate diet, leads to an increased contribution of fatty acids to total energy expenditure. A high-fat diet may lead to impaired performance as shown already in 1939 by Christensen and Hansen (33). After consumption of a high-carbohydrate diet (high FQ) higher resting RQ values were observed whereas with a high fat diet (low FQ) the RQ's were low. The intake of 100-200 g carbohydrate in the hours preceding exercise results in an increased carbohydrate oxidation at the cost of fat oxidation (3, 33, 163). With the reintroduction of the muscle biopsy technique in the late sixties, it was discovered that a high-fat, low-carbohydrate diet resulted in decreased muscle glycogen levels and this was the main factor causing lack of fatigue resistance during prolonged exercise (15, 16, 118, 119). Plasma FFA concentrations are elevated at rest and increase more rapidly when a low-carbohydrate diet is consumed (40, 152, 154). These changes in plasma FFA concentrations are attribu-

ted to changes in the rate of lipolysis. Not only plasma FFA but also plasma glycerol concentrations are elevated after a low carbohydrate diet (51, 52, 142, 149, 161, 259). Jansson and Kaijser reported that the uptake of FFA by the muscle during 25 min cycling at 65% VO_2max was 82% higher in subjects receiving a low-carbohydrate diet (5%) for 5 days, compared to subjects receiving a high-carbohydrate diet (75%) for 5 days. Plasma FFA contributed 24% and 14% respectively to energy expenditure. Increased FFA concentrations in the blood after a period of carbohydrate restriction will lead to an increased ketogenesis. After a few days of high fat feeding the ketone body production increases 5-fold (70). The arterial concentration of ketone bodies may increase 10-20-fold (70). In the first phase of light to moderate exercise, ketone body concentrations usually decline and after 30-90 min they will increase again (70, 142, 259). However, the observed concentrations under those conditions are still higher after a high fat diet compared to low fat diets. Carbohydrate restricted diets may also lead to an increased breakdown of muscle TG (40, 130, 214).

Recently, Muoio et al. (164) examined the effects of three moderate diets for 7 days in six runners. The percent energy contribution of carbohydrate, fat and protein were 61/24/14, 50/38/12 and 73/15/12 respectively. The authors conclude that the high fat diet (38% fat) increases VO_2max and running time to exhaustion. However, results may be explained by the fact that trials were not randomized. Furthermore the authors suggest that the mechanism by which performance is improved is through increased β -oxidation and fatty acid oxidation. However, no difference in R was observed between the different treatments.

In summary, short term high fat diets increase the availability of lipid substrates but reduce the storage of glycogen. As a result, although fat oxidation may be increased during exercise, fatigue resistance and exercise performance are decreased.

2.10.5 Effects of a long-term high-fat diet

It has been suggested that a 5-7 day alteration in the dietary composition is an insufficient time to induce an adaptive response to the changed diet. Jansson and Kaijser (130) concluded that a high-fat diet over a prolonged period resulted in a decreased utilization of carbohydrates and that this relative shortage of carbohydrates was compensated by an increased contribution of fat to energy metabolism (128, 129). In rats it has been shown that adaptation to a high-fat diet leads to considerable improvements of endurance capacity (161, 206). These adaptations have been attributed to the increased activity of oxidative enzymes (161, 206) and a decreased degradation of liver glycogen during exercise (206). These adaptations increase the capacity to oxidize fatty acids to an extent that not only compensates for but even outweighs the reduced glycogen stores after a high-fat diet. Only few studies looked at the effect of long term high fat diets on fat metabolism and exercise performance.

Phinney et al. (180) investigated exercise performance in obese subjects after 6 weeks of a high fat diet (90 en% fat). Before and after the diet, the intention was to have subjects exercise on 75% VO_2max until exhaustion. Subjects were able to

exercise longer on the high-fat diet as they did on their normal diet while after the diet, fat became the main substrate. Results of this study, however, may have been influenced by the fact that these subjects were not in energy balance and lost 10.6 kg of body weight. So although there were no differences in the absolute $\text{VO}_{2\text{max}}$ before and after the dietary period, there were considerable differences in the relative intensity (after the high-fat diet exercise was performed at 60% $\text{VO}_{2\text{max}}$ while the exercise intensity was 76% $\text{VO}_{2\text{max}}$ before the diet).

The observed improvement in performance may have been an artefact rather than a positive effect of the adaptation period. Therefore Phinney et al. (178, 179) performed a follow-up study in which trained subjects were studied before and after a 4 week high-fat diet (less than 20 g CHO per day). The diet drastically reduced the pre-exercise muscle glycogen concentration (143 ± 10 versus 76 ± 4 mmol glucose·kg⁻¹ wet weight muscle). However, no difference in the average time to exhaustion at 62-64% $\text{VO}_{2\text{max}}$ before and after the diet was found. However, the results are difficult to interpret because of the large variability of the subjects' performance times (times to exhaustion). One subject exercised 57% longer while other subjects showed no improvement or even decreased times to exhaustion. Also, the exercise intensity was relatively low and subjects' reliance on carbohydrates during exercise at 62-64% $\text{VO}_{2\text{max}}$ was low. In such a situation reduced carbohydrate stores may not be limiting. It is possible that at higher exercise intensities performance would have been impaired. The results of the study of Phinney (178, 179) may be supportive of this view since the subjects with the highest R values (higher rates of CHO oxidation) showed a decreased performance whereas the subjects with the lowest R values displayed an increased time to exhaustion.

Nevertheless it is remarkable that performance was not reduced in all subjects even though preexercise muscle glycogen levels were decreased by almost 50% and fat oxidation during exercise was markedly increased. These observations have been attributed to enzymatic adaptations including a 44% increase of acyl-carnitine palmitoyl transferase (CPT) activity, and a 46% decrease of the hexokinase activity (179).

Lambert et al. (145) fed 5 endurance trained cyclists 14 days a somewhat less extreme high-fat diet or high-carbohydrate diet. The high-fat diet contained 67% fat and 7% CHO whereas the high-carbohydrate diet contained 74% CHO and 12% fat. In order to evaluate exercise performance three different exercise tests were performed: a Wingate test (sprint exercise), a cycling time to exhaustion test at a high intensity (90% $\text{VO}_{2\text{max}}$) and an exhaustion test at a moderate intensity (60% $\text{VO}_{2\text{max}}$). Muscle glycogen concentrations were 44% lower after the high-fat diet. No differences were found in sprint performance and time to exhaustion during high intensity exercise. However, time to exhaustion during the moderate intensity exercise test was significantly longer (80 ± 8 min versus 43 ± 7 min). The high-fat diet also resulted in increased fat oxidation rates during exercise. Recently Helge et al. (106) studied 10 young men who ingested a high-CHO diet (65% CHO) and 10 men who ingested a high-fat diet (62% fat) for seven weeks, while endurance training was performed 3-4 times a week. Training increased $\text{VO}_{2\text{max}}$ by 11% and

time to exhaustion at 81% of pre-training VO_2max increased from 35 min to 102 min in the high-CHO group. However, although time to exhaustion also increased in the high-fat group (from 36 min to 65 min), the increase was significantly less compared to the high-CHO group. When a high CHO diet was provided during week 8 in the high-fat group, time to exhaustion was slightly restored but still lower compared to the high-CHO group (77 min vs. 104 min). The high-fat diet increased HAD activity in skeletal muscle by 25% and also the respiratory exchange ratio during exercise was lower in the high-fat group (105). These authors concluded that a fat-rich diet during an endurance training program is detrimental to improvement in endurance (106). This reduced increase in exercise capacity after training is not restored after a short-term high CHO diet.

The data concerning the effects of a long-term high-fat diet on exercise performance are not conclusive. However, studies of Lambert et al. (145) and Phinney et al. (179) used relatively low exercise intensities (60–65% VO_2max) which are far below intensities in competition. So it is not clear how these results translate into practical applications in training and competition. Helge et al. (106) on the other hand clearly displayed a negative effect of a high-fat diet during an endurance training program at an exercise level of 81% VO_2max which is close to the intensities in competition. Furthermore, from a health perspective, eating large amounts of fat has been associated with the development of obesity and cardiovascular disease.

2.11 Summary

In contrast to carbohydrate stores, fat stores are very large in humans and for any sports event practically unlimited. The main stores of fat are located in adipose tissue but also in intramuscular triglycerides (IMTG). During exercise plasma FFA (liberated from adipose tissue) and IMTG are the most important lipid substrates. IMTG stores are especially important during moderate intensity exercise and in well-trained athletes. Total fat oxidation is maximal at moderate exercise intensities. The major rate limiting step of fatty acid oxidation during exercise may be the mobilization of fatty acids from adipose tissue which is dependent on adrenergic stimulation, circulating hormone levels (in particular insulin), adipose tissue blood flow and the albumin/FFA ratio. Not only the release of FFA from adipose tissue is a limiting factor, also intramuscular factors may be involved such as, malonyl-CoA levels, FABP content, transport of fatty acids across membranes involving specific carrier proteins, the glucose-fatty acid cycle and mobilization of FFA from IMTG stores. Endurance training results in a reduced reliance on fat as an energy substrate and this is mainly due to a reduced contribution of plasma FFA. The role of IMTG may even increase with training. Ingestion of long-chain triglycerides (LCT) during exercise may reduce the gastric emptying rate and LCT will appear in the plasma only slowly. Medium-chain triglycerides (MCT) do not have these disadvantages and they are rapidly oxidized.

However, the contribution of MCT to energy expenditure is only small because they can only be ingested in small amounts without causing gastrointestinal distress. So at present, fat supplementation in the hours preceeding to or during exercise (either long chain or medium chain triglycerides) cannot be recommended. High-fat diets and fasting have been suggested to increase FFA availability and spare muscle glycogen resulting in improved performance. Both fasting and short term high-fat diets will decrease muscle glycogen content and reduce fatigue resistance. Chronic high-fat diets may provoke adaptive responses preventing the detrimental effects on exercise performance. However, at present, also from a health perspective, high fat diets should not be recommended to athletes.

2.12 References

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Chapter 3

Gastric emptying of carbohydrate – medium chain triglyceride solutions in rest

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Abstract

This study investigates the effect on gastric emptying (GE) of the addition of medium chain triglycerides (MCT) to carbohydrate (CHO) solutions. Gastric emptying (GE) is the subject of this study because it is the first regulating step in the supply of exogenous energy. The reason to choose specifically MCT as a fat source for the supply of energy is the decelerating effect long chain triglycerides (LCT) have on GE and the relative slow absorption of LCT in the intestine. If a high exogenous energy supply is important, as in long lasting exercise events, a rapid GE is important. It is speculated that MCT doesn't have the same inhibiting effects as LCT. Nine male volunteers participated in 4 GE tests of liquid mixtures of CHO (maltodextrins) and MCT (Dr1: 70% CHO - 30% MCT, Dr2: 80% - 20%, Dr3: 90% - 10%, Dr4: 100% - 0%). GE was measured in rest for 90 min according to the modified double sampling technique. GE rate, expressed as $t_{1/2}$ (SEM), was 23 (2.3), 24 (1.6), 27 (2.2) and 36 (2.9) min, respectively, from drink 1 to drink 4. Statistical analysis showed that all MCT containing drinks emptied faster than the 100% CHO drink. Two mechanisms might explain this phenomena. First, the CHO content and osmolality increases from Dr 1 to Dr 4 (both are regulators of GE). Secondly, MCT might not inhibit GE as common fat does, due to a better water solubility and absorption in the gut.

Table 3.1 Composition of various test drinks

		Drink 1	Drink 2	Drink 3	Drink 4
MCT	g/100 ml	2.40	1.60	0.80	0.00
Maldex 20	g/100 ml	12.22	14.04	15.85	17.66
Fat	Energy%	30	20	10	0
CHO	Energy%	70	80	90	100
Protein	Energy%	0	0	0	0
NaCl	mmol·l ⁻¹	20	20	20	20
Osmolality	mosm·kg ⁻¹	174	198	222	251

Daily protocol

Subjects arrived at the laboratory at 8.00 AM after an overnight fast where a naso-gastric tube (Vygon silicone gastro-duodenal tube, Levin type, Charrier 14) was placed. The stomach was emptied and rinsed until no further residue was obtained and a recovery test was performed for control of the positioning of the tube (16, 22). A bolus of 8 ml·kg⁻¹ body weight (average 585±42 ml) of one of the test meals, containing 15 mg·l⁻¹ phenolred, was administered via the tube, mixed with whatever gastric contents, and an initial sample was taken so the present gastric contents could be accounted for. Samples for the determination of GE were taken at 10 min intervals for 90 min according to George's double sampling technique (19). All GE tests in this study are performed in resting subjects, but as there is no difference in GE between rest and exercise up to 78%VO₂max (7, 16, 32,) the results can be extrapolated to exercise settings. Successive tests were at least 48 hours apart.

Calculations and statistics

As a measure of GE rate half emptying time ($t_{1/2}$) (11) was calculated from a simple exponential curve fit ($f=2-(t/t_{1/2})$) using Biomedical Computer Programs, p-series (BMDPar) (12).

For comparison of results the non-parametric Friedman test in combination with the Fischer PLSD test was used.

Results

Table 3.2a, b, c and d show the mean volumetric data for total stomach contents, test meal volume left and secretion volume, including SEM values, for all time points and all drinks.

Table 3.3 represents the $t_{1/2}$ values for the individual subjects as well as the average and SD. Figure 1 depicts the average GE curves as the percentage of the original bolus left in the stomach against time. Friedman's non-parametric test of the data showed a highly significant difference in emptying rate (p -value = 0.0056). Accord-

Figure 3.1 Average gastric emptying curves presented as % of original bolus left in the stomach.

ding to the Fisher PLSD test this difference exists at 95 % level between all MCT containing drinks as compared to the pure CHO drink.

Discussion

The results from the GE tests show that the drinks containing MCT (dr 1, 2 and 3) all empty from the stomach at a faster rate than the drink which contains only CHO (dr 4). Looking at these data, one might conclude that MCT fat has an accelerating effect on GE, a conclusion which is in contradiction with many existing opinions on the effect of fat on GE. However, Hunt (24) examined the influence of fatty acids with chain lengths from C2 to C18 on the rate of GE. Hunt's conclusion was that fatty acids with a chain length starting from C12 and higher have an inhibiting effect. The most effective inhibitors are those with 14 C atoms, while C12, C16 and C18 fatty acids are also potent in their inhibition. A chain length of C10 or less influenced GE only marginally. Moberg (29) and Houghton (23) came basically to the same conclusion. They found that liquid meals containing fat (Moberg, Intralipid®, average chain length of carbonic group C18; Houghton, Sunflower margarine, 90% chain length C18) emptied significantly slower from the stomach than those without fat. The major difference in these studies compared to ours is that in chain length of the esterified fatty acids. More than 90 % of the fatty acids of the MCT used here have a chain length of C8 and C10 and exert almost no effect on GE (24). The reason for this is probably the different way in which the gut handles fatty acids with different chain length. C8 and C10 fatty acids are moderately polar and therefore water soluble, diffuse comparatively easy trough the unstirred water layer and are mainly absorbed into the portal vein.

Gastric emptying of CHO+MCT

Table 3.2 Mean total gastric volume, test drink left and gastric secretion including standard error of the mean.

Drink 1 (70%CHO + 30%MCT)				Drink 2 (80%CHO + 20%MCT)			
Time (min)	Volume (ml)			Time (min)	Volume (ml)		
	Stomach	Test drink	Secretion		Stomach	Test drink	Secretion
0	643 (31)	585 (14)	58 (28)	0	666 (17)	585 (14)	81 (8)
10	488 (27)	397 (27)	92 (7)	10	453 (29)	375 (29)	78 (8)
20	416 (30)	310 (24)	105 (9)	20	431 (19)	333 (21)	98 (8)
30	382 (28)	264(26)	119 (12)	30	398 (32)	281 (22)	117 (17)
40	293 (28)	190 (25)	102 (9)	40	335 (43)	217 (27)	118 (21)
50	249 (27)	146 (24)	103 (10)	50	244 (19)	142 (12)	102 (13)
60	184 (23)	95 (20)	89 (14)	60	195 (18)	103 (9)	93 (14)
70	159 (30)	71 (20)	88 (19)	70	146 (23)	64 (11)	81 (14)
80	78 (17)	26 (8)	52 (11)	80	97 (19)	35 (9)	62 (11)
90	66 (18)	15 (6)	51 (14)	90	58 (14)	15 (4)	43 (10)

Drink 3 (90%CHO + 10%MCT)				Drink 4 (100%CHO + 0%MCT)			
Time (min)	Volume (ml)			Time (min)	Volume (ml)		
	Stomach	Test drink	Secretion		Stomach	Test drink	Secretion
0	650 (21)	585 (14)	65 (10)	0	642 (18)	585 (14)	57 (10)
10	464 (21)	395 (28)	68 (6)	10	445 (23)	403 (17)	42 (9)
20	454 (28)	365 (29)	90 (11)	20	427 (21)	370 (16)	57 (8)
30	388 (40)	292 (33)	96 (14)	30	412 (16)	334 (14)	78 (6)
40	338 (20)	236 (23)	102 (9)	40	381 (26)	292 (23)	89 (6)
50	293 (23)	190 (20)	103 (12)	50	315 (28)	231 (22)	84 (9)
60	231 (24)	140 (19)	91 (10)	60	269 (33)	191 (25)	78 (10)
70	174 (26)	95 (19)	80 (10)	70	227 (34)	153 (25)	74 (11)
80	129 (20)	60 (14)	69 (8)	80	193 (32)	121 (25)	72 (8)
90	69 (9)	21 (6)	48 (5)	90	124 (22)	67 (16)	56 (8)

The longer chains are apolar, lipolytic and have to pass through the lymph system (2). But, if MCT doesn't delay GE, why then do all the CHO-MCT containing drinks (dr 1, 2 and 3) empty faster than the isocaloric CHO drink (dr 4) and why is there no significant difference between the MCT drinks? Looking at Figure 3.1 and Table 3.2 one can see that there is a tendency for a faster GE if the amount of MCT increases. The reason for this may be twofold. First, CHO concentrations above 8% progressively slow GE (28, 32). The more the CHO concentration rises, the stronger the inhibiting effect will become. Secondly, with increasing CHO concentration, also the osmolality of the drinks increases (Table 3.1). Osmolality also has a restraining effect on GE (24, 29), although this effect should not be overestimated

Table 3.3 Rate of gastric emptying of various test drinks expressed as $t_{1/2}$ in minutes.

Subject	$t_{1/2}$ (min)			
	Drink 1	Drink 2	Drink 3	Drink 4
1	11.3	21.9	24.5	29.0
2	22.9	27.3	35.1	26.4
3	25.5	24.9	30.3	25.6
4	25.7	25.1	36.8	49.7
5	25.0	29.8	19.9	45.8
6	19.7	20.2	20.0	34.9
7	22.2	31.0	30.1	38.8
8	19.7	19.4	19.9	30.1
9	37.0	16.3	30.5	42.3
Average	23.2	24.0	27.5	35.8
Stdev	6.8	4.9	6.6	8.8
SEM	2.3	1.6	2.2	2.9

in this study. Inhibition occurs mostly from hypertonic solutions, not from hypotonic solutions as used in this experiment (24, 29).

The addition of progressively increasing amounts of MCT to a CHO drink, while maintaining isocaloricity, decreases both CHO concentration and osmolality. The overall conclusion of this survey is that the addition of MCT to CHO containing beverages will not negatively influence the rate of GE. On the contrary, GE of isocaloric solutions of a mixture of MCT and CHO is faster than CHO alone. This opens the possibility to combine MCT with CHO in a liquid form to provide additional energy rich substrates for exercising (ultra-) endurance athletes. Testing the metabolic effects of such a mixture is needed to verify the practical value.

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Chapter 4

Metabolic availability of oral medium chain triglycerides co-ingested with carbohydrates during prolonged exercise

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Abstract

The present study examined the metabolic response to medium chain triglycerides (MCT) ingestion with or without carbohydrates (CHO). Eight well-trained athletes cycled 4 times 180 min at 50% W_{max} (57% VO_{2max}). Subjects drank a bolus of 4 ml·kg⁻¹ at the start and 2 ml·kg⁻¹ every 20 min during exercise of either a 15% (214 g) CHO solution (CHO), an equicaloric 149 g CHO + 29 g MCT suspension (CHO+MCT), 214 g CHO + 29 g MCT (HCHO+MCT) or 29 g MCT (MCT). Exogenous MCT oxidation was measured by adding a [1,1,1-¹³C]- trioctanoate tracer to the MCT oil. ¹³CO₂-enrichment of breath samples was measured every 15 min. During the second hour (60 - 120 min period) the amount of MCT oxidized was 72% of the amount ingested with CHO+MCT whereas during the MCT trial only 33% was oxidized. The rate of MCT oxidation increased more rapidly during both CHO+MCT trials compared to MCT, yet in all three cases the oxidation rate stabilized at 0.12 g·min⁻¹ during 120-180 min of exercise. It is concluded that more MCT is oxidized when ingested in combination with CHO. Data do confirm the hypothesis that oral MCT might serve as an energy source in addition to glucose during exercise since the metabolic availability of MCT was high during the last hour of exercise with oxidation rates being about 70% of the ingestion rate. However, the maximal amount of oral MCT that can be tolerated in the gastrointestinal

tract is small (about 30 g) and limits the contribution of oral MCT to total energy expenditure to values between 3 and 7%.

Introduction

Although ingested carbohydrates can improve endurance performance, their oxidation is limited. Studies of Wagenmakers et al. (20) and of Rehrer et al. (17) showed that a two-fold increase in the amount of carbohydrates ingested, only slightly increased the rate of oxidation during exercise at 70% $\text{VO}_{2\text{max}}$. To provide more exogenous energy to the athlete, an additional energy source may be supplied. Medium chain triglycerides (MCT) with a chain length varying between 6 and 10 carbon atoms could be such a source. MCT are derived from coconut oil and are frequently used in enteral and parenteral nutrition to treat patients with fat malabsorption disorders. MCT are known to be rapidly hydrolyzed and absorbed (2). Unlike long-chain triglyceride (LCT; $>\text{C}_{12}$) which are transported in chylomicrons through the lymphatic system, MCT is converted into medium-chain fatty acids (MCFA) that directly enter the blood through the portal system. In addition MCFA can cross the mitochondrial membrane of liver and muscle independently of the acylcarnitine transfer system (4). Massicotte et al. (13) reported that the energetic contribution ($\text{kcal}\cdot\text{min}^{-1}$) of exogenous MCT was only slightly lower than that of glucose during prolonged exercise of moderate intensity. That is during the 60-120 min period of exercise, exogenous CHO was oxidized at $1.6\text{ kcal}\cdot\text{min}^{-1}$ ($0.4\text{ g}\cdot\text{min}^{-1}$) compared to $1.4\text{ kcal}\cdot\text{min}^{-1}$ ($0.16\text{ g}\cdot\text{min}^{-1}$) for MCT. Since MCT seem to be a readily available energy source for the working muscle, they could possibly enhance fat metabolism and serve as an additional substrate during prolonged endurance exercise. Therefore, addition of MCT to carbohydrate solutions, could be a useful means to further improve performance. In a preliminary study (3), MCT added to a carbohydrate solution were observed to result in enhanced delivery of energy (kcal) into the intestines, compared to an equicaloric CHO solution. Therefore the present study examined the metabolic response to CHO, CHO+MCT and MCT supplements in a randomized double blind cross-over design. To measure the oxidation rate of the MCT during exercise, a $[1,1,1\text{-}^{13}\text{C}]$ -trioctanoate tracer was incorporated in the MCT containing drinks.

Methods

Subjects

Eight male trained triathletes or cyclists aged 28.8 ± 3.8 years, weight 72.5 ± 1.7 kg, height 181.3 ± 2.3 cm, maximal work rate (W_{max}) $5.25 \pm 0.22\text{ W}\cdot\text{kg}^{-1}$, maximal oxygen uptake $64.7 \pm 2.2\text{ ml}\cdot\text{kg}^{-1}$ and a maximal heart rate of 191 ± 4.6 BPM, 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.

Pre-trials

Subjects $W_{\max}$ was attained on an electronically braked ergometer (Lode Excalibur, Groningen, The Netherlands) during an incremental exhaustive exercise test (12) one week before the first experimental trial. The results of this initial test were used to determine the 50% $W_{\max}$ which was later used in the experimental trials.

Experimental trials

Each subject performed four trials, each separated by at least seven days. A trial consisted of 180 min cycling at 50% $W_{\max}$ (57% $\dot{V}O_{2\max}$). Drinks were given in a randomized order and double blind. Drinks were all vanilla flavoured (Sandoz Nutrition, Berne, Switzerland). Subjects rested from training and were instructed to consume a comparable diet, for 3 days prior to each trial. In addition, 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 AM after an overnight fast and a standardized breakfast of two crackers with cheese was provided (14 g CHO, 4 g fat, 6 g protein). A Teflon catheter (Baxter Quick Cath Dupont, Ireland) was inserted into an antecubital vein and at 8.30 a resting blood sample was drawn. Resting breath gases were collected for the measurement of oxygen consumption (Oxycon β , Mijnhard, The Netherlands) and vacutainer tubes were filled directly from the mixing chamber in duplicate to determine the $^{13}\text{C}/^{12}\text{C}$ ratio in expired CO_2 . At 8.50 AM subjects started cycling for 10 min at 100 W as a warm-up. At 9.00 AM the exercise intensity was increased to 50% $W_{\max}$ for 180 min. Blood samples were drawn at 30 min intervals until the end of exercise. Expiratory gases were collected every 15 minutes. Two different subjects were tested on the same day, starting the protocol 10 minutes apart.

Drinks

The composition of the four test drinks as well as the amounts administered are listed in Table 4.1.

Subjects received a bolus of $4 \text{ ml} \cdot \text{kg}^{-1}$ at the start and $2 \text{ ml} \cdot \text{kg}^{-1}$ every 20 min during exercise of either a 15% CHO solution (CHO), an equicaloric CHO-MCT suspension (CHO+MCT) (70 energy% as CHO and 30 energy% as MCT), and a drink containing pure MCT in water (MCT). In order to study the effect of MCT added to the carbohydrate (CHO) in stead of the equicaloric CHO+MCT suspension, a fourth trial (HCHO+MCT) was also included in which a suspension was ingested containing the same amount of CHO (149 g) as in the CHO-trial and the same amount of MCT (29 g) as in the CHO+MCT and MCT trials. Therefore, it could be investigated whether differences between the CHO+MCT trial compared to the CHO trial are due to the MCT or due to the differential amount of CHO.

Exogenous MCT oxidation during exercise

Table 4.1 Composition of test drinks

	CHO	CHO+MCT	HCHO+MCT	MCT
<i>Drinks</i>				
CHO energy, %	100	70	77	0
MCT energy, %	0	30	23	100
Energy, kcal.l ⁻¹	626	627	806	180
CHO, g.l ⁻¹	156.2	108.6	156.2	0
MCT, g.l ⁻¹	0	21.4	21.4	21.4
NaCl, mmol.l ⁻¹	20	20	20	20
<i>Supplementation</i>				
<i>Total amounts</i>				
Water, ml	1375	1375	1375	1375
CHO, g	214	149	214	0
MCT, g	0	29	29	29
Energy, kcal	861	862	1108	248
<i>Average ingestion rates</i>				
Water, ml.min ⁻¹	7.6	7.6	7.6	7.6
CHO, g.min ⁻¹	1.19	0.83	1.19	0
MCT, g.min ⁻¹	0	0.16	0.16	0.16
Energy, kcal.min ⁻¹	4.78	4.78	4.48	1.37

CHO, carbohydrate; MCT, medium-chain triglyceride; HCHO, high-CHO. Difference between the CHO+MCT trial and the HCHO+MCT trial is the amount of CHO.

For reasons of clarity, data of the HCHO+MCT trial are presented and discussed, but not included in the graphs.

The drinks consisted of tapioca derived long chain glucose polymers of low ¹³C natural abundance (-26.12 δ per mil vs PDB, 0.0109437 ¹³C/¹²C ratio) (Sandoz Nutrition Ltd, Berne, Switzerland), and or MCT containing fatty acids with a chain length of C8 (Estasan GT8-99, Unichema, Barcelona, Spain). To all drinks 20 mmol.l⁻¹ of NaCl was added. [1,1,1-¹³C]-trioctanoate (99%), purchased from Cambridge Isotope Laboratories (Woburn, MA, USA), was incorporated in the unlabeled MCT suspension and then mixed with the CHO to form a stable suspension. Meal temperature was kept constant at 20°C. The ¹³C-enrichment of the MCT after adding the tracer was 160.61 δ per mil vs PDB (0.013042 ¹³C/¹²C ratio). The CHO trial in which carbohydrates were ingested with a similar ¹³C-enrichment as the enrichment of expired air of the subjects (-26.12 versus -26.34 δ per mil vs PDB) was used to correct for possible exercise induced background shifts.

Analysis

Blood (10 ml) was collected into EDTA containing 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 (14), free fatty acids (FFA) (Wako FFA-C test kit, Wako Chemicals, Neuss, Germany), and glycerol (Sigma, GPO-trinder 337), which were performed with the COBAS BIO analyzer (Roche diagnostics). Total energy expenditure and oxidation rates of total fat, total carbohydrates and exogenous MCT were calculated from indirect calorimetry (RQ , VO_2) and stable isotope measurements ($^{13}CO_2$ / $^{12}CO_2$) (GC continuous flow IRMS, Finnigan MAT 252). Enrichments of the substrates (in drinks) were measured with an elemental analyzer - IRMS combination (Carlo-Erba on line connected to the Finnigan MAT 252).

Calculations

From VCO_2 and VO_2 carbohydrate and fat oxidation rates were calculated using the non-protein respiratory quotient (16).

$$\text{carbohydrate oxidation} = 4.585 VCO_2 - 3.226 VO_2$$

$$\text{fat oxidation} = 1.695 VO_2 - 1.701 VCO_2$$

The isotopic enrichment of expired air was expressed as the delta per mil difference between the $^{13}C/^{12}C$ ratio of the sample and a known laboratory reference standard according to the formula:

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

($^{13}C / ^{12}C$ standard = 0.112372) The $\delta^{13}C$ was then related to the international standard Pee Dee Belemnite (PDB). The amount of medium chain triglycerides oxidized was calculated according to the formula:

$$\text{exogenous glucose oxidation} = VCO_2 \cdot \left(\frac{\delta \text{ Exp} - \delta \text{ ref}}{\delta \text{ Ing} - \delta \text{ ref}} \right) \left(\frac{1}{k} \right)$$

In which $\delta \text{ ref}$ is the ^{13}C enrichment of expired air in the reference test CHO (background), $\delta \text{ exp}$ is the ^{13}C enrichment of expired air during exercise with MCT ingestion at different time points, $\delta \text{ ing}$ 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 via oxidation of 1 gram trioctanoate ($k = 1.2369 \text{ L } CO_2 \text{ per gram MCT}$).

In the present study and in previous studies from our laboratory (17, 19, 20, 21) it was shown that instructing the subjects not to eat any products of high natural ^{13}C abundance during the experimental period was effective in reducing the background shift (change in $^{13}CO_2$) from endogenous substrate stores in European subjects (21). However, although the background shift was small in the present study, background correction was made by using the ^{13}C enrichment of breath samples in the CHO-trial.

Another methodological consideration when using $^{13}\text{CO}_2$ in expired air to calculate exogenous substrate oxidation is the trapping of exogenous $^{13}\text{CO}_2$ in the bicarbonate pool, a very large and slowly exchanging pool in which an amount of CO_2 arising from decarboxylation of energy substrates is temporarily trapped each minute (18). However, during exercise, the CO_2 production increases several fold so that a physiological steady state situation will occur and $^{13}\text{CO}_2$ in expired air will be equilibrated with the $^{13}\text{CO}_2/\text{H}^{13}\text{CO}_3^-$ pool. The dilution of $^{13}\text{CO}_2$ becomes negligible and recovery of $^{13}\text{CO}_2$ approaches 100% after 60 min of exercise (15). Therefore, in the present study, data from the initial 60 min were not used for the calculation of exogenous MCT oxidation.

Statistics

Analysis of variance for repeated measures was used to compare differences in substrate utilization and in blood related parameters among the four endurance rides. A Scheffé post hoc test was used in the event of a significant ($p < 0.05$) F-ratio.

Results

Oxygen consumption (Table 4.2) during exercise was relatively constant as the exercise intensity of the subjects was maintained at close to 57% $\text{VO}_{2\text{max}}$ and was not significantly different among the trials. Respiratory exchange ratio was also not significantly different among the trials. Background ^{13}C enrichment of the resting breath samples was -26.34 ± 0.43 δ per mil vs PDB (0.01153319 $^{13}\text{C}/^{12}\text{C}$ ratio). Changes in isotopic composition of expired CO_2 in response to exercise are shown in Table 4.3. With ingestion of CHO (of low ^{13}C natural abundance) there was a slight (although statistically not significant) increase of ^{13}C in the expired air. In the MCT containing trials the rise in ^{13}C was significant, reaching a δ per mil difference of 9–11 towards the end of 180 min exercise (compared to resting breath sample) (Table 4.3).

Table 4.2 O_2 consumption and respiratory exchange ratio

Time (min)		30	60	90	120	150	180
VO_2 ($\text{l}\cdot\text{min}^{-1}$)	CHO	2.76 $\pm$ 0.10	2.76 $\pm$ 0.07	2.76 $\pm$ 0.08	2.80 $\pm$ 0.07	2.84 $\pm$ 0.08	2.86 $\pm$ 0.08
	CHO+MCT	2.79 $\pm$ 0.10	2.80 $\pm$ 0.08	2.85 $\pm$ 0.10	2.93 $\pm$ 0.12	2.90 $\pm$ 0.10	2.90 $\pm$ 0.09
	HCHO+MCT	2.82 $\pm$ 0.07	2.81 $\pm$ 0.07	2.86 $\pm$ 0.08	2.87 $\pm$ 0.07	2.88 $\pm$ 0.09	2.93 $\pm$ 0.08
	MCT	2.71 $\pm$ 0.09	2.75 $\pm$ 0.09	2.76 $\pm$ 0.09	2.85 $\pm$ 0.09	2.86 $\pm$ 0.10	2.91 $\pm$ 0.08
RER	CHO	0.88 $\pm$ 0.01	0.86 $\pm$ 0.01	0.84 $\pm$ 0.01	0.86 $\pm$ 0.01	0.85 $\pm$ 0.02	0.82 $\pm$ 0.02
	CHO+MCT	0.87 $\pm$ 0.01	0.86 $\pm$ 0.01	0.84 $\pm$ 0.01	2.85 $\pm$ 0.01	2.85 $\pm$ 0.01	2.83 $\pm$ 0.01
	HCHO+MCT	0.87 $\pm$ 0.01	0.86 $\pm$ 0.01	0.84 $\pm$ 0.01	2.84 $\pm$ 0.01	2.83 $\pm$ 0.01	2.83 $\pm$ 0.01
	MCT	0.87 $\pm$ 0.01	0.85 $\pm$ 0.02	0.84 $\pm$ 0.01	0.82 $\pm$ 0.01	0.81 $\pm$ 0.01	0.81 $\pm$ 0.01

Values are means $\pm$ (SEM) ($n=8$). No significant differences were observed.

During the first hour, there was a sharp rise in $^{13}\text{CO}_2$ appearance in the expired air with CHO+MCT compared to the MCT trial (Table 4.3) and consequently in the estimated MCT oxidation over that period.

Table 4.3 ^{13}C enrichment in expired CO_2

Time (min)	CHO	CHO+MCT	HCHO+MCT	MCT
0	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
15	0.21 $\pm$ 0.12	3.46 $\pm$ 0.49	2.87 $\pm$ 0.48	0.79 $\pm$ 0.11
30	0.51 $\pm$ 0.13	6.85 $\pm$ 0.40	6.61 $\pm$ 0.57	1.83 $\pm$ 0.25
45	0.66 $\pm$ 0.11	8.26 $\pm$ 0.25	7.32 $\pm$ 0.70	2.33 $\pm$ 0.47
60	0.68 $\pm$ 0.09	9.31 $\pm$ 0.54	8.77 $\pm$ 0.77	2.99 $\pm$ 0.55
75	0.77 $\pm$ 0.13	9.52 $\pm$ 0.70	8.73 $\pm$ 0.65	4.21 $\pm$ 0.94
90	0.71 $\pm$ 0.10	10.57 $\pm$ 0.99	9.38 $\pm$ 0.98	5.21 $\pm$ 0.85
105	0.74 $\pm$ 0.11	10.53 $\pm$ 0.83	9.34 $\pm$ 0.97	5.75 $\pm$ 0.69
120	0.72 $\pm$ 0.09	10.34 $\pm$ 0.86	9.36 $\pm$ 0.89	7.09 $\pm$ 0.60
135	0.70 $\pm$ 0.11	9.53 $\pm$ 0.68	8.67 $\pm$ 0.99	9.53 $\pm$ 1.07
150	0.67 $\pm$ 0.10	9.22 $\pm$ 0.57	8.72 $\pm$ 1.01	10.46 $\pm$ 1.00
165	0.58 $\pm$ 0.10	9.40 $\pm$ 0.78	9.31 $\pm$ 0.90	9.97 $\pm$ 0.78
180	0.58 $\pm$ 0.08	9.60 $\pm$ 0.84	9.33 $\pm$ 0.95	10.81 $\pm$ 0.99

Values are means $\pm$ SEM in δ per mil vs Pee Dee Belemnite; n=8 subjects.

Exogenous trioctanoate oxidation showed an exponential rise during the first 60 min of CHO+MCT and then plateaued at $0.12 \pm 0.02 \text{ g}\cdot\text{min}^{-1}$ during the 120-180 min period. In the MCT trial MCT oxidation showed a linear increase (Figure 4.1).

Figure 4.1 Exogenous MCT oxidation rates over 180 min of exercise (n=8). The dotted line is the calculated average ingestion rate over the 60-180 min period. The area under this dotted line represents the total amount of MCT ingested. The area under the curves represents the amount of MCT oxidized during the 180 min period. The curve of HCHO+MCT showed the same pattern as CHO+MCT.

Peak oxidation rates were reached already after 90-120 min exercise in the CHO+MCT trial ($0.12 \text{ g}\cdot\text{min}^{-1}$) and at 150-180 min in the MCT trial ($0.12 \text{ g}\cdot\text{min}^{-1}$). During the HCHO+MCT trial, MCT oxidation rates reached peak oxidation rates of $0.11 \text{ g}\cdot\text{min}^{-1}$. In addition the pattern of exogenous MCT oxidation over time during the HCHO+MCT trial was similar to the response observed in the CHO-MCT trial: a sharp rise during the first hour and a plateau thereafter.

In Table 4.4 the amount of CHO and (exogenous and endogenous) fat oxidation during the exercise period are presented in grams. During the second hour (60-120

Figure 4.2 Relative contributions of carbohydrates (CHO), endogenous fat (endo) and exogenous medium chain triglycerides (MCT) to energy expenditure during the 60-120 min (A) and 120-180 min (B) time periods for the CHO, CHO+MCT and the MCT trials.

Table 4.4 Endogenous (endo) and exogenous (MCT) substrate oxidation during exercise.

time interval	substrate	CHO (g)	CHO+MCT (g)	HCHO+MCT (g)	MCT (g)
60-120	CHO	110.2 ± 8.0	110.1 ± 8.0	108.1 ± 7.3	99.2 ± 11.2
	Fat	41.9 ± 3.2	44.4 ± 3.5	44.5 ± 2.9	47.1 ± 4.8
	MCT	0.0 ± 0.0	$6.6 \pm 0.5^*$	$6.2 \pm 0.5^*$	3.1 ± 0.5
	endo	41.9 ± 3.2	37.8 ± 3.8	38.3 ± 2.9	44.0 ± 5.0
120-180	CHO	$111.2 \pm 9.2^*$	$111.6 \pm 7.5^*$	$102.8 \pm 7.2^*$	83.9 ± 9.4
	Fat	$43.4 \pm 4.1^*$	$45.6 \pm 2.9^*$	$48.3 \pm 3.6^*$	55.1 ± 4.1
	MCT	0.0 ± 0.0	6.4 ± 0.4	6.1 ± 0.5	6.4 ± 0.6
	endo	$43.4 \pm 4.1^*$	$39.2 \pm 2.9^*$	$42.2 \pm 3.8^*$	48.7 ± 4.4

Values are means $\pm$ SEM in g ($n=8$). * Significantly different from MCT ($p<0.05$). No differences were observed between CHO, CHO+MCT and HCHO+MCT (except for MCT oxidation in CHO trial).

min) 6.6 ± 0.5 g of exogenous MCT was oxidized in the CHO+MCT trial, 6.2 ± 0.6 g in the HCHO+MCT trial, and 3.1 ± 0.5 g in the MCT trial. This represented 76%, 71% and 33% respectively of the amount of MCT ingested. MCT oxidation during the second hour was significantly higher in the CHO+MCT and HCHO+MCT trial compared to the MCT trial ($p < 0.01$). During the last hour (120–180 min) similar amounts of MCT were oxidized in all three trials (6.4 ± 0.4 g, 6.1 ± 0.6 g and 6.4 ± 0.6 g for CHO+MCT, HCHO+MCT and MCT respectively). This implies that during the last hour in all three cases 69–71% of the amount ingested was oxidized.

During the second hour, exogenous MCT contributed 6.8% to total energy expenditure with CHO+MCT, 6.3% with HCHO+MCT and 3.2% with MCT (Figure 4.2). During the final hour the contribution were 6.3% with HCHO+MCT and 6.4% in the CHO+MCT and MCT trials.

No differences were observed in energy expenditure between the four trials. Endogenous fat utilization was significantly reduced during the CHO containing trials ($p < 0.0002$) compared to the MCT trial, while total carbohydrate utilization during the final hour was significantly higher in the CHO treatments ($p < 0.0001$).

Plasma FFA concentrations rose during exercise in all trials (Figure 4.3). This increase was most pronounced in the MCT trial in which no carbohydrate was ingested. At 30 min FFA concentrations were significantly elevated in the CHO+MCT and HCHO+MCT trial compared to the other trials ($p < 0.0001$). After 120 min FFA concentration was significantly higher in the MCT trial compared to the two other trials, reaching a level of $1.1 \text{ mmol} \cdot \text{l}^{-1}$ at the end of the exercise bout ($p < 0.01$). Toward the end of exercise glycerol concentration, were somewhat higher in the MCT trial; the difference between the treatments, however, was not statistically significant (Figure 4.3).

There was a sharp increase in β -hydroxybutyrate concentration in the CHO+MCT and HCHO+MCT trials to about $500 \text{ } \mu\text{mol} \cdot \text{l}^{-1}$ during the first 30 min; thereafter the concentration remained stable (Figure 4.3). In the CHO trial no changes in β -hydroxybutyrate were observed throughout exercise. In the MCT trial β -hydroxybutyrate rose gradually throughout exercise to a final concentration of about $800 \text{ } \mu\text{mol} \cdot \text{l}^{-1}$. With carbohydrate supplementation, euglycemia was maintained whereas in the MCT trial plasma glucose gradually declined, reaching low levels (i.e. $< 3.5 \text{ mmol} \cdot \text{l}^{-1}$) at 180 min (Figure 4.4). Plasma lactate concentrations were low during exercise (below $1.5 \text{ mmol} \cdot \text{l}^{-1}$) in all trials and there were no significant differences among the four trials (Figure 4.4), except for a small but significant increase of lactate concentration in the MCT trial ($p < 0.02$) at 180 min.

Figure 4.3 Plasma FFA, glycerol and β -hydroxybutyrate concentrations during exercise for the CHO, CHO+MCT and the MCT trials. * Significantly different from MCT ($p < 0.05$). # Significantly different from CHO+MCT ($p < 0.05$). @ Significantly different from CHO ($p < 0.05$).

Figure 4.4 Plasma glucose and lactate concentrations during exercise for the CHO ●, CHO+MCT ● and the MCT ○ trials. * Significantly different from MCT ($p < 0.05$). # Significantly different from CHO+MCT ($p < 0.05$). @ Significantly different from CHO ($p < 0.05$).

Discussion

Exogenous MCT oxidation

The most remarkable finding of this study is the more rapid increase in the oxidation rate of exogenous MCT with coingestion of carbohydrates (CHO+MCT and HCHO+MCT versus MCT). During the second hour (60 - 120 min period) the amount of MCT oxidized was 71-76% of the amount ingested when a CHO+MCT suspension was ingested, representing 6.3-6.8 % of total energy expenditure. During the MCT trial only 33% was oxidized contributing 3.2% to energy expenditure. The explanation for this phenomenon must be sought in one of following processes: gastric emptying, intestinal absorption, extraction and release of FFA by the liver or the FFA uptake and oxidation by the muscle. The sharp rise in both plasma FFA and β -hydroxybutyrate concentration with CHO+MCT or HCHO+MCT but not with MCT suggests that the CHO is allowing the medium chain fatty acids (MCFA) to get into the systemic circulation more rapidly. This implies that gastric emptying and intestinal absorption would be the most likely factors responsible for the difference in MCT oxidation with (-)CHO+MCT and MCT.

A possible explanation could be that CHO+MCT suspensions empty faster from the stomach than MCT. In a previous study from our laboratory it has been shown that gastric emptying of carbohydrate - medium chain triglyceride suspensions was very rapid at rest (3). Gastric emptying was increased by increasing amounts of MCT (up to 30 energy%) and decreasing amounts of CHO in equicaloric CHO+MCT suspensions. However, in this study no information was obtained regarding gastric emptying of MCT in the absence of CHO. Previously, it has been reported that MCT with a chain-length of C8-C10 exert almost no effect on gastric emptying (10).

Also, it cannot be excluded that MCFA are incorporated into triglycerides or other lipid pools and that this incorporation may be influenced by glucose ingestion.

The oxidation rates of MCT observed in the present study are in line with previous findings of Décombaz et al. (8) and of Massicotte et al. (13). Décombaz and colleagues (8) reported that 30% of a preexercise MCT meal (25 g) was oxidized during 120 min of exercise at a comparable exercise intensity (60% $\text{VO}_{2\text{max}}$). MCT contributed 11% to the energy yield. In a study of Massicotte et al. (13), 54% of 25 g MCT was oxidized during 120 min of exercise at 65% $\text{VO}_{2\text{max}}$. The MCT, provided in a pre-exercise meal, contributed 7% to total energy expenditure.

The relatively small contribution of MCT to energy expenditure is primarily due to the small amount of MCT (29 g in the present experiment) that was provided. This amount, however, seems to be the maximal amount that can be ingested without causing gastro-intestinal distress. A maximal tolerance of about 30 grams has also been reported by others (11). However, the metabolic availability of MCT appears to be high as the peak oxidation rates represented 77% of the ingestion rate. In the present study the peak oxidation rate was 83% of the rate of ingestion during the final hour.

Substrate/metabolite concentrations

During all trials plasma glycerol concentration rose towards the end of exercise most probably due to an increased endogenous lipolysis since the rise also occurred when no MCT were ingested (CHO-trial). No differences were observed in plasma glycerol levels in the CHO, MCT, CHO+MCT and HCHO+MCT trial. Only in the MCT trial, glycerol concentrations tended to be slightly higher during the final hour compared to the trials in which carbohydrates were ingested, but this may be due to a suppression of endogenous lipolysis by the CHO-feeding.

MCT are hydrolyzed in the intestinal lumen and both MCFAs and glycerol enter the liver directly via the portal vein. Glycerol may be converted into glucose by gluconeogenesis in the liver. The lack of a difference in plasma glycerol concentration among trials suggests that the capacity of gluconeogenesis is large and that most of the glycerol liberated upon hydrolysis of MCT liver is directly converted into glucose on the first pass through the liver. β -hydroxybutyrate data support the view that a large part of the MCFA is metabolized in the liver since ketone bodies are formed when the supply of acetyl-CoA units exceeds the oxidative capacity of the liver. MCT have been shown to be highly ketogenic (2). However if these MCFA were used for oxidative processes within 2-3 hours, either directly in the liver or indirectly via oxidation of ketone bodies in the muscle, this would always be seen as $^{13}\text{CO}_2$ production and appear in expired air.

Plasma FFA levels were higher after 30 min in the CHO+MCT trial than in the MCT-trial indicating that, indeed, MCT from CHO+MCT suspensions might be more rapidly emptied from the stomach, and hence were reaching the systemic circulation more rapidly. After 90 min, however, plasma FFA levels were lower in the CHO+MCT trial than in the MCT-trial. This might be caused by a suppression of endogenous lipolytic activity by the CHO or a reduced entrance rate of MCFA in the systemic circulation during MCT ingestion during the last hour of exercise. Similar peak oxidation rates seem to argue against the latter possibility.

With carbohydrate supplementation euglycemia could be maintained whereas in the MCT trial glucose gradually declined, reaching low levels around 3-3.5 mmol $\cdot$ l $^{-1}$ at 180 min. (Figure 4.4). Carbohydrate supplementation has repeatedly been shown to maintain plasma glucose levels, which was proposed to be the main factor responsible for the delayed onset of fatigue observed after CHO supplementation (5, 6, 7).

Lactate concentration was not affected by either MCT or CHO supplementation except at the 180 min time point where a higher lactate was observed when only MCT was supplemented. Although no ratings of perceived exertion were collected, subjects also indicated to be more fatigued during the MCT trial and had more difficulties to complete the 180 min exercise protocol. This may have resulted in a different recruitment pattern of muscle fibers or an increased contribution of other muscle groups (in legs or arms) and consequently an increased lactate production. At this time point however, subjects reached low plasma glucose levels (close to hypoglycemia) which may have resulted in increased epinephrine production and consequently higher lactate production (1).

The additional ingestion of 65 g CHO during the CHO+MCT trial did not affect the results, which indicates that the observed differences between the CHO-MCT trials and the CHO trial are due to the MCT and are not a result of the smaller amount of CHO during the CHO+MCT trial.

In summary

The data do confirm the hypothesis that oral MCT might serve as an energy source in addition to glucose during exercise. The metabolic availability of MCT was high with oxidation rates being about 70% of the ingestion rate. The maximal amount of MCT that is tolerated in the gastrointestinal tract is small (about 30 g in 3 hours) and limits the contribution of MCT to total energy expenditure to values between 3 and 7%. From this study it was concluded that orally ingested carbohydrates accelerate exogenous MCT oxidation during the initial two hours of submaximal exercise. At present we can only speculate with regard to the explanation for this accelerated oxidation, but an accelerated entrance of MCT into the systemic circulation with CHO+MCT compared to MCT may be the most likely explanation.

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Chapter 5

Carbohydrate (CHO) metabolism after ingestion of CHO and medium chain triglycerides (MCT) during prolonged exercise

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Abstract

The aim of the study was to examine CHO utilization in subjects receiving CHO or CHO+MCT supplements during 180 min of exercise at 50% $\dot{V}O_{2\max}$ (57% $\dot{V}O_{2\max}$). In a double blind cross-over design 9 trained athletes cycled 4 times. Subjects received a bolus of 4 ml·kg⁻¹ at the start and 2 ml·kg⁻¹ every 20 min during exercise of either a 15 g·l⁻¹ CHO solution (CHO trial), an equicaloric 70en% CHO-30en% MCT-suspension containing 29g MCT (CHO+MCT trial), or a 15g·L⁻¹ CHO [high CHO (HCHO)] solution plus 29g MCT (HCHO+MCT trial). A fourth trial consisted of a ¹³C-background control trial (CON). The four trials were randomized. Before and after the exercise bout, muscle biopsies were taken from the quadriceps muscle and muscle glycogen levels were determined. During exercise breath samples were collected for the estimation of exogenous and endogenous CHO oxidation. No significant differences were detected in glycogen concentration before and after the 180 min of exercise. Glycogen breakdown was similar in all three trials. In addition no changes were observed in exogenous or endogenous CHO oxidation. It is concluded that the present amount of MCT co-ingested with CHO does not influence CHO utilization or glycogen breakdown.

Introduction

Oral ingestion of carbohydrates (CHO) during exercise has been shown to maintain plasma glucose levels (7) and high levels of plasma glucose oxidation (4) resulting in improved endurance capacity (7). The rate of exogenous carbohydrate oxidation and the contribution to energy expenditure is dependent on several factors including the duration of exercise, the exercise intensity (22), the rate of ingestion (40), the time point(s) of ingestion (13), training (19) and carbohydrate availability (41). However, oxidation rates of orally ingested CHO have never been observed to be higher than 1.0-1.1 grams per minute which seems to be the maximum oxidation rate. Even ingestion rates up to ~2 grams per minute during exercise at 70% VO_2max resulted in oral glucose oxidation rates close to 1 gram per min (40). In order to supply an additional exogenous energy source for the working muscle other substrates than CHO should be ingested. One potential candidate for such an energy source could be medium chain triglycerides (MCT). MCT have been shown to be rapidly hydrolyzed, absorbed (2) and subsequently oxidized (18, 24). In addition, a preliminary study showed that MCTs co-ingested with CHO do not inhibit gastric emptying (3).

It has also been suggested that MCT ingestion may improve exercise performance by elevating plasma FFA levels and sparing glycogen (38). It has been observed that increased availability of plasma FFA reduced the rate of muscle glycogen breakdown (6, 15, 16, 34, 39) and delayed the onset of exhaustion (15).

On the other hand, increased supply and subsequent oxidation of FFA or ketone bodies have been shown to result in an inhibition of plasma glucose utilization (12, 32) as well as overall carbohydrate utilization. From these observations one might hypothesize that increasing the FFA availability by oral fat intake may also limit the utilization of the exogenous supplied carbohydrates.

Therefore the aim of the present study was to study the effects of MCT co-ingested with CHO on exogenous and endogenous CHO utilization during prolonged exercise as well as on muscle glycogen levels.

Methods

Subjects

Nine male trained triathletes or cyclists aged 26.0 ± 5.0 years, weight 75.7 ± 4.3 kg, height 185 ± 8 cm, maximal aerobic work rate (W_{max}) $5.48 \pm 0.24 \text{ W}\cdot\text{kg}^{-1}$, maximal oxygen uptake $64.7 \pm 2.3 \text{ ml}\cdot\text{kg}^{-1}$ and a maximal heart rate of $194 \pm 5.3 \text{ BPM}$, 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.

Pre-trials

Subjects $W_{\max}$ was attained on an electronically braked ergometer (Lode Excalibur, Groningen, The Netherlands) during an incremental exhaustive exercise test (20) one week before the first experimental trial. The results of this initial test were used to determine the 50% $W_{\max}$, which was later used in the experimental trials.

Experimental trials

Nine subject performed four trials, each separated by at least seven days. A trial consisted of 180 min cycling at 50% $W_{\max}$ ($57 \pm 2\%$ $\dot{V}O_{2\max}$). Drinks were given in a randomized order and double blind. Drinks were all vanilla flavored (Sandoz Nutrition, Berne, Switzerland). Subjects abstained from training and were instructed to consume a comparable diet 3 days prior to each trial. In addition, 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 AM after an overnight fast and a standardized breakfast of two crackers with cheese was provided (14 g CHO, 4 g fat, 6 g protein). A Teflon catheter (Baxter Quick Cath Dupont, Ireland) was inserted into an antecubital vein and at 8.30 AM a resting blood sample was drawn. Also a muscle biopsy was taken from the lateral part of the m. vastus lateralis. Resting breath gases were collected for the measurement of oxygen consumption (SensorMedics 2900 analyzer, Anaheim, CA, USA) and vacutainer tubes were filled directly from the mixing chamber in duplicate to determine the $^{13}\text{C}/^{12}\text{C}$ ratio in expired CO_2 . At 8.50 AM subjects started cycling for 10 min at 100 W as a warm-up. At 9.00 AM the exercise intensity was increased to 50% $W_{\max}$ for 180 min. Blood samples were drawn at 30 min intervals until the end of exercise. Expiratory gases were collected every 15 minutes. Two subjects were tested on the same day, starting the protocol 10 minutes apart. Directly after the exercise bout a second muscle biopsy was taken 2 cm proximal to the first biopsy.

Drinks

The composition of the four test drinks as well as the amounts administered are listed in Table 1. Subjects received a bolus of $4 \text{ ml}\cdot\text{kg}^{-1}$ at the start ($t=0$) and $2 \text{ ml}\cdot\text{kg}^{-1}$ every 20 min during exercise of either a 15% CHO solution (CHO) or an equicaloric CHO-MCT suspension (CHO+MCT) (70 energy% as CHO and 30 energy% as MCT). In order to study the effect of MCT added to the carbohydrate (CHO) in stead of the equicaloric CHO+MCT suspension, a third trial (HCHO+MCT) was included in which a suspension was ingested containing the same amount of CHO (149 g) as in the CHO-trial and the same amount of MCT (29 g) as in the CHO+MCT and MCT trials. Therefore, it could be investigated whether differences between the CHO+MCT trial compared to the CHO trial are due to the MCT or due to the differential amount of CHO.

Carbohydrate utilization with CHO+MCT ingestion during exercise

Table 5.1 Composition of the four test drinks including the control trial. The difference between the CHO+MCT trial and the HCHO+MCT trial is the amount of CHO. The difference between the CHO and CON trial is the source of the carbohydrates. The carbohydrates in the CHO trial were corn derived whereas the carbohydrates in the CON trial were tapioca derived.

	CHO	CHO+MCT	HCHO+MCT	CON	
<i>Drinks</i>					
% CHO energy	100	70	77	100	en%
% MCT energy	0	30	23	0	en%
Energy	626	627	806	626	kcal·l ⁻¹
CHO	156.2	108.6	156.2	156.2	g·l ⁻¹
MCT	0	21.4	21.4	0	g·l ⁻¹
NaCl	20	20	20	20	mmol·l ⁻¹
<i>Supplementation</i>					
<i>Total amounts</i>					
Water	1375	1375	1375	1375	ml
CHO	214	149	214	214	g
MCT	0	29	29	0	g
Energy	861	862	1108	861	kcal
<i>Average ingestion rates</i>					
Water	7.6	7.6	7.6	7.6	ml·min ⁻¹
CHO	1.19	0.83	1.19	1.19	g·min ⁻¹
MCT	0	0.16	0.16	0	g·min ⁻¹
Energy	4.78	4.78	6.15	4.78	kcal·min ⁻¹

The carbohydrates in these trials were corn derived long-chain glucose polymers of high natural ¹³C-abundance (-11.31 δ per mil vs PDB, 0.0111101 ¹³C/¹²C ratio). To be able to correct for possible shifts in background ¹³C-enrichment during exercise a fourth trial was included in which tapioca derived long chain glucose polymers of low ¹³C natural abundance (-26.12 δ per mil vs PDB, 0.0109437 ¹³C/¹²C ratio; San doz Nutrition Ltd, Berne, Switzerland) (CON) were ingested. This CON trial was used only for ¹³C background measurements, no other measurements are presented. The four trials were conducted in random order. MCT contained fatty acids with a chain length of C8 (Estasan GT8-99, Unichema, Barcelona, Spain) and had a ¹³C-enrichment of -29.81 δ per mil vs PDB (0.0109437 ¹³C/¹²C ratio). To all drinks 20 mmol·L⁻¹ of NaCl was added. Meal temperature was kept constant at 20°C.

Analysis

Blood (10 ml) was collected into EDTA containing 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 (11), β-hydroxybutyrate (27), free fatty acids (FFA) (Wako FFA-C test kit, Wako Chemi-

cals, Neuss, Germany), and glycerol (Sigma, GPO-trinder 337), which were performed with the COBAS BIO analyzer (Roche diagnostics). Muscle biopsies were freeze dried and glycogen content was assayed spectrophotometrically after hydrolysis with HCl according to Passenneau et al. (30). Glycogen concentration was expressed as mmol glycosyl units per kg dry weight of tissue. Total energy expenditure and oxidation rates of total fat, total carbohydrates and exogenous MCT were calculated from indirect calorimetry (RQ , VO_2) and stable isotope measurements ($^{13}\text{CO}_2/^{12}\text{CO}_2$) (GC continuous flow IRMS, Finnigan MAT 252). Enrichments of the substrates (in drinks) were measured with an elemental analyzer - IRMS combination (Carlo-Erba on line connected to the Finnigan MAT 252).

Calculations

From VCO_2 and VO_2 carbohydrate and fat oxidation rates were calculated (31).

$$\text{carbohydrate oxidation} = 4.585 \text{ VCO}_2 - 3.226 \text{ VO}_2$$

$$\text{fat oxidation} = 1.695 \text{ VO}_2 - 1.701 \text{ VCO}_2$$

The isotopic enrichment of expired air was expressed as the delta per mil difference between the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and a known laboratory reference standard according to the formula:

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

The $\delta^{13}\text{C}$ was then related to the international standard Pee Dee Bellemnitella (PDB). The amount of carbohydrates oxidized was calculated according to the formula:

$$\text{exogenous glucose oxidation} = \text{VCO}_2 \cdot \left(\frac{\delta \text{ Exp} - \delta \text{ ref}}{\delta \text{ Ing} - \delta \text{ ref}} \right) \left(\frac{1}{k} \right)$$

In which $\delta \text{ ref}$ is the ^{13}C enrichment of expired air in the reference test CON (background), $\delta \text{ exp}$ is the ^{13}C enrichment of expired CO_2 during exercise with CHO(+MCT) ingestion at different time points, $\delta \text{ ing}$ is the ^{13}C enrichment of the carbohydrates in the ingested CHO+MCT suspension and k is the amount of CO_2 (in liters) produced via oxidation of 1 gram glucose ($k = 0.7466 \text{ L CO}_2$ per gram glucose).

In the present study and in previous studies from our laboratory (18, 33, 36, 40, 42) it was shown that instructing the subjects not to eat any products of high natural ^{13}C abundance during the experimental period was effective in reducing the background shift (change in $^{13}\text{CO}_2$) from endogenous substrate stores in European subjects (42). However, although the background shift was small in the present study, background correction was made by using the ^{13}C enrichment of breath samples in the CON-trial.

Another methodological consideration when using $^{13}\text{CO}_2$ in expired air to calculate exogenous substrate oxidation is the trapping of exogenous $^{13}\text{CO}_2$ in the bicarbonate pool, a very large and slowly exchanging pool in which an amount of CO_2 arising from decarboxylation of energy substrates is temporarily trapped (35). However, during exercise, the CO_2 production increases several fold so that physiological steady state situation will occur and $^{13}\text{CO}_2$ in expired air will be rapidly equilibrated with the $^{13}\text{CO}_2/\text{H}^{13}\text{CO}_3^-$ pool.

The dilution of $^{13}\text{CO}_2$ becomes negligible and recovery of $^{13}\text{CO}_2$ approaches 100% after 60 min of exercise (29). Therefore, in the present study, data from the initial 60 min were not used for the calculation of exogenous MCT oxidation.

Gastrointestinal discomfort

A questionnaire concerning experienced gastrointestinal discomfort was provided after each exercise test. Subjects had to score the following items on a scale from 1 to 5 (1 = not at all, 5 = very severe): nausea, intestinal cramps, belching, vomiting, diarrhea, flatulence, stomach ache, abdominal pressure, and eructation.

Statistics

Analysis of variance for repeated measures was used to compare differences in substrate utilization and in blood related parameters among the CHO, CHO+MCT and HCHO+MCT trial. A Scheffé post hoc test was used in the event of a significant ($p < 0.05$) F-ratio. All results are expressed as mean $\pm$ SEM.

Results

Breath analysis

Oxygen consumption (Table 2) during exercise was relatively constant as the exercise intensity of the subjects was maintained at close to 57% $\text{VO}_{2\text{max}}$ and was not significantly different among the trials. Respiratory exchange ratio was also not significantly different among the trials.

The average ^{13}C enrichment of the resting breath samples was -26.36 ± 0.25 δ per mil vs PDB (0.0109410 $^{13}\text{C}/^{12}\text{C}$ ratio). Changes in isotopic composition of expired CO_2 in response to exercise are shown in Table 3.

Table 5.2 Values are means $\pm$ (SEM) (n=8). Oxygen consumption (VO_2) and respiratory exchange ratio (RER) during 3 hours of cycling exercise at 57% $\text{VO}_{2\text{max}}$ with ingestion of CHO, CHO+MCT or HCHO+MCT. No significant differences were observed among the trials or over time.

Time (min)		30	60	90	120	150	180
VO_2 ($\text{L}\cdot\text{min}^{-1}$)	CHO	2.78 (0.08)	2.79 (0.10)	2.79 (0.10)	2.77 (0.10)	2.80 (0.08)	2.75 (0.10)
	CHO+MCT	2.76 (0.08)	2.76 (0.09)	2.79 (0.07)	2.76 (0.08)	2.77 (0.09)	2.73 (0.09)
	HCHO+MCT	2.77 (0.07)	2.80 (0.07)	2.80 (0.08)	2.84 (0.09)	2.73 (0.08)	2.80 (0.09)
RER	CHO	0.89 (0.01)	0.88 (0.01)	0.87 (0.01)	0.88 (0.01)	0.87 (0.01)	0.86 (0.02)
	CHO+MCT	0.86 (0.02)	0.87 (0.01)	0.86 (0.01)	0.86 (0.01)	0.87 (0.01)	0.86 (0.01)
	HCHO+MCT	0.87 (0.02)	0.88 (0.01)	0.87 (0.01)	0.86 (0.01)	0.87 (0.01)	0.88 (0.01)

Table 5.3 Delta per mil enrichment during 3 hours of exercise versus the enrichment of the resting sample (mean $\pm$ SEM) (n=8).

Time	CHO	CHO+MCT	HCHO+MCT	CON
0	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
15	0.04 $\pm$ 0.14	0.01 $\pm$ 0.02	0.06 $\pm$ 0.08	0.10 $\pm$ 0.09
30	1.29 $\pm$ 0.32	1.20 $\pm$ 0.22	1.42 $\pm$ 0.32	0.37 $\pm$ 0.12
45	2.40 $\pm$ 0.37	2.25 $\pm$ 0.24	2.47 $\pm$ 0.30	0.51 $\pm$ 0.11
60	3.02 $\pm$ 0.36	2.70 $\pm$ 0.27	3.11 $\pm$ 0.31	0.53 $\pm$ 0.09
75	3.61 $\pm$ 0.30	3.06 $\pm$ 0.26	3.48 $\pm$ 0.24	0.59 $\pm$ 0.10
90	3.84 $\pm$ 0.37	3.26 $\pm$ 0.25	3.73 $\pm$ 0.27	0.58 $\pm$ 0.09
105	4.08 $\pm$ 0.36	3.50 $\pm$ 0.24	3.91 $\pm$ 0.26	0.56 $\pm$ 0.08
120	4.32 $\pm$ 0.34	3.76 $\pm$ 0.20	4.11 $\pm$ 0.26	0.55 $\pm$ 0.10
135	4.34 $\pm$ 0.35	3.84 $\pm$ 0.19	4.09 $\pm$ 0.24	0.53 $\pm$ 0.11
150	4.37 $\pm$ 0.34	3.80 $\pm$ 0.22	4.35 $\pm$ 0.26	0.50 $\pm$ 0.11
165	4.43 $\pm$ 0.37	3.82 $\pm$ 0.18	4.56 $\pm$ 0.26	0.41 $\pm$ 0.12
180	4.28 $\pm$ 0.38	3.75 $\pm$ 0.18	4.80 $\pm$ 0.17	0.34 $\pm$ 0.07

During CON (with ingestion of CHO of low ^{13}C natural abundance) there was a small increase of ^{13}C in the expired air (0.2-0.6 δ per mil vs PDB). In the CHO(+MCT) containing trials the rise in ^{13}C was significant, reaching a δ per mil difference of 3-4 δ per mil vs PDB towards the end of 180 min exercise (compared to resting breath sample) (Figure 5.1). Exogenous carbohydrate oxidation increased during the first hour and leveled off during the final 90 min (Figure 5.2).

Figure 5.2 Relative contributions of endogenous (CHO_{endo}) and exogenous carbohydrates (CHO_{exo}), and total fat oxidation to energy expenditure during the 60-120 min and 120-180 min time periods for the CHO, CHO+MCT and the HCHO+MCT trials.

At the end of the exercise bout exogenous carbohydrate oxidation rates were 0.89 g·min⁻¹, 0.73 g·min⁻¹ and 0.91 g·min⁻¹ for CHO, CHO+MCT and HCHO+MCT respectively. Mean oxidation rates over the 60-180 min period were 0.79 g·min⁻¹, 0.63 g·min⁻¹ and 0.73 g·min⁻¹ respectively. Exogenous carbohydrate oxidation tended to be slightly lower in the CHO+MCT trial than in the CHO trial. This difference, however, did not reach statistical significance. It was estimated that during the 60-180 min period in the CHO trial, 94.5 g of exogenous carbohydrate was oxidized versus 75.7 g in CHO+MCT and 87.1 g in HCHO+MCT. The amounts of CHO- (exogenous and endogenous) and fat oxidation during the second and third hour of exercise are presented in Table 5.4.

Figure 5.2 Exogenous MCT oxidation rates over 180 min of exercise.

Table 5.4 Substrate oxidation of endogenous (endo) and exogenous (exo) substrates. Expressed as grams over the 60-120 min and the 120-180 min period (mean $\pm$ SEM). No significant differences in substrate utilization were observed among the trials or time periods.

Time interval	CHO	CHO+MCT	HCHO+MCT
60-120			
CHO	132.2 $\pm$ 11.4	122.5 $\pm$ 10.3	127.3 $\pm$ 10.3
exo	44.2 $\pm$ 3.7	34.2 $\pm$ 3.73	8.6 $\pm$ 5.0
endo	88.0 $\pm$ 8.3	88.3 $\pm$ 7.38	8.7 $\pm$ 9.3
Fat	34.3 $\pm$ 3.7	37.5 $\pm$ 3.73	6.7 $\pm$ 3.2
120-180			
CHO	124.1 $\pm$ 12.8	119.6 $\pm$ 9.8	130.8 $\pm$ 9.9
exo	50.3 $\pm$ 3.2	41.5 $\pm$ 5.0	48.5 $\pm$ 4.8
endo	73.8 $\pm$ 9.9	78.1 $\pm$ 6.5	82.3 $\pm$ 9.3
Fat	33.8 $\pm$ 4.2	38.7 $\pm$ 3.0	35.1 $\pm$ 3.1

Muscle glycogen

Preexercise muscle glycogen concentrations were comparable among the trials: 516 $\pm$ 32, 518 $\pm$ 30 and 505 $\pm$ 26 mmol glucose $\cdot$ kg dw $^{-1}$ for CHO, CHO+MCT and HCHO+MCT respectively. Muscle glycogen levels decreased significantly in the three trials (Figure 5.3) (expressed in mmol glucose $\cdot$ kg dw $^{-1}$) 277 $\pm$ 14 for CHO, 249 $\pm$ 20 for CHO+MCT and 240 $\pm$ 18 for HCHO+MCT. Glycogen breakdown was not significantly different among the three trials.

Figure 5.3 Pre and post exercise glycogen concentrations and glycogen breakdown for the CHO, CHO+MCT and the HCHO+MCT trials. No significant differences were observed among the trials.

Plasma variables

Plasma FFA were decreased compared to rest after 30 minutes of exercise and were significantly elevated during the final 30 minutes of exercise (Figure 5.4a). No differences in plasma FFA levels were observed among the trials. There was a significant increase in plasma glycerol levels already after 30 min in all trials, but no differences could be observed among the three trials (Figure 5.4b). Plasma β -hydroxybutyrate concentrations increased to about $500 \mu\text{mol}\cdot\text{l}^{-1}$ during the first 30 min in the CHO+MCT and HCHO+MCT trials; thereafter the concentration remained stable (Figure 5.4c). In the CHO trial no changes in β -hydroxybutyrate were observed throughout exercise. Plasma glucose levels were maintained during exercise (Figure 5.5a), whereas plasma lactate concentrations tended to decrease compared to the resting value (Figure 5b). No differences were observed in plasma glucose and lactate concentrations among the trials.

Gastrointestinal discomfort

Subjects reported some gastro intestinal discomfort in all tests. The occurrence and the severity (average score 2.8) of the complaints was not different among the trials. Most frequently reported were intestinal cramps, nausea and belching.

Figure 5.4 Plasma FFA, glycerol and β -hydroxybutyrate concentrations during exercise for the CHO, CHO+MCT and the HCHO+MCT trials. * Significant difference between CHO and CHO+MCT ($p < 0.05$).

Figure 5.5 Plasma glucose and lactate concentrations during exercise for the CHO, CHO+MCT and the HCHO+MCT trials. * Significant difference between CHO and CHO+MCT ($p < 0.05$). @ Significant difference between CHO and HCHO+MCT ($p < 0.05$).

Discussion

Plasma concentrations

Increased plasma glycerol and ketone body concentrations have been frequently observed after MCT feeding (1, 8, 17, 18, 24, 37). In the present study glycerol concentrations were not significantly elevated after MCT ingestion. Plasma β -hydroxybutyrate levels were elevated to moderate levels (400–500 mmol·l⁻¹) after MCT ingestion. This may indicate that MCT is metabolized in the liver, resulting in the production of ketone bodies while the glycerol from hydrolysis of MCT is rapidly utilized for gluconeogenesis.

Muscle glycogen

It has been reported in several studies that increased availability of plasma FFA resulted in muscle glycogen sparing (6, 9, 15, 16, 34, 39) and hence increased performance (15). In humans, plasma FFA levels have been elevated by injecting heparin which stimulates lipoprotein lipase activity, after feeding subjects a high fat meal (long chain fatty acids, LCFA) or infusion of a triglyceride emulsion (Intralipid) (6, 9, 39). In these studies a glycogen sparing effect was observed when FFA availability was high. While the infusion of fats and the consumption of triglycerides in combination with an injection of heparin are interesting approaches to test the interaction between CHO and fat metabolism during exercise, this method has little practical value. Therefore it has been suggested to increase FFA availability by ingesting MCTs (1, 8, 17, 18, 24, 37, 38). MCTs are delivered into the blood more rapidly than ingested long chain triglycerides (LCTs) (2, 10, 25), and they can cross the mitochondrial membrane without carnitine (5). Therefore it has been argued that MCT might be a readily available energy source for the working muscle. In addition it has been suggested that MCT might spare muscle glycogen and improve time trial cycling performance (38). In the present study and in a recent study with a similar experimental setup (18) MCT ingestion did not affect plasma FFA levels. Hence, no effect on muscle glycogen utilization was observed. This is in agreement with the only other available study in which muscle glycogen concentrations determined after MCT ingestion (8). In this study MCTs were ingested 1 hour pre exercise and did not result in glycogen sparing during 1 hour exercise at 60% $\dot{V}O_2$ max. It may be that the amount of MCT provided was too small to influence plasma FFA concentrations or that MCFAs are oxidized rapidly in the liver and/or in skeletal muscle so that the plasma FFA concentrations remains the same.

Substrate utilization

As in a previous study with a similar experimental set-up (18), MCT ingestion did not affect total carbohydrate and fat utilization. Peak oxidation rates of the oral ingested long chain glucose polymer were 0.80–0.94 g·min⁻¹ which is in line with previous studies (14, 21, 23, 26, 28, 33, 36, 40). Peak oxidation rates of glucose, glucose polymers and starch have been found to be between 0.8 and 1.0 g·min⁻¹ with

comparable ingestion rates, feeding schedule and exercise intensity (4, 36, 40). The ingested CHO contributed 15-25% to energy expenditure during the 120-180 min period.

The observation that MCT ingestion did not affect muscle glycogen breakdown and carbohydrate utilization might be explained by several factors:

First, as in our previous study (18), MCT ingestion did not increase plasma FFA levels, whereas in those studies that reported muscle glycogen sparing (6, 39) plasma FFA concentrations were elevated to very high levels by ingesting long chain fatty acids or infusing a long chain triglyceride emulsion and heparin.

Also, the amount of MCT provided in the present study (29 grams) may have been too small to alter substrate utilization significantly. With ingestion of 29 grams of MCT over a 180 min period, MCT contributes maximally about 7% to energy expenditure (18).

Gastrointestinal discomfort

The MCT (29 grams) seemed to have no influence on the palatability of the beverages. However, since in all tests some GI-discomfort was reported this may be attributed to the long chain glucose polymers which were co-ingested with the MCT. Ivy et al (17) reported that administration of 30 grams MCT, in combination with cereal caused some minor distress in 10% of the subjects.

In summary

29 grams of MCT co ingested with CHO during 180 min of exercise at 57% VO_2max does not influence exogenous or endogenous CHO utilization and does not influence muscle glycogen breakdown.

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Chapter 6

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

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Abstract

The present study examined the medium chain triglyceride (MCT) oxidation rate of oral 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 4 times 90 min at 50% $\dot{V}O_{2\max}$ (57% $\dot{V}O_{2\max}$). In 2 trials they followed a LG-protocol to achieve low glycogen stores in the leg muscles the evening prior to the experiment and in 2 trials they followed a HG-protocol. Subjects received a bolus of 4 ml·kg⁻¹ at the start and 2 ml·kg⁻¹ 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 [1,1,1-¹³C]- trioctanoate tracer to the MCT oil and measuring ¹³CO₂ production in the breath. The results show that 85% of MCT ingested was oxidized in LG and 69% in HG during the 60-90 min period. There was no statistically significant difference in MCT utilization in LG and HG. Peak oxidation rates were 0.15 g·min⁻¹ and 0.13 g·min⁻¹ respectively. MCT contributed 7.6% (LG) and 6.5% (HG) to total energy expenditure during the 60-90 min period. Total fatty acid oxidation was significantly elevated in the LG-trial, but was not influenced by MCT inges-

tion. Concomitantly carbohydrate oxidation was reduced in LG, but no effect of MCT was observed. We conclude 1. that the contribution of MCT to total energy expenditure was small and 2. that strenuous exercise the day prior to the experiment followed by a low CHO intake, leading to a low CHO availability, substantially increased total fat oxidation but did not significantly increase MCT oxidation.

Introduction

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 blood stream 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 co-ingested with carbohydrates (12).

As exercise progresses muscle glycogen levels decline, and this decline is accompanied by a shift in substrate utilization from carbohydrate to fat. Plasma FFA-uptake and oxidation increases 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 especially be 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 and normal to high muscle glycogen stores, in a randomized cross-over design. To study the oxidation rate of exogenous MCT during exercise, a [1,1,1-¹³C]-trioctanoate tracer was incorporated in the drink.

Methods

Subjects

Eight male highly trained elite triathletes or cyclists (age 28.9 ± 2.5 , weight 77.9 ± 3.0 , height 184.5 ± 3.1 , maximal work rate 434 ± 14 W and VO_2max 70 ± 2 ml·kg⁻¹·min⁻¹), competing at 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.

Pre-trials

Subjects' maximal workload (W_{max}) was attained on an electronically braked ergometer (Lode Excalibur Sport, Groningen, The Netherlands) during an incremental exhaustive exercise test (14) one week before the first experimental trial. The results of this initial test were used to determine the 50% W_{max} which was later used in the experimental trials. Subjects randomly performed two glycogen depletion trials to achieve low glycogen stores (LG) and two glycogen loading trials (HG). The depletion trial was always performed on the evening (8-10 PM) preceding the experimental trial. An intermittent exercise protocol was employed, consisting of 2-min bouts at 90% W_{max} , interspersed with 2 min at 50% W_{max} . When the subjects were unable to complete the 2 min 90% W_{max} , the high workload was lowered to subsequently 80%, 70%, and 60% W_{max} . The exercise was stopped when the 2 min at 60% W_{max} could not be completed any more. This protocol has previously been shown to lead to very low muscle glycogen levels (below 150 mmol·g dry weight⁻¹) (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 (4000-5000 kJ; $\pm 80\%$ CHO, $\pm 10\%$ fat, $\pm 10\%$ protein) at the laboratory, the evening prior to the experimental test (8-10 PM), to ensure a high carbohydrate intake and concomitant normal to high glycogen stores.

Experimental trials

Each subject performed four trials, each separated by at least seven days. A trial consisted of 90 min cycling at 50% W_{max} ($\sim 57\%$ VO_2max) VO_2 data are presented in Table 6.1.

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 ¹³C during the entire experimental period.

Protocol

Subjects reported to the laboratory at 8.00 AM after an overnight fast and a standardized breakfast of two crackers with cheese (14 g CHO, 4 g fat, and 6 g pro-

MCT oxidation and glycogen availability during exercise

Table 6.1 Steady state oxygen uptake and carbon dioxide production at different time points during 90 min of exercise in subjects ingesting carbohydrates (CHO) or a carbohydrate-medium chain triglyceride mixture (CHO+MCT) with normal to high glycogen (HG) or low glycogen (LG) levels.

Time (min)	15	30	45	60	75	90
VO ₂						
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
VCO ₂						
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±0.08	2.66±0.08	2.70±0.08	2.74±0.11

Values are mean ± SEM, n=8

tein) was provided. A teflon catheter (Baxter Quick Cath, Dupont, Ireland) was inserted into an antecubital vein and at 8:30 AM a resting blood sample was drawn. Resting breath gases were collected for the measurement of oxygen consumption (SensorMedics 2900 analyser, Anaheim, CA, U.S.A.) and vacutainer tubes were filled directly from the mixing chamber in duplicate to determine the ¹³C/¹²C ratio in expired CO₂. At 8:50 AM a 10 min warm-up began at 100W. At 9:00 AM subjects started cycling at 50% Wmax for 90 min and in the first minute they drank an initial bolus (4 ml·kg⁻¹) of either one of the test drinks. Thereafter every 20 min a beverage volume of 2 ml·kg⁻¹ was given. Blood samples were drawn at 5, 10, and 15, and every 15 minutes thereafter. Expiratory gases were collected every 15 minutes. Two subjects were tested on the same day, starting the protocol 4 minutes apart.

Drinks

The drinks consisted of tapioca derived long chain glucose polymers of low ¹³C natural abundance (Sandoz Nutrition Ltd, Berne, 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⁻¹ of NaCl was added.

The composition of the drinks is listed in Table 6.2. On average subjects ingested 146.0 g of 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.

Table 6.2 Beverage composition. Subjects ingested 12 ml·kg⁻¹ over the 90 min period.

	CHO	CHO+MCT
CHO (energy%)	100	60
MCT (energy%)	0	40
CHO (g·l ⁻¹)	156.2	93.1
MCT (g·l ⁻¹)	0	28.5
Energy kJ·l ⁻¹ (kcal·l ⁻¹)	2620 (627)	2620 (627)

Tracer methodology

A[1,1,1-¹³C]-trioctanoate tracer (99%), purchased from Cambridge Isotope Laboratories (Woburn, MA, USA), was incorporated in the unlabeled MCT suspension and then mixed with the CHO to form a stable suspension. The ¹³C-enrichment of the MCT was +160.61 δ per mil (0.013042 ¹³C/¹²C ratio) whereas the ¹³C-enrichment of the CHO was -26.12 δ per mil (0.0112372 ¹³C/¹²C ratio). The enrichment of the CHO was about the same as the average enrichment of the subjects resting expired air (-27.18 δ per mil).

In the present study and in previous studies from our lab (12, 20, 24, 27, 28) we have shown that instructing the subjects not to eat any products of high ¹³C abundance during the experimental period was effective in reducing the background shift (change in ¹³CO₂) from endogenous substrate stores (28). We nevertheless decided to correct the background with the change of the ¹³C enrichment of breath samples observed in the CHO-trial (Table 6.3).

Table 6.3 Resting enrichments values and change in enrichment of breath samples at different time points versus rest sample (in delta per mil vs PDB) (mean ± SEM).

	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

MCT oxidation and glycogen availability during exercise

During the initial phases of exercise some retention of $^{13}\text{CO}_2$ in the bicarbonate pool will occur (21) and thus could lead to an underestimation of the calculated exogenous oxidation rates. However, during exercise, the CO_2 production increases eight to tenfold leading to a physiological steady state situation in which $^{13}\text{CO}_2$ in expired air will be in equilibrium with the $^{13}\text{CO}_2/\text{H}^{13}\text{CO}_3^-$ pool. It has been shown that the dilution of $^{13}\text{CO}_2$ becomes negligible and recovery of $^{13}\text{CO}_2$ approaches 100% after 60 min of exercise (18). Therefore, in the present study, data of exogenous MCT oxidation are presented for the 60-90 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), free fatty acids (FFA) (Wako NEFA-C test kit, Wako Chemicals, Neuss, Germany), glycerol (Sigma, GPO-trinder 337), on a COBAS BIO analyzer. From breath samples (VCO_2 , VO_2) and stable isotope measurements (IRMS, Finnigan MAT 252), total energy expenditure and oxidation rates of total fat, total carbohydrates 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 VCO_2 and VO_2 carbohydrate and fat oxidation rates were calculated using stoichiometric equations (19).

$$\text{glucose oxidation} = 4.585 \text{ VCO}_2 - 3.226 \text{ VO}_2$$

$$\text{fat oxidation} = 1.695 \text{ VO}_2 - 1.701 \text{ VCO}_2$$

The isotopic enrichment was expressed as the delta per mil difference between the ^{13}C and ^{12}C ratio of the sample and a known laboratory reference standard according to the formula:

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

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

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

$$\text{exogenous glucose oxidation} = \text{VCO}_2 \cdot \left(\frac{\delta_{\text{exp}} - \delta_{\text{bkg}}}{\delta_{\text{ing}} - \delta_{\text{bkg}}} \right) \left(\frac{1}{k} \right)$$

In which 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 gram trioctanoate ($k = 1.2369 \text{ L CO}_2$ per gram MCT).

Statistics

Analysis of variance for repeated measures was used to compare differences in substrate utilization and in blood related parameters between the four endurance rides. A Scheffé post hoc test was used in the event of a significant ($p < 0.05$) F-ratio.

Results

VO_2 was relatively constant throughout the experiments and there were no differences among the four trials (Table 6.1). Average background ^{13}C enrichment measured from the resting breath samples was -27.21 ± 0.61 (Table 6.3). Changes in isotopic composition of expired CO_2 in response to exercise are presented in Table 6.3. 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 per mill difference of ≥ 10 –13 towards the end of 90 min exercise (compared to CHO experiment breath samples). There was no difference in the rates of $^{13}\text{CO}_2$ appearance in expired air between the HG and LG trial. Exogenous MCT oxidation showed a gradual increase over time both in the LG and HG state (Figure 6.1). Peak oxidation rates were reached at the end of exercise (90 min) and were $0.15 \text{ g}\cdot\text{min}^{-1}$ (LG) and $0.13 \text{ g}\cdot\text{min}^{-1}$ (HG) respectively. No differences were observed between the LG and HG trials.

Figure 6.1 Exogenous trioctanoate oxidation (MCT) from CHO+MCT suspensions during exercise in the high glycogen (HG) and low glycogen (LG) trials. No significant differences between LG and HG.

MCT oxidation and glycogen availability during exercise

In Table 6.4 the amount of CHO and exogenous and endogenous fat oxidation during the exercise period is presented. Over the 60-90 min period 4.24 ± 0.27 g of 5 grams exogenous MCT were oxidized in the LG trial and 3.45 ± 0.26 in the HG trial. This represented 85% and 69% of the total amount of MCT ingested. Exogenous MCT contributed 7.6% to total energy expenditure in the LG trial and 6.5% in the HG trial (during the 60-90 min period). These differences, however, were not statistically significant.

Table 6.4 Total carbohydrate oxidation, endogenous fat and exogenous MCT oxidation during the 60-90 min period in the low glycogen (LG) and high glycogen trials (HG) in grams. * Significant difference between LG and HG ($p < 0.05$).

	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

No differences in energy expenditure between the four trials were observed (Figure 6.2). There were large differences in substrate utilization between the HG and LG trials, but differences between the CHO and the CHO+MCT trials were not statistically significant. Total carbohydrate utilization over 90 min was significantly higher in the HG trials, 63% (CHO) and 53% (CHO+MCT) of total energy expenditure compared to 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 (Figure 6.3). 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 to the CHO trials and in the LG trials compared to the HG trials. Glycerol concentrations were not significantly different in the resting situation and increased during exercise in all trials (Figure 6.3). 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 (Figure 6.3). There was no significant difference between HG and LG. There were no large changes in plasma glucose concentrations (Figure 6.3). At 15 and 30 min however, a slight but significant higher glucose concentration was observed in the HG-CHO trial compared to the LG- trials.

Figure 6.2 Total energy expenditure, carbohydrate oxidation and fat oxidation over 90 min of exercise with ingestion of carbohydrates (CHO) or a carbohydrate-medium chain triglyceride suspension (CHO+MCT) in the high glycogen (HG) and low glycogen (LG) trials.
[#] Significant difference ($p < 0.001$) between LG and HG.

Figure 6.3 Plasma free fatty acids (FFA), β -hydroxybutyrate, glycerol and glucose concentrations during 90 min of exercise in the low glycogen (LG) and high glycogen (HG) trials, with carbohydrate (CHO) or carbohydrate-medium chain triglyceride (CHO+MCT) ingestion.

Significant difference ($p < 0.05$) between LG and HG until the end of exercise.

* Significant difference ($p < 0.05$) between CHO and MCT until the end of exercise.

Discussion

Exogenous MCT oxidation

The amount of MCT oxidized was about 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 $^{13}\text{CO}_2$ 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 oxi-

died during 120 min of exercise at 65% VO_2max . The MCT, provided in a pre-exercise meal, contributed 7% to total energy expenditure. Décombaz et al. (7) reported that 30% of a pre-exercise MCT meal (25 g) was oxidized during 120 min of exercise at a comparable exercise intensity (60% VO_2max). MCT contributed 1% 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 $^{13}\text{CO}_2$ 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 MCT while 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 to LCT, 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 is comparable to those of glucose, which is reflected in a similar time course of $^{13}\text{CO}_2$ 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 (Rd) 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 is hydrolyzed in the lumen and both MCFAs 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 will be 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 MCFAs 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 MCT 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 pre-exercise feeding (1, 7, 11, 16, 25).

In 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 MCT's that were co-ingested with carbohydrates during 90 min of exercise.

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Chapter 7

A new validated endurance performance test

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Abstract

The extensive use of performance tests in diet intervention studies mirrors the importance of such a measurement. Although many different endurance performance tests have been used in the past, the majority of these different protocols has never been validated. In this study reproducibility of three different endurance performance tests was evaluated. Thirty well-trained subjects were divided into three subgroups matched for age, weight and maximal workload (W_{max}) during an incremental exercise protocol. Each group of subjects performed five times one of three exercise protocols: protocol A (A) consisted of cycling at 75% W_{max} until exhaustion on an electromagnetically braked ergometer. In protocol B (B) subjects received a preload of 45 min 70% W_{max} and then performed as much work as possible during 15 min with the ergometer set in the pedaling rate dependent mode. Protocol C (C) consisted of a time trial, in which subjects had to complete a pre-set amount of work as quickly as possible, the ergometer being set in the pedaling rate dependent mode. All protocols lasted about 1 hour in order to keep energy expenditure comparable among the trials. Each subject performed one learning trial, which was not included in the data evaluation, followed by five test trials. Coefficient of variation (CV) was calculated for the three protocols. CV (A) was 26.6%, CV (B) 3.5% and CV (C) 3.4%. It was concluded that reproducibility of a performance test at 75% W_{max} till exhaustion is poor and that this test is

unreliable at this exercise intensity. Reproducibility of protocol B and C was good and these protocols seem to be reliable performance measurements. Therefore time trial protocols may result in better performance evaluation.

Introduction

In exercise physiology methods to evaluate aerobic and anaerobic power of athletes have been studied extensively, but so far only scarce literature is available on the reproducibility of endurance performance tests. On the other hand there appears to be an increasing demand to evaluate the effects of nutrition, ergogenic aids, drugs or training programmes on endurance performance. A quick review through some recent literature shows that no uniform "endurance performance test" exists, but a wide variety of different exercise protocols is used. Testing methodologies have traditionally included sub-maximal performance rides at a fixed percentage of maximal oxygen uptake ($\text{VO}_{2\text{max}}$) or maximal power output (W_{max}). Coyle et al. (2) reported that time to exhaustion during prolonged exercise at a constant work output was highly correlated with 40-km time trial performance. However Krebs and Powers (5) reported poor test-retest reliability of time to fatigue.

Because of the lack of a standard and well validated performance test, it is often concluded that "there is no effect", whereas with the performance test applied it was not possible to detect "the effect". Therefore the aim of this study was to investigate the reproducibility of three different endurance performance tests under carefully controlled conditions.

Methods

Subjects

In order to investigate the reproducibility of different endurance performance tests 30 well-trained competitive or recreational male triathletes or cyclists were recruited. These subjects were all actively cycling for at least 3 years and involved in endurance training on a regular basis (> 4 times/week). The study was approved by the local medical ethical committee and subjects signed an informed consent. Subjects characteristics are listed in Table 7.1.

Pre test

In a pre test maximal workload (W_{max}) was determined using a graded exercise test according to Kuipers et al. (6). After a warm-up period of 5 min at 100 W, workload was increased by 50 W every 2.5 min till a heart rate was reached of 160 bpm. Then workload was increased by 25 W every 2.5 min. The W_{max} was determined by the following formula:

$$W_{\max} = W_{\text{out}} + (t / 150) * 25$$

where W_{out} is the workload of the last completed stage, t is the time in seconds in the final stage.

Heart rate was continuously measured by a Sporttester® (Polar, Finland). Like $\dot{V}O_{2\max}$, $W_{\max}$ is a good predictor of aerobic capacity (7, 3, 1).

The ergometers used (Lode Excalibur Sport, Lode, Groningen The Netherlands) are electromagnetically braked, but are able to switch from a pedaling rate independent mode (hyperbolic mode) to a pedaling rate dependent mode.

In the hyperbolic mode a certain work rate can be imposed to a subject and this load will be constant, independently of the subjects pedaling rate. In the pedaling rate dependent mode the ergometer acts like a mechanically braked ergometer: with increasing pedaling rate, the work rate will increase according to the following formula:

$$W = L \cdot (\text{RPM})^2$$

In which RPM is the pedaling rate and L is a (constant) factor which is dependent on a subjects' $W_{\max}$. The ergometers are calibrated yearly and deviation is $< 0.5\%$ even at very high work rates.

Protocol

Thirty subjects were randomly divided into three groups of ten subjects, matched for age, weight and $W_{\max}$. Each subgroup was assigned to one of three test protocols: A, B or C (Table 7.1).

Each subject performed the protocol to which he was assigned, six times, including a learning trial. This learning trial was not included in the statistical analysis.

To avoid any influence of circadian variance, subjects performed their tests at the same time of the day. Subjects were not allowed to train the day before the test.

Two days before the test, training was not allowed to be exhaustive.

Additionally, all subjects were instructed to eat the same meal the evening prior to the test, preferably a high carbohydrate meal such as pasta. In the hour preceding the test, subjects were not allowed to eat. To further control carbohydrate and energy intake, food records were provided for the day prior to the test and the day of the test. During all tests environmental conditions were standardized, temperature was kept at 20°C , relative humidity varied between 50 and 60 %.

Subjects were encouraged by the same person every test and water was available ad libitum. Tests were designed in such a way that they all lasted about 1 hour in an attempt to keep total energy expenditure comparable.

Protocol A: Continuous exercise at 75% $W_{\max}$

After a short warming-up (5 min, 100 W), subjects exercised at 75% of their maximal power output until exhaustion on an electromagnetically braked ergometer. After the first test, workload was adjusted if necessary to ensure that all subjects

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Table 7.1 Characteristics of the 30 subjects and the 3 matched groups.

	<i>Age (yrs)</i>	<i>Weight (kg)</i>	<i>Length (cm)</i>	<i>Wmax (W)</i>	<i>Wmax · kg⁻¹ (W · kg⁻¹)</i>
Protocol A					
1A	24	84	194	412	4.90
2A	37	75	187	405	5.40
3A	22	89	188	400	4.49
4A	22	76	189	367	4.83
5A	26	63	184	326	5.17
6A	29	75	180	410	5.47
7A	20	78	196	435	5.58
8A	20	68	186	360	5.29
9A	25	71	184	390	5.49
10A	26	58	173	380	6.55
<i>mean</i>	25	75	188	389	5.18
<i>stdev</i>	5	8	5	33	0.37
Protocol B					
1B	20	69	178	435	6.30
2B	24	69	187	400	5.80
3B	42	82	189	413	5.04
4B	20	75	183	352	4.69
5B	26	66	182	375	5.68
6B	21	78	187	450	5.77
7B	40	75	180	350	4.67
8B	25	87	191	400	4.60
9B	22	69	188	413	5.99
10B	19	72	182	403	5.60
<i>mean</i>	27	74	185	399	5.39
<i>stdev</i>	8	7	4	34	0.65
Protocol C					
1C	24	79	177	362	4.58
2C	26	72	180	372	5.17
3C	19	80	193	435	5.44
4C	20	65	175	375	5.77
5C	29	68	179	400	5.88
6C	18	65	182	350	5.38
7C	19	70	186	425	6.07
8C	36	82	179	350	4.27
9C	23	73	182	361	4.95
10C	26	79	179	355	4.49
<i>mean</i>	24	73	181	381	5.28
<i>stdev</i>	6	6	5	32	0.60
Overall:					
<i>mean</i>	25.0	73.7	184.0	388.7	5.31
<i>stdev</i>	6.3	7.3	5.6	31.7	0.58

would perform approximately one hour. By doing this the inter-subject variability was minimized and the (subjective) experienced intensity of exercise became the same for all subjects. During the test, subjects were unaware of time or any other variable (like heart rate) and the only information subjects received was the pedaling rate. The test was aborted when pedaling rate dropped below 60 rpm. The time from the start of the experiment till the point of exhaustion, expressed in minutes was the measure of performance.

Protocol B: pre-loaded time trial

These test was started with a pre-load of 45 min at 70% W_{max} . Thereafter a 15 min time trial followed in which subjects had to perform as much work as possible. During the first 45 min the electromagnetically braked ergometers were in the hyperbolic mode, so that the work rate (70% W_{max}) was independent of pedaling rate. During the 15 min time trial the ergometer was in the pedaling rate dependent mode so that with increasing pedaling rate the work rate increased. In this way the subject could pace himself and try to maintain a high cadence over 15 min in order to maximize power output.

The subjects were motivated to perform as much work as possible in 15 min. The total amount of work (power output over 15 min) was taken as a measure of performance. To avoid test re-test influence, the subject received no information about the amount of work performed, heart rate or pedaling rate. The subjects were only aware of time. The cycle ergometer was connected to a computer which recorded the work rate every second and immediately calculated the total amount of work performed.

Protocol C: One hour time trial

A time trial was simulated in which the ergometer was set in the pedaling rate dependent mode. After a short warming up (5 min 100W) subjects were asked to perform a certain amount of work (equal to about 1 hour cycling) as fast as possible. The measure of performance was the time to complete the target amount of work.

This target amount of work was based on the maximal workload (W_{max}). Because the aim of the test was to provoke the same energy expenditure as a test at 75% W_{max} until exhaustion which will last about one hour (3600 sec), the target amount of work was calculated according to the formula:

$$\text{Target amount of work (J)} = 0.75 \cdot W_{max} \cdot 3600$$

The ergometer was set in the pedaling rate dependent mode according to the formula: $W = L \cdot (\text{RPM})^2$

L was chosen in a way which would cause a pedaling rate of 90 RPM at 70% W_{max} . In other words the constant " L " was dependent on the subjects W_{max} . This would mean that 75% W_{max} could be achieved at about 100 RPM which

appeared to be the preferential pedaling rate of most cyclists.

The same adaptations to the workload adjuster had to be made as in protocol B: workload and time were registered from which the amount of work could be calculated.

During this test the subject was informed about the percentage of the total preset work that had already been performed. He did not receive information on workload, pedaling rate, time and heart rate.

Statistics

The presence of an order effect was tested within each protocol with a repeated measures analysis of variance (ANOVA). Individual coefficients of variation (CV's) were calculated for each subject and averaged within protocols to obtain an overall CV. Equality of the CV's between protocols was tested with the Mann-Whitney test.

Results

No differences existed between the three groups with respect to age, height, weight, Wmax and Wmax per kg body weight (Table 7.1).

Results are shown in Tables 7.2, 7.3 and 7.4.

Table 7.2 Results of protocol A: 75% Wmax until exhaustion. Values represent the time until exhaustion in seconds. The learning trial was not included in the calculation of the coefficient of variation (CV). No significant changes between the tests could be observed.

	learning trial (s)	test 1 (s)	test 2 (s)	test 3 (s)	test 4 (s)	test 5 (s)	mean (s)	stdev (s)	CV (%)
1A	1890	4002	3039	5871	6070	4837	4764	1274	26.7
2A	1958	3710	3060	3541	4445	5353	4022	895	22.3
3A	1599	3518	1872	3355	2946	4118	3162	835	26.4
4A	1710	3587	*	4827	4435	5493	4586	796	17.4
5A	2670	5478	2626	3958	6141	6705	4982	1670	33.5
6A	1891	3039	1558	3085	3092	1890	2533	748	29.5
7A	2503	1620	2174	1497	1751	1200	1648	358	21.7
8A	4000	5734	3795	4208	5653	3593	4597	1026	22.3
9A	3270	1605	1928	4245	3945	3549	3054	1207	39.5
10A	3651	*	*	*	*	*			
mean	2388	3588	2507	3843	4275	4082	3705	979	26.6
stdev	809	1435	757	1214	1513	1757			

Protocol A

During the learning trial of protocol A subjects exercised at 291 ± 24 W. After this test the workload was slightly adjusted, so that during the 5 experimental trials, the test would last approximately 1 hour. The work rate of 6 subjects was reduced (10–20 W) whereas 3 subjects remained at the original workload. The original workload was 291 ± 22 W and the work rate after the adjustments was 282 ± 23 W. One subject dropped out.

Times until exhaustion during the 5 experimental trials in chronological order were: 60, 41, 64, 71 and 68 min respectively (For accuracy, the results are expressed in seconds in Table 7.2). This resulted in a coefficient of variation (CV) of 26.6%. Individual CV's ranged from 17.4% to 39.5%. No test order effects were observed.

Protocol B

The $W_{\max}$ dependent factor "L" varied within the range of 0.035–0.044 depending on the subjects $W_{\max}$. The amount of work performed during the 15 min time trial ranged from 272 ± 24.2 W to 282.2 ± 31.1 W during the five experimental trials (Table 7.3).

Individual CV's ranged from 1.7% to 5.8%. This resulted in a mean CV of 3.5%. No significant differences between the tests were observed so there appeared to be no test order effects. Even when the learning trial was included in the analysis, there was no test order effect.

Table 7.3 Results of protocol B: 45 min 65% $W_{\max}$ followed by 15 min time trial. Values are average workloads for the 15 min time trial (in W). The learning trial was not included in the calculation of the coefficient of variation (CV). No significant changes between the tests could be observed.

	learning trial	test 1	test 2	test 3	test 4	test 5	mean	stdev	CV
	(W)	(W)	(W)	(W)	(W)	(W)	(W)	(min)	(%)
1B	320.0	292.0	320.6	327.5	321.7	323.4	317.0	14.2	4.5
2B	294.0	278.2	297.1	287.8	299.6	288.4	290.2	8.5	2.9
3B	261.0	262.0	300.1	302.5	286.0	293.8	288.9	16.3	5.7
4B	237.7	233.0	227.8	219.7	236.6	219.7	227.4	7.7	3.4
5B	231.7	269.0	280.2	277.7	275.7	270.9	274.7	4.7	1.7
6B	295.0	306.0	277.9	325.2	316.2	307.0	306.5	17.8	5.8
7B	227.5	240.4	251.3	258.6	242.3	251.4	248.8	7.4	3.0
8B	273.0	277.4	281.9	280.9	284.4	286.0	282.1	3.3	1.2
9B	221.0	293.1	285.0	304.0	271.8	298.9	290.6	12.6	4.4
10B	266.9	295.8	284.1	299.2	297.6	303.0	295.9	7.1	2.4
mean	262.3	272.3	280.2	287.1	281.6	282.2	282.2	10.0	3.5
stdev	35.3	24.2	27.2	33.7	29.3	31.1			

Reproducibility of endurance performance tests

Table 7.4 Results of protocol C: Time trial with a fixed amount of work to be performed. Values represent the time to complete the fixed amount of work (in seconds). The learning trial was not included in the calculation of the coefficient of variation (CV). No significant changes between the tests could be observed.

	learning trial	test 1	test 2	test 3	test 4	test 5	mean	stdev	CV
	(s)	(s)	(s)	(s)	(s)	(s)	(s)	(s)	(%)
1C	3774	4014	3880	3820	3636	3530	3776	193	5.1
2C	-	3830	3804	3833	3754	3816	3807	32	0.8
3C	3816	3483	3553	3460	3550	3324	3474	93	2.7
4C	3515	3634	3697	3525	3790	3512	3632	117	3.2
5C	3814	3718	3461	3454	3630	3512	3555	115	3.2
6C	4033	4174	3797	4227	3754	3800	3950	230	5.8
7C	3464	3700	3524	3515	3536	3494	3554	83	2.3
8C	3866	3762	3766	4025	3690	3644	3777	148	3.9
9C	4223	4123	3929	3921	3809	3934	3943	113	2.9
10C	4152	3958	3854	3909	3881	3613	3843	134	3.5
mean	3813	3826	3712	3753	3683	3618	3731	126	3.4
stdev	248	232	165	279	101	195			

Protocol C

The time to complete the preset amount of work varied between 3618 ± 195 sec ($= 60 \pm 3$ min; 5) and 3826 ± 232 sec ($= 64 \pm 4$ min; 1) (see Table 7.4). As in protocol A and B, no significant differences between the tests were observed so there appeared to be no test order effects. Even when the learning trial was included in the analysis, there was no test order effect. Individual CV's ranged from 0.8% to 5.8%. Mean CV for this test was 3.4%. Both protocols B and C show significantly lower CV's than protocol A (both p-values < 0.0001). The CV's for protocols B and C were not significantly different ($P=0.88$).

Discussion

A continuous protocol at a submaximal load until exhaustion is the classical and most common performance test. In the past most endurance performance tests have been performed at exercise intensities ranging from 60 to 90% $\text{VO}_{2\text{max}}$ until exhaustion. The most remarkable finding of the present study is the poor reproducibility of a continuous exercise protocol until exhaustion (CV 26.6%). This is in close agreement with Krebs and Powers (5) who were the first to mention that reports concerning the reliability of performance tests were noticeably absent from the literature. Hence they examined the reliability of cycling in a laboratory at approximately 80% $\text{VO}_{2\text{max}}$. They maintained the VO_2 at a constant level by

adjusting the workload. Subjects were tested twice, 4-hours postprandial at the same time of the day and they were verbally encouraged to exercise as long as possible. The interclass correlation coefficient for the two trials was $r=0.51$ ($r^2=0.26$). Furthermore within subject test-retest variability ranged from 5.2 - 55.9% (mean = 20.3%).

We therefore agree with Krebs and Powers (5) that this kind of performance test at intensities of about 80% VO_2 max is not reliable. The observed poor reproducibility, might be explained by the fact that psychological factors may contribute to significant changes of performance independent of the experimental manipulation. Because these tests have an "open end", psychological factors that might influence performance, such as motivation, monotony, and boredom are more pronounced than in the time trial oriented approach where the "endpoint is known".

Endurance performance tests with a known endpoint (a certain target amount of work or time), such as the time trial protocols in the present study, appear to be highly reproducible.

Time trial protocols B and C had CV's of 3.5% and 3.4% respectively. In a study of Coyle et al (2) performance of elite-national class cyclists was evaluated by determining the highest average work rate that each subject could maintain for 1 hour using a Monark ergometer. The subjects were allowed to vary both the resistance on the flywheel and pedal cadence, which were monitored continuously, in selecting the power output. The subjects cycled intermittently for 1-2 min periods at the approximate performance work rate, as a part of their warming-up. The initial work rate was set upon a performance prediction from the results of the previous day's lactate evaluation as well as from the subjects perception during the warm-up. The subjects were instructed to maintain this initial work rate for 8 min, after which it could be adjusted up and down. During the test the subjects had a visual display of the elapsed time, pedal cadence, flywheel resistance, work rate and their heart rate. They were strongly encouraged by the investigators. It was concluded that their actual road racing 40 km time trial performance was highly correlated with average absolute power during the 1 hour laboratorium performance test ($r=-0.88$; $p < 0.001$).

Hickey et al (4) validated a time trial cycling performance test consisting of a test on a computerized cycle ergometer in an isokinetic mode. Subjects were instructed to complete a fixed work output as fast as possible and were allowed to adjust cadence in 5 rpm increments. Eight subjects completed 12 trials involving 4 successive performance rides at three total work outputs (1600, 200 and 14 kilojoules), comparable to 40, 5 and 0.5 miles. A shorter time to complete the task indicated improved performance.

Performance time in the LT was approximately 105 min, in the MT 12 min and the ST 0.5 min. The mean coefficient of variation (CV) for the medium (MT, 200 kJ) and long trial (LT, 1600 kJ) rides were 0.95% and 1.01% respectively. The CV for

the short trial (ST, 14 kJ) ride was significantly greater than the CV for MT and LT respectively.

However trial 4 in the LT was significantly faster than the other trials. The authors attributed this to the knowledge of the subjects that this was their last test. It was suggested that trained cyclists are able to reproduce endurance performance in a time trial protocol as described with a coefficient of variation of approximately 1.0%.

This is also in agreement with the present findings where a CV of 3.5% was found for a preloaded time trial (protocol B) and a CV of 3.4% for protocol C. Furthermore the protocols B and C are more practice oriented whereas protocol A is a rather unusual type of performance. The fact that the CV was much smaller in this type of trial might be related to the fact that subjects know when they finish in contrast to trial A where there is an open end.

There appeared to be no learning effect or any other test order effect during the three different trials. This implicates that it is not necessary to perform an extra learning trial prior to the start of a study with one of the performance tests described. However, the subjects in the present study were already customized to the procedures at the lab and had performed exercise tests before.

In conclusion, the classical exercise protocol for measuring endurance performance (exercise time until exhaustion) in cyclists is not reliable at intensities of 75-80% VO_2 max unless the workload is related to the lactate threshold (2). Time trial protocols, such as described above, appear to be much more reliable since their reproducibility is higher. In these tests there appears to be no learning effect or time trend in well-trained cyclists or triathletes.

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Chapter 8

Effect of MCT and carbohydrate ingestion during exercise on substrate utilization and subsequent cycling performance

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Abstract

The aim of the present study was to investigate the effect of MCT ingestion during exercise on subsequent time-trial cycling performance. Seven well trained cyclists performed 4 exercise trials consisting of 2 h at 60% VO_2max followed by a simulated time-trial (i.e. completion of a preset amount of work as fast as possible) of approximately 15 min duration. During the trials, subjects ingested (1) a 10% carbohydrate solution (CHO; 170 ± 6 g glucose), (2) a 10% carbohydrate electrolyte solution with 5% MCT (CHO+MCT; 85 ± 3 g MCT), (3) 5% MCT (MCT) or (4) artificially colored and flavored water (PLAC). CHO or CHO+MCT ingestion had no effect on performance compared to water PLAC, while MCT had a negative effect. Average work rate during the time-trial was 314 ± 19 W, 314 ± 13 W and 312 ± 18 W with CHO, CHO+MCT and PLAC respectively and was 17-18% lower in the MCT trial (263 ± 22). In addition MCT ingestion compared to PLAC had no effect on total rates of fat or CHO oxidation nor on exogenous or endogenous CHO utilization. This negative effect of MCT was associated with increased gastrointestinal complaints (i.e. intestinal cramping). These data suggest that large amounts of MCT (85 g) ingested during prolonged submaximal exercise may provoke gastro-intestinal problems leading to decreased exercise performance.

Introduction

Carbohydrate (CHO) supplementation has received much attention in exercise physiology since the late 1960's. Carbohydrate feedings have been shown to increase performance during prolonged exercise lasting 1 hour or longer (4, 6, 17). Ingested carbohydrates may be oxidized at rates up to $1 \text{ g} \cdot \text{min}^{-1}$, which appears to be the maximal oxidation rate of this exogenous substrate (13, 32). Therefore we argued that medium chain triglycerides (MCT) may be a valuable additional substrate to be ingested during exercise (19-21). MCT have a smaller molecular size compared to long chain triglycerides (LCT), they have a lower melting point and are more soluble in water. Because of these different physical properties, MCT are rapidly absorbed and enter the liver directly through the portal vein whereas LCT have to be transported through the relatively slow lymphatic system (2). Studies with stable isotopes have shown that MCFA are rapidly oxidized at rest (24) and during exercise (19, 23). We showed that CHO coingested with MCT accelerated the oxidation of MCT during the first 90 min of exercise (19). By adding MCT to a carbohydrate solution, the oxidation rates of these carbohydrates will not be impaired (21) and the MCT can serve as an additional fuel for the working muscle (19, 20). However, in recent studies we found that MCT, although rapidly oxidized, contributed only marginally to total energy expenditure (19, 20) and did not spare muscle glycogen (21). In these studies the amount of MCT ingested was small (about 30 grams) and no rise in plasma FFA was observed after MCT ingestion. Studies in which high plasma FFA were induced by giving either a high fat meal or an intravenous infusion of a triglyceride emulsion (Intralipid®) in combination with heparin have reported reduced muscle glycogen utilization during exercise compared to control conditions (7, 11, 31). This has lead to the suggestion that ingestion of sufficient amounts of MCT may elevate plasma FFA levels, spare muscle glycogen and improve performance. Van Zeyl et al (30) gave large amounts of MCT (86 grams) during 2 hours of cycling exercise at $60\% \text{VO}_{2\text{max}}$ followed by a 40 km time-trial. It was observed that plasma FFA levels were elevated and 40 km time-trial performance was improved when MCT was coingested with CHO. However, performance was decreased when only MCT was ingested compared to CHO even though in the MCT trial plasma FFA levels were elevated. Unfortunately the study had no water control group (without CHO and MCT ingestion), and thus the separate effect of MCT could not be studied. We have previously observed gastrointestinal discomfort when MCTs were ingested in large quantities, which of course may negatively affect performance.

Accordingly, the aim of this study was to examine the metabolic effects of ingesting a large quantity of MCT (85 g), alone and in combination with CHO during 2 h of cycling compared to CHO or water ingestion. A second purpose was to investigate the effect of CHO, MCT or CHO+MCT or water ingestion on subsequent time-trial cycling performance.

Methods

Subjects

Seven endurance trained male cyclists who trained regularly more than 2 hours a day, 4-7 days a week, were recruited for this study. Five to seven days prior to the first experimental trial, maximal oxygen consumption ($\text{VO}_{2\text{max}}$), maximal work rate (W_{max}) and peak heart rate (HR_{peak}) were determined in a stepwise incremental exercise test to exhaustion as described by Kuipers et al. (22). After a warm-up period of 5 min at 100 W, the work rate was increased by 50 W every 2.5 min until a heart rate of 160 bpm was reached. Then the work rate was increased by 25 W every 2.5 min. The W_{max} was determined by the following formula:

$$\text{W}_{\text{max}} = \text{W}_{\text{out}} + (t / 150) * 25$$

where W_{out} is the last completed stage, t is the time in the final stage. All exercise tests were performed on an Excalibur Sport electromagnetically braked cycle ergometer (Lode, Groningen, The Netherlands). Heart rate was measured continuously by a Sporttester® (Polar, Kelmele, Finland). Mean $\text{VO}_{2\text{max}}$, W_{max} and HR_{peak} were $74 \pm 1 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $401 \pm 17 \text{ W}$ and $187 \pm 1 \text{ BPM}$ respectively. The study was approved by the local Medical Ethical Committee.

Procedure

On the day of the test subjects reported to the laboratory at 12:00 PM after a standardized breakfast in the morning. At the laboratory a standardized lunch was provided. At 13:15 PM a teflon catheter (Baxter Quick Cath Dupont, Ireland) was inserted into an antecubital vein and connected to a threeway stopcock (Discofix-3 Braun Meisungen, Germany). At 13:30 PM a resting blood sample was drawn. Resting breath gases were collected for the measurement of oxygen consumption and carbon dioxide production (Oxycon β , Mijnhardt, The Netherlands). At 13:55 AM subjects rode 5 min at 100W as a warm-up. At 14:00 PM subjects started cycling at 60% $\text{VO}_{2\text{max}}$ for 2 h and in the first minute they had to drink an initial bolus ($8 \text{ ml} \cdot \text{kg}^{-1}$) of either one of the test drinks. Thereafter every 15 min a beverage volume of $2 \text{ ml} \cdot \text{kg}^{-1}$ was provided. Blood samples (10 ml) were collected every 30 min and expiratory gases were collected every 15 minutes in 15 ml vacutainers (Becton & Dickenson).

Time trial

After 120 min of exercise at 60% $\text{VO}_{2\text{max}}$, a simulated time-trial of approximately 15 min duration was performed as previously described (18). The ergometer was set in the pedaling rate dependent mode (work rate exponentially increases as the pedaling rate increases). Subjects were asked to perform a certain amount of work (equal to about 15 min cycling at 75% of their maximal work load) as fast as possible. The measurement of performance was the time to complete the set amount of work. This total amount of work was based on the maximal work rate (W_{max}) and was calculated according to the formula:

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Total amount of work = $0.75 \cdot W_{\max} \cdot 900 \text{ s}$

The ergometer was set in the pedaling rate dependent mode according to the formula

$$W = L \cdot (\text{RPM})^2$$

In which RPM is the pedaling rate and L is a constant factor which is dependent on a subjects' $W_{\max}$. L was chosen in a way, which at a pedaling rate of 90 RPM, elicited 70% $W_{\max}$. The ergometer was connected to a computer which on line recorded work rate and time and calculated the amount of work performed as previously described (18). The only information the subjects received was the amount of work performed and the percentage of work performed relative to the pre set amount of work (0% at the start, 100% at completion of the trial). Subjects did not receive information regarding work rate, pedaling rate, time or heart rate. During all tests environmental conditions were standardized, temperature was kept at 20°C and an electric fan circulated air in order to minimize thermal stress. Subjects were encouraged by the same person every test. This performance test has been shown to produce highly reproducible results: coefficient of variation of this test was 3.35% (18).

To avoid any influence of circadian variance, subjects performed their tests at the same time of the day. Subjects were not allowed to train the day before the test. Two days before the test, training was not allowed to be exhaustive. Additionally, all subjects were instructed to eat the same meal the evening prior to the test, which was controlled by a registration procedure. They were advised to eat a carbohydrate rich meal such as pasta the evening prior to the test. To further control carbohydrate and energy intake, food records were provided for the day prior to the test and the day of the test.

Beverages

Subjects received four drinks on four different occasions, subsequent tests being separated by at least 5 days. The drinks consumed were (1) a 10% carbohydrate solution (CHO; $170 \pm 6 \text{ g}$ glucose), (2) a 10% CHO solution with 5% MCT (CHO+MCT; $170 \pm 6 \text{ g}$ glucose, $85 \pm 3 \text{ g}$ MCT), (3) 5% MCT ($85 \pm 3 \text{ g}$ MCT) or (4) placebo consisting of artificially colored and flavored water (PLAC). The carbohydrates in the CHO+MCT and CHO drink consisted of corn derived glucose (Amylum, Belgium) and the ^{13}C -enrichment of this CHO was $-11.3 \text{ \delta}$ per mil vs PDB as determined by combustion-IRMS (Carlo Erba - Finnigan MAT 252, Bremen, Germany). MCT contained fatty acids with a chain length of 99% C8 (Triocanoate, Estasan GT8-99, Unichema, Barcelona, Spain). To each liter of drink 5 g fructose, 730 mg citric acid, 160 mg aspartame and 640 mg of a flavor substance (orange) was added to make the taste comparable in all trials.

Subjects ingested $8 \text{ ml} \cdot \text{kg}^{-1}$ of either one of the drinks during the first minute of the warming up and $2 \text{ ml} \cdot \text{kg}^{-1}$ every 15 min thereafter. Such a drinking pattern has pre-

viously been reported to result in high rates of gastric emptying throughout exercise (27). Drinks were given in a randomized order and subjects were unaware of the content of the drink.

During the CHO and CHO+MCT trials, subjects ingested a glucose solution with a high natural abundance of ^{13}C to study oral glucose oxidation. The PLAC trial was used to allow correction for changes in breath $^{13}\text{CO}_2$ background enrichment during exercise. To avoid background shifts standard procedures were followed (19, 20, 32, 33). Subjects were instructed not to consume any products with a high natural abundance of ^{13}C during the entire experimental period. This was done to minimize a shift in background enrichment due to changes in endogenous substrate utilization. Furthermore subjects were instructed to keep their diet as constant as possible the days before the trials.

Analysis

Blood (5 ml) was collected into EDTA containing tubes and was centrifuged for 4 min at 4°C . Aliquots of plasma were frozen immediately in liquid nitrogen and stored at -40°C until analysis of glucose (Roche, Uni Kit III, 0710970), lactate (12), β -hydroxybutyrate (25), free fatty acids (FFA) (Wako FFA-C test kit, Wako Chemicals, Neuss, Germany), and glycerol (Sigma, GPO-trinder 337) which were performed with the COBAS BIO semi automatic analyzer. From indirect calorimetry (RQ , VO_2) and stable isotope measurements ($^{13}\text{CO}_2$ / $^{12}\text{CO}_2$) (continuous flow-IRMS, Finnigan MAT 252, Bremen, Germany), total energy expenditure and oxidation rates of total fat, total carbohydrates and exogenous glucose were calculated.

Calculations

From VCO_2 and VO_2 carbohydrate and fat oxidation rates were calculated using stoichiometric equations (26).

$$\text{glucose oxidation} = 4.585 \text{ VCO}_2 - 3.226 \text{ VO}_2$$

$$\text{fat oxidation} = 1.695 \text{ VO}_2 - 1.701 \text{ VCO}_2$$

The isotopic enrichment was expressed as the delta per mil difference between the ^{13}C and ^{12}C ratio of the sample and a known laboratory reference standard according to the formula of Craig (9):

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

The $\delta^{13}\text{C}$ was then related to an international standard Pee Dee Bellemnitella (PDB-1).

The amount of glucose oxidized was calculated according to the formula:

$$\text{exogenous glucose oxidation} = \text{VCO}_2 \cdot \left(\frac{\delta \text{ Exp} - \delta \text{ bkg}}{\delta \text{ Ing} - \delta \text{ bkg}} \right) \left(\frac{1}{k} \right)$$

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In which δ bkg is the ^{13}C enrichment of expired air in the background trial (PLAC served as the background correction trial), 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 ingested glucose and k is the amount of CO_2 (in liters) produced by the oxidation of 1 gram glucose ($k = 0.7467$ liter CO_2 per gram glucose).

Total carbohydrate oxidation minus exogenous glucose oxidation was used to calculate endogenous glycogen utilization.

endogenous glycogen utilization = total CHO oxidation - exogenous glucose oxidation

Questionnaires

At 0, 60 and 120 min and at completion of the time-trial subjects were asked to fill out a questionnaire. This questionnaire contained questions about the presence of gastro-intestinal discomfort or problems at that moment. Specifically these questions addressed the following possible complaints: nausea, bloated feeling, belching, gastro-intestinal cramping, vomiting, diarrhea, urge to defaecate, headache and dizziness. These items were scored on a 10-point scale.

Statistics

The presence of an order effect and the effect of the drinks on performance was tested with a repeated measures analysis of variance (ANOVA). Statistical significances were located with a Scheffé's post-hoc test.

Results

Time trial cycling performance

Times to complete the preset amount of work in the four trials are presented in Table 1 and were not significantly different between CHO, CHO+MCT or placebo.

Table 8.1 Results time-trial (TT): Time to complete the preset amount of work, mean and peak heart rate (HR) and ratings of perceived exertion (RPE) during the time-trial. * denotes a significant difference ($P < 0.05$) between MCT and the other trials (mean $\pm$ SEM; $n=7$).

Trial	time TT (min)	average work rate (W)	mean HR TT (BPM)	max HR TT (BPM)	RPE (0-10)
CHO	14:11 $\pm$ 0:34	314.4 $\pm$ 18.5	180 $\pm$ 2	187 $\pm$ 2	9.14 $\pm$ 0.34
CHO+MCT	14:01 $\pm$ 0:19	313.9 $\pm$ 13.0	181 $\pm$ 2	187 $\pm$ 2	9.29 $\pm$ 0.29
MCT	17:20 $\pm$ 1:06*	263.1 $\pm$ 22.4*	170 $\pm$ 4*	182 $\pm$ 6	9.00 $\pm$ 0.44
PLAC	14:26 $\pm$ 0:42	311.6 $\pm$ 17.8	182 $\pm$ 2	187 $\pm$ 2	8.57 $\pm$ 0.81

Time to accomplish the preset amount of work in the MCT trial was significantly longer compared to the other trials. There was no test order effect. Average work rate during the time-trial was 17-18% lower in the MCT trial compared to the other trials (Figure 8.1). Mean heart rate was also significantly lower during the MCT trial whereas the mean heart rate in the other trials was comparable. No statistical differences were observed in peak heart rate during the time-trial and in ratings of perceived exertion.

Figure 8.1 Average work rate during the time-trial with ingestion of carbohydrates (CHO), carbohydrates and medium chain triglycerides (CHO+MCT), MCT or placebo (PLAC). * denotes a significant difference between MCT and the other trials ($P < 0.05$) (mean $\pm$ SEM; $n=7$).

Blood parameters

During the 120 min of steady state exercise, plasma glucose concentration initially rose from resting values of 4.6-5.1 mM to 5.8 mM during CHO and CHO+MCT, whereas in both PLAC and MCT plasma glucose concentrations showed a tendency to decline (Figure 8.2).

Plasma glucose concentrations during MCT (and PLAC at 60 min) were significantly ($P < 0.05$) lower during the two hours of exercise compared to the two CHO trials (CHO and CHO+MCT). Plasma glucose concentration increased during the time-trial but this increase was only significant in the MCT and PLAC trial (Table 2).

Plasma lactate concentrations during the 120 min of exercise were stable and similar in all trials and average values are below 1 mM (0.7-0.9 mM; Figure 8.2). Plasma lactate increased during the time-trial to high levels during CHO, CHO+MCT and PLAC (8.0-10.3 mM) and increased less during MCT (3.6 mM; Table 2).

Plasma FFA concentrations increased from resting values between 213 and 292 μ M to 406 $\pm$ 87 μ M during CHO, 506 $\pm$ 87 μ M during CHO+MCT and increased to values above 700 μ M at the end of 2 h. exercise when no carbohydrates were ingested (722 $\pm$ 146 during PLAC and 820 $\pm$ 134 during MCT). During exercise plasma FFA concentrations were significantly higher ($P < 0.05$) with MCT compared to the CHO and CHO+MCT trial (except at 120 min). Also during the time-trial plasma

Figure 8.2 Plasma glucose, lactate, free fatty acids (FFA), glycerol and β -hydroxybutyrate during 120 min exercise at 60% VO_2max with ingestion of CHO, CHO+MCT, MCT or placebo. * denotes a significant ($P < 0.05$) difference between CHO and MCT. † denotes a significant ($P < 0.05$) difference between CHO+MCT and MCT. # denotes a significant ($P < 0.05$) difference between CHO and PLAC. § denotes a significant ($P < 0.05$) difference between CHO+MCT and PLAC. @ denotes a significant ($P < 0.05$) difference between MCT and PLAC.

FFA were higher in the MCT trial (Table 2).

Glycerol concentration rose gradually during exercise in all trials. Although with CHO feeding glycerol concentration seemed to be somewhat lower by the end of 2 h. exercise, this difference was not statistically significant. Only at 120 min glycerol concentration during PLAC was significantly ($P<0.05$) higher compared to CHO and CHO+MCT. During the time-trial glycerol concentrations further increased and concentrations remained lower in the carbohydrate feeding trials ($P<0.05$; Table 8.2).

β -hydroxybutyrate concentrations displayed a small rise from 200–250 μM to 222–380 μM during CHO, CHO+MCT and PLAC but increased markedly with MCT (883 μM ; $P<0.05$). During the time-trial β -hydroxybutyrate concentrations significantly increased to high levels (1.1–1.7 mM; Table 2).

Table 8.2 Plasma glucose, lactate, FFA, glycerol and β -hydroxybutyrate after 120 min of exercise at 60% VO_2max (i.e. the start of the time-trial) and directly after the time-trial. * denotes a significant ($P<0.05$) difference before and directly after the time-trial. † denotes a significant ($P<0.05$) difference compared to CHO and CHO+MCT. # significantly different from PLAC ($P<0.05$). § significantly different from CHO ($P<0.05$) (mean $\pm$ SEM; $n=7$).

beverage	time	glucose (mmol·l ⁻¹)	lactate (mmol·l ⁻¹)	FFA ($\mu\text{mol}\cdot\text{l}^{-1}$)	glycerol ($\mu\text{mol}\cdot\text{l}^{-1}$)	β -hydroxy- butyrate (mmol·l ⁻¹)
CHO	120 min.	4.87 $\pm$ 0.16	0.8 $\pm$ 0.1	406 $\pm$ 87	159 $\pm$ 33	222 $\pm$ 27
	end TT	5.24 $\pm$ 0.35	8.0 $\pm$ 1.9*	442 $\pm$ 47	274 $\pm$ 44	1085 $\pm$ 107*
CHO+MCT	120 min.	4.73 $\pm$ 0.21	0.7 $\pm$ 0.1	506 $\pm$ 87	134 $\pm$ 22	376 $\pm$ 69
	end TT	5.29 $\pm$ 0.60	9.4 $\pm$ 1.2*	533 $\pm$ 128	217 $\pm$ 29	1739 $\pm$ 186§*
MCT	120 min.	4.00 $\pm$ 0.10†	0.9 $\pm$ 0.1	819 $\pm$ 134†#	233 $\pm$ 29†	883 $\pm$ 223†
	end TT	4.54 $\pm$ 0.27*	3.6 $\pm$ 0.8†#*	950 $\pm$ 219†#	324 $\pm$ 25†	1253 $\pm$ 111†*
PLAC	120 min.	4.38 $\pm$ 0.13	0.9 $\pm$ 0.1	722 $\pm$ 146	271 $\pm$ 42†	383 $\pm$ 52
	end TT	5.49 $\pm$ 0.20*	10.3 $\pm$ 1.9*	592 $\pm$ 118	362 $\pm$ 47†	1652 $\pm$ 193*

Indirect calorimetry

During the second hour of exercise R-values averaged 0.88–0.91 in CHO and CHO+MCT and was significantly lower with MCT and PLAC (0.83–0.88). Carbohydrate ingestion reduced fat oxidation in both CHO and CHO+MCT compared to PLAC and MCT and maintained CHO oxidation rates above 2 g·min⁻¹ (Figure 8.3). Without CHO ingestion, CHO oxidation rates decreased to 1.5 g·min⁻¹ (Figure 8.3). No differences were observed between CHO and CHO+MCT and between PLAC and MCT.

Figure 8.3 Total carbohydrate and fat oxidation during 120 min exercise at 60% $\dot{V}O_{2\max}$ with ingestion of CHO, CHO+MCT, MCT or placebo.

* denotes a significant difference between CHO and MCT and between CHO and PLAC ($P < 0.05$). † denotes a significant difference between CHO+MCT and MCT and PLAC ($P < 0.05$).

Exogenous glucose oxidation

Background ^{13}C enrichment measured from the resting breath samples was -26.1 ± 0.2 δ per mil vs. PDB. With ingestion of the corn-derived glucose in the CHO and CHO+MCT trial the rise in ^{13}C was significant, reaching a δ per mil vs. PDB difference of approximately 3.5 towards the end of 120 min exercise (compared to resting breath sample). The changes in background enrichment during exercise in the C trial were about 10% of the ^{13}C -enrichment provoked by the exogenous glucose in the CHO and CHO+MCT trials. Therefore a background

correction was made for the calculation of exogenous glucose oxidation, using the data from the PLAC trial. No differences were observed in background enrichment between MCT and PLAC. Exogenous glucose oxidation showed a gradual increase over time and levelled off after approximately 75 min (Figure 8.4).

Figure 8.4 Exogenous carbohydrate oxidation during CHO and CHO+MCT and endogenous carbohydrate oxidation during 120 min exercise at 60% VO_2max with ingestion of CHO, CHO+MCT, MCT or placebo.

Maximal oxidation rates were $0.81 \pm 0.03 \text{ g}\cdot\text{min}^{-1}$ (CHO) and $0.78 \pm 0.03 \text{ g}\cdot\text{min}^{-1}$ (CHO+MCT), respectively. No differences in oral CHO oxidation between CHO and CHO+MCT were observed. Endogenous CHO (liver and muscle glycogen) oxidation rates as calculated from total CHO oxidation minus exogenous glucose oxidation was similar in CHO and CHO+MCT (Figure 8.4).

Gastro-intestinal discomfort

Gastro-intestinal complaints were registered by a questionnaire. Selected complaints are displayed in Table 8.3. Vomiting and diarrhea were not reported during exercise. Two subjects vomited after the MCT trial, 3 complained of diarrhea afterwards. Nausea, headache and dizziness were not significantly different among the trials. Belching and a bloated feeling in the stomach was more often reported when CHO, CHO+MCT or MCT was ingested compared to PLAC. Gastro-intestinal cramps occurred significantly more when MCT was ingested and this was the most serious complaint reported during exercise.

Table 8.3 Gastro-intestinal (GI) discomfort after 120 min of exercise at 60% VO_2max (i.e. the start of the time-trial) and directly after the time-trial. * denotes a significant ($P < 0.05$) difference compared to PLAC. † denotes a significant ($P < 0.05$) difference compared to CHO.

beverage	time point	nausea	belching	bloated feeling	headache and/or dizziness	GI-cramping
CHO	120 min. end TT	1.0±1.0 1.0±1.0	1.4±1.0* 0.0±0.0	1.4±1.4* 1.4±1.4*	0.7±0.7 1.0±0.1	0.0±0.0 0.0±0.0
CHO+MCT	120 min. end TT	1.3±1.2 1.2±1.1	1.2±0.7* 0.3±0.3*	2.5±1.5* 1.7±1.1*	1.5±0.9 1.3±1.2	2.0±1.3*† 1.8±1.2*†
MCT	120 min. end TT	0.1±0.1 1.1±1.1	1.7±0.7* 1.7±0.8*	1.7±1.1* 1.1±1.1*	1.5±0.9 1.1±1.1	2.0±1.0*† 4.1±1.3*†
PLAC	120 min. end TT	0.0±0.0 0.0±0.0	0.0±0.0 0.0±0.0	0.0±0.0 0.0±0.0	0.6±0.4 0.0±0.0	0.0±0.0 0.0±0.0

Discussion

Fat supplementation during exercise is believed to be undesirable for several reasons. First of all, endogenous fat stores are very large and therefore fat ingested during exercise would not add appreciable triglyceride to the total body content. Secondly, the digestion and absorption of fat is slow and the appearance of ingested LCT in the blood stream may take 3-4 hours. Dietary fat contains long chain triglycerides (LCT) which have to be hydrolyzed first in the intestine, after which long chain fatty acids diffuse into the intestinal mucosa cells for reesterification to triglycerides. These long chain triglycerides will then be incorporated into chylomicrons and transported through the lymphatic system, which ultimately drains in the systemic circulation. A third reason why fat supplementation is not recommended is the fact that fat will reach the circulation in chylomicrons which are generally believed to be no major energy source for the working muscle. Besides this, LCT is known to be a potent inhibitor of gastric emptying. For these reasons, fat supplementation during exercise is not recommended. However, it has been shown that medium chain triglycerides do not slow gastric emptying (3), are rapidly hydrolyzed and absorbed, and they enter the systemic circulation directly through the portal vein (2). It has also been shown that ingested MCT was oxidized at high rates relative to their rate of ingestion (10, 19, 20, 23). In addition to this, we recently showed that MCT coingested with CHO were more rapidly oxidized (i.e. reached maximal MCT oxidation rates more quickly) than MCT alone (19). Furthermore, we showed that 30 g MCT coingested with CHO did not significantly affect exogenous CHO oxidation during 3 hours of exercise at ~60%

$\text{VO}_{2\text{max}}$ (21). Only few studies investigated the effect of MCT ingestion on exercise performance. Satabin et al. (29) have studied the effect of preexercise ingested MCT on exercise capacity and found no effect of a single 45 g bolus MCT or equal caloric amount of glucose on times to fatigue at 60% $\text{VO}_{2\text{max}}$. Recently, van Zeyl et al. (30) reasoned that the amount of MCT provided was too small to detect performance effects of MCT ingestion and they gave their subjects 86 g of MCT in repeated small boluses during exercise. After 120 min of submaximal exercise at 60% $\text{VO}_{2\text{max}}$, their subjects performed a simulated 40 km time trial. Performance was decreased with MCT ingestion compared to CHO, but simultaneous ingestion of CHO and MCT resulted in an increased performance compared to CHO (i.e. average speed during the time trial). One of their hypothesis was that CHO+MCT ingestion improved performance compared to CHO due to an increased availability of plasma FFA and a subsequent sparing of muscle glycogen (30). This has been suggested to favorably influence exercise capacity (14, 28). However, van Zeyl et al (30) had no placebo control group that received no MCT or CHO and the separate effect of MCT compared to control was not investigated. Therefore the present study was aimed at investigating the effect of ingestion of large amounts of MCT (85g) with or without carbohydrates compared to the ingestion of CHO or water on time trial cycling performance. As in the study of van Zeyl et al (30) drinks were ingested during two hours of submaximal exercise at 60% $\text{VO}_{2\text{max}}$ and thereafter a time trial was performed. Interestingly we found no differences in performance when subjects ingested CHO, CHO+MCT or water. However, when subjects ingested MCT, performance was significantly decreased.

One of the possible explanations for the fact that in this study MCT added to a glucose solution did not have the positive effect reported by van Zeyl et al (30) is the difference in the duration of the time trial. We applied a 15 min time trial in contrast to the 60 min time trial used by van Zeyl et al (30) which was chosen after pilot studies showed that subjects developed serious gastro-intestinal discomfort when the time trial lasted longer than 15 min. However, the test to test coefficient of variation of a time-trial lasting approximately 1 h was similar to the coefficient of variation of a 15 min time trial (18). Both performance tests have shown to be highly reproducible (18).

Other differences between the study of van Zeyl et al (30) and ours may include the type of CHO (short chain glucose polymers versus glucose), the type of MCT (a mixture of C8 and C10 versus pure C8) used and the time of the day at which experiments were performed (morning versus early afternoon). How these differences would explain the different findings, however, is unclear.

The most likely explanation for the differences between the study of van Zeyl et al (30) and the present study, may be the differences in gastro-intestinal discomfort. In the present study subjects more often reported gastro-intestinal cramping with MCT which was negatively correlated to their performance. Because of this gastro-intestinal discomfort, subjects reported that they could not push as hard as they could without gastro-intestinal discomfort. This is supported by a lower mean heart rate (Table 8.1) and lower plasma lactate and β -hydroxybutyrate concentra-

tions (Figure 8.1) which are the result of a lower absolute work rate (Table 8.1, Figure 8.1). Similar gastro-intestinal complaints have been reported by Ivy et al (16). PLAC provoked the least gastro-intestinal discomfort, whereas CHO given in these relatively large quantities (on average 85 grams per hour) resulted in a bloated feeling. This may be the explanation for the lack of performance effect after CHO ingestion. Van Zeyl et al (30) however, did not report major gastro-intestinal problems during exercise when CHO+MCT was ingested.

Interestingly, we did not observe a positive effect on time trial performance of CHO compared to water PLAC. Previously we have shown that CHO ingestion during exercise may improve time trial performance lasting approximately 1 h (17). Here the time trial was relatively short (14 min) which may have prevented a performance effect. However we strongly feel that the bloated feeling as reported by the subjects after CHO ingestion was the major reason of a lack of time trial performance improvement. The amount of CHO provided in the previous trials was 75g per 60 min (17) and 170g per 2 h in the present study, after which the time trial was performed. This larger total amount of CHO ingested may be responsible for the "bloated feeling" reported by the subjects and the lack of a performance effect compared to PLAC.

In the present study, the exercise intensity increased drastically during the time trial compared to the 120 min submaximal exercise at 60% VO_2max . Glycolysis was stimulated and lactate was formed and accumulated in plasma (Table 2). Plasma glucose concentration increased (significantly in the MCT and PLAC trial) as a result of the high exercise intensity. This has been shown before and has been attributed to increased circulating plasma epinephrine levels which increase hepatic glucose production (5). Apparently the availability of acetyl-CoA units in the liver increased more than the utilization which caused the large increase in plasma β -hydroxybutyrate concentration. Responses seem to be somewhat less pronounced in the MCT trial where the absolute work rate was somewhat less.

As reported previously (4, 8, 19), the CHO markedly blunted plasma FFA and total fat oxidation and maintained high rates of CHO oxidation. Although the amount of MCT ingested was threefold higher compared to previous studies from our lab (19-21) and from others (10, 16, 23), in agreement with these studies, we found no significant differences in total fat and CHO utilization with or without MCT ingestion (Figure 8.2).

Increases in plasma FFA and glycerol were blunted by CHO ingestion but MCT ingestion had no effect on the plasma FFA or glycerol concentration. We have reported before that glycerol concentrations were not significantly elevated after MCT ingestion and we suggested that this was due to the capacity of the liver to use glycerol for gluconeogenesis (19).

Also, in agreement with previous findings (21), MCT did not influence exogenous or endogenous CHO oxidation rates. It has been suggested that MCT ingestion may elevate the plasma FFA concentration and subsequently spare muscle glycogen (7, 11, 31). However several studies have shown that ingestion of 25-30 g of MCT did not lower the rate of muscle glycogen breakdown (1, 10, 15, 21). Howev-

er, van Zeyl et al (30) suggested that the amount of MCT in the above mentioned studies was too small to affect muscle glycogen breakdown. Therefore they gave their subjects large amounts of MCT and tracer data suggested that glycogen breakdown was reduced. Although we did not measure muscle glycogen concentrations, tracer methods do not show a sparing of endogenous CHO stores (liver and muscle glycogen) after MCT ingestion. Because van Zeyl et al (30) reported no differences in plasma glucose oxidation with ingestion of MCT, it is unlikely that we would have observed glycogen sparing in this study since total CHO oxidation rates were similar.

In conclusion, the present study showed that ingestion of large amounts of MCT (85 g) coingested with CHO did not significantly affect total rates of CHO and fat oxidation and had no positive effect on performance. When only MCT was ingested performance decreased by 17-18% probably as a result of gastro-intestinal distress.

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Chapter 9

Reduced oxidation rates of ingested glucose during prolonged exercise with low endogenous CHO availability

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Abstract

This study investigated the effect of endogenous carbohydrate availability on oxidation rates of ingested glucose during moderate intensity exercise. Seven well-trained cyclists performed two trials of 120 min of cycling exercise in random order at 57% $\text{VO}_{2\text{max}}$. Preexercise glycogen concentrations were manipulated by glycogen lowering exercise in combination with CHO restriction (low glycogen; LG) or carbohydrate loading (moderate to high glycogen; HG). In the LG and HG trial, subjects ingested 4 ml·kg⁻¹ bw of an 8% corn-derived glucose solution of high natural ¹³C-abundance at the start, followed by bolusses of 2 ml·kg⁻¹ every 15 min. The third trial, in which potato derived glucose was ingested served as a control test (C) for background correction. Exogenous glucose oxidation rates were calculated from the ¹³C-enrichment of the ingested glucose and of the breath CO₂. Total CHO oxidation was lower in LG than in HG during 60-120 min of exercise (84±7 versus 116±8 g; mean±SEM; P<0.05). Exogenous CHO oxidation in this period was 28% lower in LG compared to HG. Maximal exogenous oxidation rates were also lower (P<0.05) in the LG trial (0.64±0.05 g·min⁻¹) than in the HG trial (0.88±0.04 g·min⁻¹). This decreased utilization of exogenous glucose was accompanied by increased plasma FFA levels (2-3 times higher) and lower insulin concen-

trations. It is concluded that glycogen lowering exercise, performed the evening before an exercise bout, in combination with CHO-restriction leads to a reduction of the oxidation rate of ingested glucose during moderate intensity exercise.

Introduction

Carbohydrate ingestion has become common practice for endurance athletes during prolonged exercise in order to improve performance. Carefully conducted studies showed that glucose ingestion during exercise improved performance both in intermittent (35) and in prolonged continuous exercise (3, 7). During intermittent exercise, the increased time to exhaustion with carbohydrate (CHO) ingestion has been attributed to a sparing of muscle glycogen stores (35). However, during prolonged continuous exercise, muscle glycogen breakdown is not affected by CHO ingestion and the enhanced performance is attributed to a maintenance of blood glucose levels and high rates of CHO oxidation during the late phases of exercise (3). It has been shown that the ingested CHOs reduce hepatic glucose output (i.e. spare liver glycogen) (2, 23). A larger contribution of exogenous carbohydrates is believed to be more beneficial to endurance performance since endogenous substrates are more conserved. In this context it is important to know the factors that affect exogenous carbohydrate oxidation.

Therefore the oxidation of ingested CHOs has been intensively investigated since the late seventies, using stable isotope techniques involving the ingestion of naturally labeled ^{13}C -glucose (21, 25, 33). From a large number of studies it appeared that the extent to which these CHOs are oxidized is dependent on several factors including the amount and type of CHO ingested, the feeding schedule and the exercise intensity. However, at present the effect of variation of preexercise glycogen levels on exogenous glucose oxidation is unclear.

To our knowledge only two studies attempted to address this question. Ravussin et al (28) observed no significant differences in the oxidation rates of ingested carbohydrates in a group of glycogen depleted subjects versus a control group during low intensity exercise at 40% VO_2max . However, the study had no cross-over design, which, taking individual variation into account, weakens the results. Massicotte et al (22) studied exogenous carbohydrate oxidation during 2 hours of exercise at moderate intensity (52% VO_2max) in fed and fasted conditions and did not find differences in the oxidation rates of ingested glucose. However, the primary effect of overnight fasting is emptying of the liver glycogen stores, while muscle glycogen stores change little. Therefore, the fact that these studies did not show an effect of glycogen availability on exogenous CHO oxidation may be caused by experimental or methodological limitations of these studies.

Although little information is available on effect of preexercise glycogen levels on the oxidation rates of exogenous carbohydrates, there are some reports on the effect of muscle glycogen on blood glucose uptake by the muscle, in situations without carbohydrate ingestion. Gollnick et al (10) and Maehlum (19), observed a

higher glucose uptake in a leg with a low muscle glycogen content compared to a normal glycogen leg by measuring a-v differences across the exercising leg. On the other hand, others showed decreased muscle glucose uptake or CHO oxidation when plasma FFA levels were elevated (6, 12, 30, 32). Such an elevation of plasma FFA always occurs when the glycogen depletion is achieved by exercise the night before, followed by fasting or a low CHO diet, until the final exercise the morning after.

Therefore this study investigated the effect of glycogen lowering exercise versus carbohydrate loading on oxidation rates of ingested glucose during 2 h cycling exercise at 57% $\text{VO}_{2\text{max}}$.

Material and Methods

Subjects

Seven well-trained cyclists or triathletes (25 ± 1.4 (SEM) yr, 72.2 ± 1.2 kg) 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 Ethical Committee of the University of Limburg.

Pre testing

Subjects' $\text{VO}_{2\text{max}}$ was measured on an electronically braked cycle ergometer (Lode Excalibur, Groningen The Netherlands) during an incremental exhaustive exercise test (18) one week before the first experimental trial. W_{max} averaged 414 ± 13.7 W and $\text{VO}_{2\text{max}}$ 5.1 ± 0.2 l·min⁻¹. The results of this initial test were used to determine the 50% W_{max} workload which was later used in the experimental trials.

Experimental trials

To obtain a situation with low endogenous carbohydrate availability (i.e. glycogen levels) and a situation with moderate to high endogenous carbohydrate availability a similar protocol was applied as previously described (15). Each subject performed three trials, a low glycogen trial (LG), a normal to high glycogen trial (HG) and a control trial (C), each separated by at least seven days. The trials consisted of 120 min cycling at 50% W_{max} (~ 57% $\text{VO}_{2\text{max}}$). The order of the trials was determined by counterbalancing.

The evening prior to the low glycogen trial (LG) subjects followed a glycogen depletion protocol (13 h preexercise). The depletion trial, starting at 8.00 PM consisted of an intermittent cycling exercise protocol, consisting of 2-min bouts at 90% W_{max} , interspersed with 2 min at 50% W_{max} . When the subjects were unable to complete the 2 min 90% W_{max} , the high workload was lowered to subsequently 80%, 70% and finally 60% W_{max} . The exercise was stopped when the 2 min at 60% W_{max} could not be completed anymore. This protocol has previously been shown to be effective in lowering glycogen levels (<35 $\mu\text{mol} \cdot \text{g}^{-1}$) (17).

Reduced oral glucose oxidation with low glycogen

During the low glycogen trials, the subjects were allowed to consume water *ad libitum*. Subjects were allowed to eat two crackers with cheese (14 g CHO, 4 g fat, 6 g protein) and to drink a cup of decaffeinated coffee or tea in the time between completion of the glycogen depletion protocol and going to sleep.

During the high glycogen (HG) trial subjects were instructed to refrain from any vigorous exercise the day before the experimental trial. The evening preceeding the experimental trials (13 h pre-exercise) subjects received a CHO-rich meal predominantly consisting of pasta (4000-5000 kJ; 250-300g CHO, 15g fat, 20g protein) at the laboratory to ensure a high carbohydrate intake and concomitant optimal glycogen stores.

A third test was employed which served as a control trial (C) to be able to correct for changes in breath $^{13}\text{CO}_2$ background enrichment during exercise. In addition, subjects were instructed not to consume any products with a high natural abundance of ^{13}C during the entire experimental period. This was done to minimize a shift in background enrichment due to changes in endogenous substrate utilization (29, 33, 34). Furthermore subjects were instructed to keep their diet as constant as possible the days before the trials. Furthermore, in order to prevent underestimation of calculated exogenous glucose oxidation rates due to isotopic dilution of $^{13}\text{CO}_2$ in the bicarbonate pool, results of the first 60 min were neglected and all calculations were performed for the 60-120 min exercise period.

Protocol

Subjects reported to the laboratory at 8.00 AM after an overnight fast and before all trials a small standardized breakfast of two crackers with cheese was provided (14 g CHO, 4 g fat, 6 g protein). A Teflon catheter (Baxter Quick Cath Dupont, Ireland) was inserted into an antecubital vein and at 8.30 a resting blood sample was drawn. Resting breath gases were collected (Oxycon β , Mijnhardt, The Netherlands) and duplicate vacutainer tubes were filled directly from the mixing chamber to determine the $^{13}\text{C}/^{12}\text{C}$ ratio in expired CO_2 . At 8.50 AM subjects started a warming-up of 5 min at 100 W followed by 5 min at 40% W_{max} . At 9.00 AM the workload was increased to 50% W_{max} for 120 min. During the first min subjects drank an initial bolus ($8 \text{ ml} \cdot \text{kg}^{-1} = 46.2 \pm 0.7 \text{ g}$) of an 8% glucose solution. Thereafter every 15 min a beverage volume of $2 \text{ ml} \cdot \text{kg}^{-1} (= 11.5 \pm 0.2 \text{ g})$ was provided. The average amount of glucose provided during the 120 min of exercise was $127.1 \pm 5.0 \text{ g}$. Blood samples were drawn at 30 min intervals until the end of exercise. Expiratory gases were collected every 15 minutes. Two subjects were tested on the same day, starting the protocol 10 min apart.

Glucose solutions

In order to quantify exogenous glucose oxidation, solutions were prepared from corn-derived glucose (Amylum, Belgium) which has a high natural abundance of ^{13}C . The ^{13}C -enrichment of the glucose was $-11.2 \text{ \textperthousand}$ vs. PDB and was determined by on-line combustion-IRMS (Carlo Erba-Finnigan MAT 252, Bremen, Germany). During the control trial, subjects ingested an 8% solution pre-

pared from potato derived glucose (AVEBE, The Netherlands). This glucose had a ^{13}C -enrichment of -26.1 δ per mil vs. PDB (Pee Dee Bellimnitella) which is similar to the ^{13}C -enrichment of expired air of Europeans (33).

Analysis

Blood (5 ml) was collected into EDTA containing tubes and was centrifuged for 4 min at 4°C . Aliquots of plasma were frozen immediately in liquid nitrogen and stored at -40°C until analysis of glucose (Roche, Uni Kit III, 0710970), lactate (11) and free fatty acids (FFA) (Wako NEFA-C test kit, Wako Chemicals, Neuss, Germany) which were performed with the COBAS BIO semi-automatic analyzer. Insulin was analyzed by radioimmunoassay (Linco Inc.; St Louis). From indirect calorimetry (RQ , VO_2) and stable isotope measurements ($^{13}\text{CO}_2$ / $^{12}\text{CO}_2$) (IRMS, Finnigan MAT 252), total energy expenditure and oxidation rates of total fat, total carbohydrates and exogenous glucose were calculated.

Calculations

From VCO_2 and VO_2 carbohydrate and fat oxidation rates were calculated using stoichiometric equations (24). The isotopic enrichment was expressed as the delta per mil difference between the ^{13}C and ^{12}C ratio of the sample and a known laboratory reference standard according to the formula of Craig (8):

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

The $\delta^{13}\text{C}$ was then related to an international standard Pee Dee Bellimnitella (PDB).

The amount of glucose oxidized was calculated according to the formula:

$$\text{exogenous glucose oxidation} = \text{VCO}_2 \cdot \left(\frac{\delta \text{ Exp} - \delta \text{ C}}{\delta \text{ Ing} - \delta \text{ C}} \right) \left(\frac{1}{k} \right)$$

In which $\delta \text{ C}$ is the ^{13}C enrichment of expired air in the control test C (background), $\delta \text{ Exp}$ is the ^{13}C enrichment of expired air during exercise at different time points, $\delta \text{ Ing}$ is the ^{13}C enrichment of the ingested glucose and k is the amount of CO_2 (in liters) produced by the oxidation of 1 gram glucose ($k = 0.7467$ liter CO_2 per gram glucose).

Statistics

Analysis of variance (ANOVA) for repeated measures was used to compare differences in substrate utilization and in blood related parameters between the three trials. A Scheffé post hoc test was used in the event of a significant ($p < 0.05$) F-ratio.

Results

Blood parameters

No differences were observed in resting plasma glucose concentrations between the LG and HG trial (Figure 9.1). Plasma glucose tended to be lower in the LG trial and tended to decrease towards the end of exercise. However, these differences were not statistically significant. Plasma insulin levels were significantly lower at the start of exercise in LG compared to HG (Figure 9.1). Although in both conditions plasma insulin concentrations decreased to very low levels the difference between the two trials remained, except at timepoint 120 min.

Figure 9.1 Plasma glucose (A), insulin (B), FFA (C), and lactate (D) concentrations during exercise at 57% VO_2max after CHO depletion and a CHO restricted diet (LG; open squares) and after glycogen loading (HG; black squares). Values are mean $\pm$ SEM. * Significantly different from LG, $P<0.05$.

Plasma FFA levels were significantly higher at rest and during exercise in the LG trial compared to the HG trial (Figure 9.1). Plasma FFA did not increase during HG and C (concentrations were approximately $200 \mu\text{mol}\cdot\text{l}^{-1}$) but increased gradually during exercise in LG, reaching moderately high FFA levels ($700\text{--}900 \mu\text{mol}\cdot\text{l}^{-1}$). At all time points plasma FFA concentrations were significantly higher in the LG trial. Whereas at rest no differences could be observed between the trials, during exercise plasma lactate concentrations were significantly lower in LG versus HG, except time point 120 (Figure 9.1).

Indirect calorimetry

During exercise fat oxidation was significantly higher in the LG trial and CHO oxidation concomitantly lower. Carbohydrate oxidation over the entire exercise period was $164 \pm 15 \text{ g}$ in the LG trial and $219 \pm 14 \text{ g}$ in the HG trial indicating that CHO contributed 25% less to energy expenditure in the glycogen depleted state. This decreased utilization of CHO was completely compensated by fat oxidation since no differences were observed in total energy expenditure between the trials. An overview of substrate utilization in the HG and LG trials during the 60–120 min period is listed in Table 9.1. The relative contribution of substrates to total energy expenditure is depicted in Figure 9.2.

Table 9.1 Substrate utilization during exercise. Exogenous carbohydrate oxidation (CHO exo), endogenous carbohydrate oxidation (CHO endo) and fat oxidation (Fat) are calculated for the 60–120 min of exercise at 57% $\text{VO}_{2\text{max}}$ after CHO depletion and CHO restricted diet (LG) or after glycogen loading (HG). Values are mean $\pm$ SEM. * Significantly different from LG, $P < 0.05$.

	HG	LG
CHO exo (g)	49.3 ± 1.6 *	35.7 ± 3.3
CHO endo (g)	67.0 ± 6.6 *	48.3 ± 6.9
Fat (g)	70.0 ± 5.1 *	91.1 ± 8.2

Exogenous glucose oxidation

Background ^{13}C enrichment measured from the resting breath samples was -26.5 ± 0.6 δ per mil vs. PDB. Changes in isotopic composition of expired CO_2 in response to exercise are shown in Figure 9.3. With ingestion of the corn-derived glucose in trial LG and HG the rise in ^{13}C was significant, reaching a δ per mil vs. PDB difference of approximately 4 towards the end of 120 min exercise (compared to resting breath sample). The changes in background enrichment during exercise in the C trial were about 7–10% of the ^{13}C -enrichment provoked by the exogenous

Figure 9.2 Relative contributions of substrates to total energy expenditure. This relative contribution of exogenous carbohydrate oxidation (CHO exo), endogenous carbohydrate oxidation (CHO endo) and fat oxidation (Fat) were calculated for the 60-120 min of exercise at 57% VO_2max after CHO depletion and CHO restricted diet (LG) or after glycogen loading (HG). * Significantly different from LG, $P < 0.05$.

glucose in the LG and HG trials. Therefore a background correction was made for the calculation of exogenous glucose oxidation, using the data from the C trial. Exogenous glucose oxidation showed a gradual increase over time both in the glycogen depleted and carbohydrate loaded state (Figure 9.3). Oxidation rates leveled off after approximately 75 min and maximal oxidation rates of $0.64 \pm 0.05 \text{ g} \cdot \text{min}^{-1}$ (LG) and $0.88 \pm 0.04 \text{ g} \cdot \text{min}^{-1}$ (HG), were respectively observed. Exogenous glucose oxidation was significantly lower in the LG compared to the HG experiment (35.7 ± 3.3 and $49.3 \pm 1.6 \text{ g}$ during 60-120 min respectively; Table 9.1).

Discussion

The most remarkable finding of this study is that exogenous glucose oxidation was lower when the glycogen stores were reduced by strenuous exercise the day before the exercise trial and kept low by ingestion of a CHO-restricted diet. Exogenous glucose oxidation was 28% lower in LG compared to HG: $36 \pm 3 \text{ g}$ of glucose was oxidized during 60-120 min of exercise with low glycogen stores whereas $50 \pm 2 \text{ g}$ was oxidized with normal to high glycogen stores. The glycogen depletion protocol and CHO restriction caused large changes in substrate utilization. Fat contributed 57 % to energy supply when subjects were carbohydrate loaded (Figure

Figure 9.3 Upper panel. Breath ^{13}C enrichment during exercise at 57% $\text{VO}_{2\text{max}}$ after CHO depletion and a CHO restricted diet (LG), after glycogen loading (HG) both with ingestion of corn derived glucose, and a control trial (C) in which potato derived glucose was ingested. Lower panel. Exogenous glucose oxidation in the HG and LG trial. Values are mean $\pm$ SEM.

* Significantly different from LG, $P < 0.05$.

9.2). With reduction of the glycogen stores by a glycogen depletion protocol the night before the test, fat became an even more important fuel, contributing 70% to energy expenditure. When taking these major shifts in substrate utilization into account, exogenous glucose oxidation, expressed as a percentage of total carbohydrate oxidation remained remarkably constant: 42.5% in LG and 42.6% in HG. An explanation for the decreased oral glucose oxidation with low glycogen stores in the muscle may be the elevated plasma FFA concentration in the LG trial. Increased FFA availability is suggested to reduce carbohydrate oxidation via the glucose-fatty acid cycle as first described by Randle et al (27). However, others

showed that the classical Randle-cycle may not be involved in the regulation of CHO and fat oxidation during exercise in man (4, 9).

Another explanation for the decreased exogenous glucose oxidation rates observed in the present study may be the lower insulin levels in the LG trial as a result of the glycogen lowering exercise the night before in combination with the CHO restricted diet. Insulin concentrations were 30% lower at the start of exercise and remained lower in the LG trial. Since, even small changes in insulin may provoke marked changes in plasma glucose utilization (personal communication E Coyle), the lower plasma insulin concentrations after the glycogen depletion protocol may at least partly explain the decreased exogenous glucose oxidation. Unfortunately little information is available on the effect of small variations in insulin levels on glucose utilization during exercise.

Ravussin et al (28) studied oxidation rates of exogenous carbohydrates in a group of glycogen depleted subjects and a control group and found no significant differences. Unfortunately, the study had no cross-over design and the glycogen depletion protocol may have been less exhaustive than the one applied here. Muscle glycogen concentrations were not measured in this study but plasma insulin and FFA concentrations were comparable.

Massicotte et al (22) studied exogenous carbohydrate oxidation during 2 hours of exercise in fed and fasted conditions and could not find differences in the exogenous oxidation rates of ingested glucose. This may be due to the fact that carbohydrate stores may not have been depleted. Fasting will predominantly empty the liver glycogen stores, while muscle glycogen stores change little.

Often, increased availability of muscle glycogen is associated with decreased glucose uptake by the muscle (14, 31) whereas low muscle glycogen levels are associated with increased muscle glucose uptake (14). Recently, Hargreaves et al (13) studied Rd glucose in subjects who performed glycogen-lowering exercise either 24 or 48 h before a trial at 65-70% VO_2max in combination with either a high or a low carbohydrate diet. No differences were observed in the (^3H -glucose) tracer determined Rd glucose. Because no plasma glucose oxidation was measured in this study, it cannot be excluded that part of the glucose was used for glycogenesis in the glycogen depleted muscle. However, it is usually assumed that all the glucose taken up by the muscle is also oxidized. If that assumption is true, there were no differences in plasma glucose oxidation between the glycogen depleted and loaded trials in the study of Hargreaves et al (13).

Bosch et al (1) compared high with normal glycogen stores by investigating carbohydrate loaded versus non-carbohydrate loaded subjects. They did not observe differences in blood glucose oxidation. In both these studies (1, 13) differences between pre-exercise glycogen concentrations of the high and normal to low glycogen groups were probably smaller than in the present study. From previous studies we estimate that muscle glycogen concentrations were around 160-190 $\mu\text{mol}\cdot\text{g}^{-1}\text{ ww}^{-1}$ in the HG trial and below 35 $\mu\text{mol}\cdot\text{g}^{-1}\text{ ww}^{-1}$ in the LG trial (17). Hargreaves et al (13) report pre-exercise values of 96 $\mu\text{mol}\cdot\text{g}^{-1}\text{ ww}^{-1}$ after exercise and 24 h on a high CHO diet and 54 $\mu\text{mol}\cdot\text{g}^{-1}\text{ ww}^{-1}$ after exercise and 24 h on a low

CHO diet. Bosch et al (1) investigated trained cyclist who were either CHO loaded or non-loaded. Pre-exercise muscle glycogen concentrations varied from $94 \mu\text{mol}\cdot\text{g}^{-1}$ in loaded to $124 \mu\text{mol}\cdot\text{g}^{-1}$ in non-loaded subjects. Although we did not measure muscle glycogen concentrations in the present study, our estimations indicate that differences in muscle glycogen were larger than in other studies. The different results of the present study compared to other studies are likely attributed to the extent to which muscle glycogen concentrations were reduced and the hormonal disturbances provoked by the exhausting exercise trial the day before. In the present study we used an extremely exhausting exercise protocol that leads to very low muscle glycogen concentrations ($<35 \mu\text{mol}\cdot\text{g}^{-1}$) (17), high plasma FFA and low plasma insulin levels.

A third possible mechanism could be that ingested glucose is trapped in the liver when glycogen is depleted. Since the amount of glucose ingested was the same in both the LG and HG trial and exogenous glucose oxidation rates were lower in the LG trial, the question arises: were did the non-oxidized glucose go in the LG trial. It is unlikely that differences in the rate of gastric emptying and/or intestinal absorption in the LG trial compared to the HG trial. A more plausible explanation may be storage. Since the size of the plasma glucose pool did not change (similar plasma glucose concentrations), the glucose must have been stored elsewhere, possibly in the liver. It has also been suggested that muscle glycogen synthesis can occur during low-intensity exercise if the muscle is glycogen depleted and if CHO is ingested (5, 16). However, the exercise intensity at which this muscle glycogen synthesis is observed during exercise is usually lower ($<50\% \text{VO}_2\text{max}$ (16, 35)) than the $57\% \text{VO}_2\text{max}$ in the present study. At present, we can only speculate on the fate of this non-oxidized glucose in the LG-trial as we did not estimate the rate of appearance of glucose from the gut or liver glycogen synthesis.

When comparing the above mentioned studies, we may not forget, however, that the studies of Hargreaves et al (13) and Bosch et al (1) measured blood glucose oxidation, which may be closely correlated to exogenous carbohydrate oxidation, but not necessarily comparable. Exogenous carbohydrates have to pass the intestinal wall and enter the liver before appearing in the main circulation from which they can be extracted by the muscle. The absolute oxidation rates of oral glucose in the present study are somewhat lower than the oxidation rates of maltodextrins in a previous study obtained with the exact same feeding regimen and amount (33). This may be explained by the lower exercise intensity used in the present study. It has been shown that the exogenous substrate utilization is closely related to the energy expenditure and thus to the exercise intensity in the range of $45\text{--}70\% \text{VO}_2\text{max}$ (20, 26). In the study of Wagenmakers et al. (33) the exercise intensity was $65\% \text{Wmax}$ (i.e. $70\% \text{VO}_2\text{max}$) whereas in the present study the exercise intensity was $50\% \text{Wmax}$ ($57\% \text{VO}_2\text{max}$). This workload of $50\% \text{Wmax}$, however, was the highest possible workload to complete a 2h exercise bout after the glycogen depletion trial.

In conclusion, the present study shows decreased exogenous glucose oxidation during prolonged moderate intensity exercise ($57\% \text{VO}_2\text{max}$) after glycogen low-

ering exercise the evening prior to the exercise bout and carbohydrate restriction. It is suggested that incorporation of ingested glucose into liver glycogen, increased plasma FFA levels and decreased insulin concentrations may be responsible for the observed differences. At present, we can only speculate on the underlying mechanisms.

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Chapter 10

Exogenous glucose oxidation during exercise in endurance trained and untrained subjects

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Abstract

To investigate the effect of training status on the oxidation of orally ingested glucose during exercise 7 endurance trained cyclists (T, VO_2max $67 \pm 2.3 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and 8 untrained subjects (UT, $48 \pm 2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) were studied during 120 min of exercise at $\sim 60\% \text{VO}_2\text{max}$. At the onset of exercise, $8 \text{ ml} \cdot \text{kg}^{-1}$ of an 8% naturally labeled $[^{13}\text{C}]$ -glucose solution was ingested and $2 \text{ ml} \cdot \text{kg}^{-1}$ every 20 min thereafter. Energy expenditure was higher in T compared to UT (3404 kJ versus 2630 kJ; $P < 0.01$). Fat contributed significantly more to energy expenditure in T (42.3%) compared to UT (30.2%) while CHO contributed 58.7% and 69.8% respectively. No differences were observed in exogenous glucose oxidation which contributed 25.0% in T and 27.5% in UT respectively. Peak exogenous glucose oxidation rates were not significantly different among the two groups ($0.95 \pm 0.07 \text{ g} \cdot \text{min}^{-1}$ in T and $0.96 \pm 0.03 \text{ g} \cdot \text{min}^{-1}$ in UT). It was concluded that the state of training had no influence on exogenous glucose utilization during submaximal exercise at the same relative intensity.

Introduction

It is now well-established that CHO ingestion during exercise can increase exercise time to exhaustion at moderate exercise intensities ($60\text{--}70\% \text{VO}_2\text{max}$) (6, 7). A decreased rate of carbohydrate oxidation during exercise has been recognized as a

major determinant of fatigue (2). The mechanism by which exogenous CHO ingestion exerts its effect is probably maintenance of normoglycemia and high rates of carbohydrate oxidation (6). The contribution of ingested carbohydrates has been measured by applying stable isotope techniques. This method was first established by Pirnay et al (33) in the early seventies who gave subjects an oral load of naturally labeled [^{13}C]-glucose and demonstrated that the ingested glucose was an effective metabolic substrate. Ever since, this tool has repeatedly been used to follow the oxidation of ingested carbohydrates. Attempts have been made to determine the factors affecting exogenous carbohydrate oxidation. The factors studied include the amount and type of carbohydrate ingested (11, 25, 27, 28, 37), the feeding schedule (10), glycogen availability (17, 29) and the exercise intensity (26, 34).

At present, the effect of training on exogenous CHO oxidation is largely unknown. Training provokes a large shift in substrate utilization during submaximal exercise (4, 13, 14). After training the reliance on carbohydrate as an energy source is markedly decreased as indicated by a decreased R (4, 13, 14) and a slower rate of muscle glycogen breakdown (21). In rats (1) and humans (4) training also decreases blood glucose turnover during moderate intensity exercise, indicating training improves the ability to maintain plasma glucose during prolonged exercise. In addition, Jansson and Kaijser (16) reported lower leg glucose uptake in trained compared to untrained men during exercise at 65% $\text{VO}_{2\text{max}}$ as determined by arterial-venous concentration difference of glucose and estimated blood flow. In contrast, it has been reported that training decreases the intramuscular glucose-6-phosphate concentration (5, 16), increases insulin sensitivity (22) and insulin concentrations seem to decrease less during exercise (40). These factors may favor glucose uptake by the muscle. In addition, it has been reported that exogenous glucose oxidation increases with increasing exercise intensities (26, 34). When trained and untrained subjects exercise at the same relative intensity the absolute work rate of the trained will be higher and when the relationship between exercise intensity and exogenous CHO oxidation is true, exogenous CHO oxidation should increase.

To our knowledge there is only one study that investigated the effect of physical training on exogenous glucose oxidation. Krzentowski et al (23) measured the oxidation of orally ingested glucose during exercise at the same absolute intensity (40% of the pretraining $\text{VO}_{2\text{max}}$), before and after a 6 week training program. Although endurance training is known to cause a large shift from predominantly carbohydrate oxidation towards fat oxidation (4, 13, 14) in their study, Krzentowski et al (23) observed no changes in total carbohydrate or fat oxidation. Yet, exogenous glucose oxidation was increased by 17% by the training.

Until now, no study has investigated the effect of training on exogenous glucose oxidation during exercise at the same relative exercise intensity. Therefore the present study aimed at investigating the differences in exogenous carbohydrate oxidation between endurance trained and untrained subjects at the same relative, moderate intensity.

Material and Methods

Subjects

Two groups of subjects with different training backgrounds participated in this study: eight well-trained cyclists or triathletes and seven healthy untrained subjects. The trained subjects (T) trained at least four times a week 2 hours or more and had a training history of at least 5 years. The untrained subjects (U) were healthy young adults who were not active in any sport and had no history of endurance training. Subjects' characteristics are listed in Table 10.1. After explaining the nature and the risks of the experimental procedures to the subjects, their written informed consent was obtained. The study was approved by the local Ethical Committee.

Table 10.1 Subjects' Characteristics.

	Untrained (n=7)	Trained (n=8)	
Age	20.9 ± 0.5	25.1 ± 1.4	years
Height	183 ± 3	182 ± 2	cm
Weight	75.4 ± 3.8	72.3 ± 1.2	kg
BMI	22.6 ± 0.9	22.0 ± 0.5	m·kg ⁻²
Wmax	291 ± 12	414 ± 14 ***	W
VO ₂ max	3.5 ± 0.1	5.2 ± 0.1 ***	l·min ⁻¹

***Significant differences ($P < 0.001$) between trained and untrained subjects (mean ± SEM).

Pre testing

Subjects' VO₂max was measured on an electronically braked cycle ergometer (Lode Excalibur, Groningen The Netherlands) during an incremental exhaustive exercise test (24) one week before the first experimental trial. The results of this test were used to determine the 50% Wmax (~60% VO₂max) workload which was later used in the experimental trials.

Experimental trials

Each subject performed two exercise trials, each separated by at least seven days. The order of the trials was counterbalanced. Each trial consisted of 120 min cycling at 50% Wmax (~55-60% VO₂max). During one test subjects ingested a glucose solution with a high natural abundance of ¹³C to study oral glucose oxidation. The second test was employed with ingestion of a glucose solution with a low natural abundance of ¹³C to allow correction for changes in breath ¹³CO₂ background enrichment during exercise. To avoid background shifts standard procedures were followed (18, 19). Subjects were instructed not to consume any products with a high natural abundance of ¹³C during the entire experimental period. This was done to minimize a shift in background enrichment due to changes in endogenous

substrate utilization. Furthermore subjects were instructed to keep their diet as constant as possible the days before the trials.

Protocol

Subjects reported to the laboratory at 8.00 AM after an overnight fast, and before all trials a small standardized breakfast of two crackers with cheese was provided (14 g CHO, 4 g fat, 6 g protein). A Teflon catheter (Baxter Quick Cath Dupont, Ireland) was inserted into an antecubital vein and at 8.30 AM a resting blood sample was drawn. Two subjects were tested on the same day, starting the protocol 10 min apart (i.e. the second subjects started the protocol 10 min later). Resting breath gases were collected (Oxycon β , Meinhardt, Mannheim, Germany) and vacutainer tubes were filled directly from a mixing chamber in duplicate to determine the $^{13}\text{C}/^{12}\text{C}$ ratio in expired CO_2 . At 8.50 AM the first subject started a warming-up of 5 min at 100 W followed by 5 min at 40% Wmax. At 9.00 AM the workload was increased to 50% Wmax for 120 min. During the first min subjects drank an initial bolus (8 ml·kg⁻¹) of an 8% glucose solution. Thereafter every 15 min a beverage volume of 2 ml·kg⁻¹ was provided. The average amount of glucose provided during the 120 min of exercise was 127 ± 2 g in T and 132 ± 7 in U. Blood samples were drawn at 30 min intervals until the end of exercise. Expiratory gases were collected every 15 minutes.

Glucose solutions

In order to quantify exogenous glucose oxidation, solutions were prepared from corn-derived glucose (Amylum, Belgium) which has a high natural abundance of ^{13}C . The ^{13}C -enrichment of the glucose was $-11.2 \delta \text{ pm}$ vs PDB (-0.01259 APE) and was determined by on-line combustion-IRMS (Carlo Erba-Finnigan MAT 252). During the control trial, subjects ingested an 8% solution prepared from potato derived glucose (AVEBE, The Netherlands). This glucose had a ^{13}C -enrichment of $-26.1 \delta \text{ pm}$ vs PDB (-0.02934 APE) which is similar to the ^{13}C -enrichment of expired air of Europeans (39).

Analysis

Blood (5 ml) was collected into EDTA containing tubes and was centrifuged for 4 min at 4°C. Aliquots of plasma were frozen immediately in liquid nitrogen and stored at -40°C until analysis of glucose (Uni Kit III, 0710970, La Roche, Basel, Switzerland), lactate (9) and free fatty acid concentrations (FFA) (Wako NEFA-C test kit, Wako Chemicals, Neuss, Germany) which were performed with the COBAS BIO semi automatic analyzer (Roche, Basel, Switzerland). Insulin was analyzed by radio-immuno-assay (Linco Ultra sensitive Human Insulin RIA kit). From indirect calorimetry (RQ , VO_2) and stable isotope measurements (breath $^{13}\text{CO}_2 / ^{12}\text{CO}_2$ ratio) (IRMS, Finnigan MAT 252, Bremen, Germany), total energy expenditure and oxidation rates of total fat, total carbohydrates and exogenous glucose were calculated.

Calculations

From VCO_2 and VO_2 carbohydrate and fat oxidation rates were calculated (32).

$$\text{glucose oxidation} = 4.585 \text{ VCO}_2 - 3.226 \text{ VO}_2$$

$$\text{fat oxidation} = 1.695 \text{ VO}_2 - 1.701 \text{ VCO}_2$$

The isotopic enrichment was expressed as the delta per mil difference between the ^{13}C and ^{12}C ratio of the sample and a known laboratory reference standard according to the formula of Craig (8):

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

The $\delta^{13}\text{C}$ was then related to an international standard Pee Dee Bellemnitella (PDB).

The amount of glucose oxidized was calculated according to the formula:

$$\text{exogenous glucose oxidation} = \text{VCO}_2 \cdot \left(\frac{\delta \text{ Exp} - \delta \text{ C}}{\delta \text{ Ing} - \delta \text{ C}} \right) \left(\frac{1}{k} \right)$$

In which $\delta \text{ C}$ is the ^{13}C enrichment of expired air in the control test C (background), $\delta \text{ Exp}$ is the ^{13}C enrichment of expired air during exercise at different time points, $\delta \text{ Ing}$ is the ^{13}C enrichment of the ingested glucose and k is the amount of CO_2 (in liters) produced by the oxidation of 1 gram glucose ($k = 0.7467$ liter CO_2 per gram glucose).

In the present study and in previous studies from our laboratory (20, 35, 37, 39) it was shown that instructing the subjects not to eat any products of high natural ^{13}C abundance during the experimental period was effective in reducing the background shift (change in $^{13}\text{CO}_2$) from endogenous substrate stores in European subjects (39). However, although the background shift was small in the present study, background correction was made by using the ^{13}C enrichment of breath samples in the CHO-trial.

It should be noted that when using $^{13}\text{CO}_2$ in expired air to calculate exogenous substrate oxidation is the trapping of exogenous $^{13}\text{CO}_2$ in the bicarbonate pool, a very large and slowly exchanging pool in which an amount of CO_2 arising from decarboxylation of energy substrates is temporarily trapped each minute (36). However, during exercise, the CO_2 production increases several fold so that a physiological steady state situation will occur and $^{13}\text{CO}_2$ in expired air will be equilibrated with the $^{13}\text{CO}_2/\text{H}^{13}\text{CO}_3^-$ pool. The dilution of $^{13}\text{CO}_2$ becomes negligible and recovery of $^{13}\text{CO}_2$ approaches 100% after 60 min of exercise (31). Therefore, in the present study, data from the initial 60 min were not used for the calculation of exogenous glucose oxidation.

Statistics

An unpaired t-test was used to compare the differences in substrate utilization and blood parameters between the trained and untrained. Analysis of variance for repeated measures was performed to study differences over time within each group. A Scheffé post hoc test was applied in case of a significant ($P < 0.05$) F-ratio to locate the differences.

Results

O_2 uptake and heart rate

The workload at 50% W_{max} was 207 ± 7 W in the trained subjects and 146 ± 6 W in the untrained subjects. Over the entire exercise period VO_2 was significantly higher in the trained compared to untrained subjects ($P < 0.01$). On average VO_2 was $38 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in trained subjects and $29 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in untrained subjects which corresponded to 57% and 60% $VO_2 \text{ max}$ respectively. Despite the higher absolute work rate, the trained subjects exercised at a significantly lower heart rate (HR). In trained subjects HR increased from 127 ± 3 BPM to 130 ± 3 bpm and in untrained subjects from 129 ± 4 BPM to 147 ± 4 BPM towards the end of exercise.

Figure 10.1 The absolute ($\text{kJ}\cdot 60 \text{ min}^{-1}$) and relative (%) contributions of substrates to energy expenditure in trained and untrained subjects.

* significant difference between the two groups ($P < 0.05$).

Carbohydrate and fat oxidation

After 15 min of exercise R was 0.87 ± 0.01 in trained subjects and 0.91 ± 0.01 in untrained subjects ($P < 0.05$) and after 120 min of exercise the R was 0.85 ± 0.01 and 1.89 ± 0.01 in trained and untrained respectively ($P < 0.05$). Total fat oxidation calculated over the second hour of exercise (60–120 min) was significantly higher in trained compared to the untrained subjects (37.4 ± 2.3 g in T versus 22.5 ± 1.4 g in UT; Figure 10.1). No differences were observed in the absolute amounts of carbohydrate oxidized in this period (116.3 ± 7.5 g versus 113.7 ± 3.6 in UT; Figure 10.1).

Exogenous glucose oxidation

Background ^{13}C enrichments measured from the resting breath samples was comparable for the trained and untrained (-26.2 ± 0.2 δ pm vs PDB in T and -25.6 ± 0.2 δ pm vs PDB). Changes in isotopic composition of expired CO_2 in response to exercise are shown in Figure 10.2.

With ingestion of the corn-derived glucose in CHO tests the rise in ^{13}C was significant, reaching an enrichment difference of approximately 4 δ pm towards the end of 120 min exercise (compared to resting breath sample). The changes in background enrichment during exercise in the control trial were minimal and not statis-

Figure 10.2 Upper panel Breath $^{13}\text{CO}_2/^{12}\text{CO}_2$ (δ pm vs PDB). Lower panel No difference in exogenous glucose oxidation in endurance trained and untrained subjects at the same relative exercise intensity.

tically significant. A background correction was made for the calculation of exogenous glucose oxidation, using the data from the control trial. In line with the breath isotopic enrichment, exogenous glucose oxidation showed a gradual increase over time (Figure 10.2). Peak oxidation rates were reached at the end of exercise (120 min) and were $0.95 \pm 0.07 \text{ g} \cdot \text{min}^{-1}$ and $0.96 \pm 0.03 \text{ g} \cdot \text{min}^{-1}$ in T and UT respectively. This difference was not statistically significant. The amount of exogenous glucose oxidized over the 60-120 min period was slightly, but not significantly, higher in trained compared to untrained ($49.5 \pm 1.5 \text{ g}$ versus $44.8 \pm 2.6 \text{ g}$; Figure 10.1). Endogenous glucose oxidation as calculated from total CHO oxidation minus exogenous glucose oxidation was also similar in trained and/or untrained ($68.9 \pm 2.7 \text{ g}$ in T and $66.8 \pm 6.8 \text{ g}$ in UT; Figure 10.1).

Blood parameters

Plasma glucose concentrations remained stable during exercise between 4.5 and 4.9 $\text{mmol} \cdot \text{l}^{-1}$. No differences between the two groups were observed (Figure 10.3a). Plasma FFA levels were also similar, although there was a tendency for the trained subjects to have slightly higher plasma FFA concentrations (Figure 10.3b). Plasma

Figure 10.3 Plasma glucose (a), FFA (b) and lactate (c) and (d) insulin concentrations in trained ■ and untrained □ subjects during 120 min of exercise.

*significant difference between the two groups ($P < 0.05$).

lactate concentrations were significantly higher in the untrained subjects compared to the trained throughout the entire exercise period (Figure 10.3c). In both trials plasma insulin levels decreased during exercise from values around 10–11 $\mu\text{U}\cdot\text{ml}^{-1}$ to values around 4–5 $\mu\text{U}\cdot\text{ml}^{-1}$. There were no differences between the trained and untrained (Figure 10.3d).

Discussion

The oxidation of orally ingested CHO has been extensively investigated by using either stable or radioactive tracers. Both methods revealed information regarding the factors that are of influence on exogenous CHO oxidation rates (see review Hawley et al (10)). Among these factors are the type (11, 25, 27, 28, 30, 37) and the amount of CHO ingested (35, 38), the feeding schedule (10), glycogen availability (17, 29) and the exercise intensity (26, 34). However, the effects of training on the utilization of these ingested CHO has received little attention. Therefore the purpose of this study was to investigate exogenous CHO oxidation rates in trained and untrained subjects at a comparable relative exercise intensity (i.e. 57–60% $\dot{V}\text{O}_{2\text{max}}$).

Subjects had different training backgrounds and also showed large differences in maximal work rate and $\dot{V}\text{O}_{2\text{max}}$ (Table 10.1). Therefore endurance trained subjects exercised at a 42% higher absolute work rate, while relative exercise intensity was the same. As a result energy expenditure was also 29% higher in the trained group. One of the main adaptations to endurance training is a reduction in the rate of carbohydrate oxidation during exercise and a concomitant increased rate of fat oxidation. This effect has been observed both at the same absolute and relative exercise intensity (3, 4, 12, 14–16). Also in the present study a higher rate of fat oxidation and an increased relative contribution of fat was observed in trained subjects compared to untrained when they exercised at the same relative exercise intensity. However, despite markedly higher rates of fat oxidation, and despite a smaller percent contribution of carbohydrate oxidation, trained subjects had similar rates of exogenous carbohydrate oxidation. Although the absolute work rate in the trained was higher, endogenous CHO utilization was similar in trained and untrained.

A more appropriate way to look at the data during exercise at the same *relative* exercise intensity may be expressing substrate utilization *relative* to energy expenditure (Figure 10.1). The contribution of fat is higher in trained (42.3% versus 30.2%). The reduction in the relative contribution is mostly explained by a lower contribution of endogenous glucose (33.7% versus 42.3%), whereas exogenous glucose contribution is about equal in the trained and untrained subjects (25.0% versus 27.5%).

To our knowledge, there is only one other study (23) that investigated the effect of training on exogenous carbohydrate oxidation rates. When comparing our data with the findings of that study of Krzentowski et al. (23) there seem to be some striking differences. In their study, 6 subjects trained 60 min, 5 times a week at a

low intensity (30-40% $\text{VO}_{2\text{max}}$) and oral CHO oxidation was investigated at the same absolute exercise intensity before and after a 6-week training program. A training induced increase in $\text{VO}_{2\text{max}}$ of 29% was reported while carbohydrate and fat oxidation changed only little. Fat oxidation seemed to be slightly higher after training but during the later stages of exercise this difference was not statistically significant. Exogenous carbohydrate oxidation, however, was increased by 17% after training.

The difference in $\text{VO}_{2\text{max}}$ between the trained and untrained were much larger in the present study (46% in this study versus 29% reported by Krzentowski et al (23)) and the exercise intensity was higher (56-60% $\text{VO}_{2\text{max}}$ in this study versus 40% $\text{VO}_{2\text{max}}$ reported by Krzentowski et al (23)). Despite the larger difference in training status, the trained subjects did not have an increased exogenous glucose oxidation, so that we cannot confirm the suggestion brought about by Krzentowski et al (23) that training leads to an increased capacity to oxidize ingested CHO. For both groups in our study peak oxidation rates of the ingested glucose were 0.95-0.96 $\text{g}\cdot\text{min}^{-1}$. These values are close to the maximum oxidation rate of orally ingested CHO, which is believed to be about 1.0 $\text{g}\cdot\text{min}^{-1}$ (10, 38). It should be noted, however, that for a more complete comparison between a group of trained and untrained subjects, data should be collected at both the same absolute and relative exercise intensity. In this study we investigated only exogenous CHO oxidation at the same relative exercise intensity.

Of interest are also a paper of Massicotte et al (26) and one of Pirnay et al (34). They looked at the effect of increasing exercise intensity and the effect on exogenous glucose oxidation. With increasing exercise intensity, the rate of oral CHO oxidation increases (26, 34), although at high work rates oxidation rates may level off (34). Apparently the present study is not in line with these findings: we found slightly but not significantly higher oxidation rates for subjects exercising at VO_2 of 38 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and a VO_2 of 29 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. The work rate in the untrained in the present study was below the value at which leveling off of the relationship between work rate and oral glucose oxidation, as reported by Pirnay et al (34), occurred. So, training seems to change the relationship between exercise intensity and exogenous glucose oxidation.

In conclusion, the present study demonstrates that exogenous glucose oxidation rates during similar relative submaximal exercise intensities were not different in trained and untrained subjects, even with large differences in the relative contribution of carbohydrate and fat. This may indicate that glucose appearance from the gut into the circulation is one of the main limiting factors for exogenous CHO oxidation. Lastly, one of the most profound observations in this study was the higher fat oxidation rates in the endurance trained subjects.

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Chapter 11

Carbohydrate-electrolyte feedings improve 1 H time trial cycling performance

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Abstract

Carbohydrate-electrolyte (CE) feedings have been shown to improve endurance performance at moderate intensities (60-75% $\text{VO}_{2\text{max}}$) and of more than 2 h duration. The effects of CE feedings during high intensity exercise (i.e. $\geq 80\%$ $\text{VO}_{2\text{max}}$) of shorter duration (~1 h) are less clear. Therefore the purpose of the present study was to investigate the effect of the ingestion of a 7.6% CE solution during exercise on time trial cycling performance of approximately 1 h. This type of performance testing has been shown to be more reproducible (coefficient of variation 3.35%) than the traditional exercise test to exhaustion. On two occasions and in random order nineteen endurance trained cyclists completed an exercise test requiring the accomplishment of a set amount of work as fast as possible (time trial) under strictly standardized conditions. At the start and during the trials they drank in total 14 $\text{ml}\cdot\text{kg}^{-1}$ of either a 7.6% CE solution or artificially flavored and colored water (placebo). Time to complete the set amount of work was significantly reduced and thus performance was significantly increased ($P<0.001$) with the CE drink by 2.3%. Time to complete the set amount of work was 58.74 ± 0.52 min with CE and 60.15 ± 0.65 min with placebo ($p<0.001$). Average workload during the time trials was 297.5 ± 1.4 W and 291.0 ± 10.3 W respectively. Subjects exercised at $76.4 \pm 0.7\%$ of their maximal work rate (W_{max}) with CE and at 74.8% W_{max} with placebo ($p<0.001$). It was concluded that also in relative short term (1 h) high intensity

(75% Wmax) cycling exercise ingestion of a carbohydrate-electrolyte solution compared to placebo improves performance.

Introduction

Carbohydrate (CHO) feedings during exercise have been repeatedly shown to increase performance during exercise lasting longer than 2 hours at intensities of 50-70% $\text{VO}_{2\text{max}}$ (3, 4, 7-9, 13, 24, 26, 27). This has been attributed to maintenance of blood glucose levels and high rates of carbohydrate oxidation (4, 9). Numerous investigators reported increased performance with both simple and complex carbohydrate feedings when subjects were fed several hours prior to exercise or at the onset and during exercise (7, 24, 26, 27). Carbohydrate electrolyte solutions have been formulated to guarantee an optimal replacement of both water and carbohydrates. Very few studies however, investigated the effect of carbohydrate-electrolyte solutions with concentrations similar to available commercial sports drinks on performance during high intensity exercise ($\geq 80\% \text{VO}_{2\text{max}}$; (1, 2, 19)). Little information is available on the effect of carbohydrate ingestion on performance during intense exercise lasting about 1 hour.

In the present study we therefore investigated the effect of CE feedings on time trial cycling performance (comparable to a 40 km time trial) under strictly standardized conditions, without additional measurements, to avoid disturbing effects on the subjects performance ability. Previously we have shown that performance testing using a time trial approach (subjects have to accomplish a set amount of work as fast as possible) gives a much better reproducibility on repeated testing than the traditional time to exhaustion test (11). We and others (10, 22) believe that blood sampling and measurements of respiratory gas exchange increases variability (subjects ability to concentrate is diminished and they can not push as hard as they could without any measurements) and therefore is avoided here during performance testing.

Methods

Subjects

17 Endurance trained male and 2 female cyclists who trained regularly more than 2 hours a day, 4-7 days a week, volunteered for this study which was approved by the local Medical Ethical Committee. Mean age, height and body mass of the male and female subjects are shown in Table 11.1.

5-7 Days prior to the first experimental trial, maximal oxygen consumption ($\text{VO}_{2\text{max}}$), maximal workload (Wmax) and maximal heart rate (HRmax) were determined in a stepwise incremental exercise test to exhaustion as described by Kuipers et al (12). After a warm-up period of 5 min at 100 W, the workload was increased by 50 W every 2.5 min until a heart rate was reached of 160 bpm. Then

workload was increased with 25 W every 2.5 min. The $W_{\max}$ was determined by the following formula:

$$W_{\max} = W_{\text{out}} + (t / 150) \cdot 25$$

where W_{out} is the last completed stage, t is the time in the final stage. All exercise tests were performed on an Excalibur Sport electromagnetically braked ergometer (Lode, Groningen, The Netherlands). Heart rate was continuously recorded by a sporttester® (Polar, Kelmele, Finland).

Table 11.1 Characteristics of the male ($n=17$) and female subjects ($n=2$). Maximal oxygen consumption (VO_2max) and maximal power output ($W_{\max}$) were measured during the incremental exercise test to exhaustion.

	male ($n=17$) mean $\pm$ SEM	female ($n=2$) mean $\pm$ SEM
Age (yrs)	23 $\pm$ 1	21 $\pm$ 2
Height (m)	182 $\pm$ 2	173 $\pm$ 0.1
Body mass (kg)	70.4 $\pm$ 1.5	56.1 $\pm$ 11.2
VO_2max ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	72.9 $\pm$ 1.4	64.2 $\pm$ 0.34
$W_{\max}$ (W)	396 $\pm$ 11	288 $\pm$ 38

Procedure

16 Subjects were familiar with the exercise protocol. The other 3 subjects performed a familiarization trial. An exercise trial consisted of a simulated time trial in which the ergometer was set in the pedaling rate dependent mode (workload increases as the pedaling rate increases). After a short warming up (5 min 100W) subjects were asked to perform a certain amount of work (equal to about 1 hour cycling) as fast as possible. The measurement of performance was the time to complete the set amount of work. This performance test has previously been shown to produce highly reproducible results (11). Coefficient of variation of this test in 5 repeated tests was 3.35%. In addition, Palmer et al (18) recently confirmed that this kind of test is highly reproducible. This total amount of work was based on the maximal workload ($W_{\max}$) and the total amount of work to be performed was calculated according to the formula:

$$\text{Total amount of work} = 0.75 \cdot W_{\max} \cdot 3600$$

The ergometer was set in the pedaling rate dependent mode according to the formula

$$W = L \cdot (\text{RPM})^2$$

CE feedings improve cycling performance

In which RPM is the pedaling rate and L is a $W_{\max}$ dependent constant factor which can be adjusted before the test by the experiment leader. This factor was chosen in a way which would evoke a pedaling rate of 90 RPM at 70% $W_{\max}$. This would mean that 75% $W_{\max}$ could be achieved at about 95 RPM which appeared to be the preferential pedal cadence of most cyclists. The ergometer was connected to a computer which on line recorded workload and the elapsed time and calculated the amount of work performed. The only information the subject received was the amount of work performed and the percentage of work performed relative to the pre set amount of work (0% at the start, 100% at completion of the trial). This information was displayed on a computer screen in front of the ergometer. Subjects did not receive information on workload, pedaling rate, time or heart rate. A fan was placed in front of the subjects to provide some cooling during the tests.

To avoid any influence of circadian variance, subjects performed their tests at the same time of the day. Subjects were not allowed to train the day before the test. Two days before the test, training was not allowed to be exhaustive. Additionally, all subjects were instructed to eat quantitatively and qualitatively the same diet during the 24 h prior to the test. This was controlled by a registration procedure. They were advised to eat a carbohydrate rich meal such as pasta the evening prior to the test. In the hour preceding the test, subjects were not allowed to eat. To further control carbohydrate and energy intake, food records were provided for the day prior to the test and the day of the test. During all tests environmental conditions were standardized, temperature was kept at 20°C, relative humidity varied between 50 and 60 %. Subjects were encouraged by the same person every test.

Beverages

Subjects received two types of drinks on different occasions, subsequent tests being separated by at least 5 days. The drinks consumed were (1) a 7.6% (76 g·l⁻¹) carbohydrate-electrolyte solution (Isostar, Sandoz Nutrition, Switzerland) or (2) artificially colored and flavored water to which a small amount of fructose (5 g·l⁻¹) was added (for composition of test drinks see Table 11.2).

Table 11.2 Test drink composition per 100 ml.

	CE solution	Placebo
Carbohydrate (g)	7.6	0.5
Organic acid (g)	0.5	0.073
Sodium (mg)	69	-
Potassium (mg)	18	-
Calcium (mg)	1	-
Chloride (mg)	30	-
Vitamin C (mg)	10	-
Vitamin E (mg)	0.6	-
Beta carotene (mg)	0.33	-
Sweeteners (mg)	-	0.016
Flavor orange natural (mg)	0.032	0.064

The drinks were indistinguishable to the subjects and provided in random order; 10 subjects receiving the CE solution as the first drink, 9 subjects receiving placebo as the first drink. Subjects ingested $8 \text{ ml} \cdot \text{kg}^{-1}$ of the 7.6% CE solution or placebo as a bolus during the 5 min warm-up period. Thereafter $2 \text{ ml} \cdot \text{kg}^{-1}$ was ingested at achievement of 25%, 50% and 75% of the set amount of work. Such a drinking pattern has previously been reported to result in high rates of gastric emptying throughout exercise (21).

Statistics

The presence of an order effect and the effect of the drinks on performance were tested by paired t-tests. All results are expressed as mean $\pm$ SEM.

Results

Analysis of dietary records revealed that the meals ingested the day before the two experimental trials and also the diets on the day of the experiment were similar for each subject. Mean daily carbohydrate intake was from $367 \pm 18 \text{ g}$ before the CE trial and $341 \pm 22 \text{ g}$ before the placebo trial. Carbohydrates provided $55 \pm 1\%$ of total energy intake the day before the CE experiment and $57 \pm 2 \text{ energy}\%$ CHO the day before the placebo experiment. As previously reported we did not observe a test order effect, meaning that subjects rode the first test as fast as the second. Performance, however, was significantly improved by the CE feedings by 2.3% (Figure 11.1).

Figure 11.1 Time trial performance: differences in the time to complete a set amount of work (min) between CE and placebo. Subjects were significantly faster with ingestion of a 7.6% CE solution compared to placebo. 17 Subjects responded with increased performance whereas 2 subjects responded with decreased performance. Male subjects are indicated by ● and female subjects by ○.

CE feedings improve cycling performance

In Figure 11.1 individual changes in the times to complete the set amount of work are presented. With placebo, subjects needed 60.15 ± 0.65 min, and with CE they needed 58.74 ± 0.52 min to complete the set amount of work. 17 Subjects responded with improved performance to the CE solution whereas 2 subjects showed decreased performance. Individual data are shown in Table 11.2.

The average workload during the time trial was 291.0 ± 10.3 and 297.5 ± 10.3 with placebo, and CE respectively (Table 11.3). Subjects exercised at $76.4 \pm 0.7\%$ of their maximal work rate ($W_{\max}$) with CE and at $74.8 \pm 0.8\%$ $W_{\max}$ with placebo. No differences in heart rate were observed among the two experiments (Figure 11.2).

Table 11.3 Average workload during the time trial and differences with CE or placebo ingestion

Subject	Average Workload (W)		Difference
	CE	placebo	
1	290.2	281.3	8.9
2	321.1	316.8	4.3
3	325.9	305.9	20.0
4	310.3	295.0	15.4
5	309.4	303.3	6.1
6	308.0	310.5	-2.5
7	368.7	359.6	9.2
8	297.1	294.2	2.9
9	307.2	303.6	3.6
10 §	183.4	172.8	10.7
11	351.7	336.2	15.5
12 §	228.7	220.7	8.0
13	311.6	306.6	5.0
14	301.6	295.9	5.7
15	330.5	325.7	4.8
16	230.6	223.5	7.0
17	253.5	248.9	4.7
18	298.9	297.7	1.2
19	324.6	330.8	-6.1
Mean	297.5	291.0	6.5
SEM	10.3	10.3	1.41

Individual values. § denotes female subject.

Discussion

In this investigation, 19 endurance trained subjects (Table 11.1) performed a set amount of work as fast as possible (about 1 h duration) in a moderate environment (20°C) while receiving either a 7.6% CE solution or placebo. The purpose of the

experiment was to investigate the effect of CE feedings on performance during intense exercise of relative short duration (1 hour) using a previously validated endurance performance test. Therefore we decided to only measure time to complete the set amount of work and heart rate because other measurements might have interfered with the subjects' ability to concentrate during cycling at high intensity ($\sim 85\%$ VO_2max) as suggested by Coyle and Hamilton (10) and Robinson et al (22).

Figure 11.2 Heart rate during time trial (mean $\pm$ SEM). No significant differences were observed between the CE and the placebo trial. ($n=19$)

Although several studies have shown that performance is increased after carbohydrate ingestion during moderate intensity exercise ($50\text{--}70\%$ VO_2max) of long duration (>2 h), little information is available on the effect of carbohydrates on high intensity exercise of about 1 h duration. We found that carbohydrate ingestion during exercise (~ 1 h) decreased time to complete a set amount of work by 2.3%. Results are in agreement with a study of Below et al (2) who showed a 6.3% increased performance when subjects had to accomplish a set amount of work as fast as possible (approximately 10 min) after 50 min of intense exercise (85% VO_2max). In a study of Powers et al (19) subjects exercised at an initial workload of 85% VO_2max until exhaustion. In this experiment exhaustion was defined as a 10% decline in the power output below the initial value. Subjects ingested either a beverage with carbohydrates and electrolytes, with electrolytes only, or a placebo. Although 7 of 9 subjects worked longer with the carbohydrate drink, the difference was not statistically significant. Neuffer et al (16) showed improved performance in short term high-intensity exercise following carbohydrate ingestion. Well-trained cyclists exercised 45 min at 80% VO_2max followed by an exercise bout requiring the accomplishment of the most possible work in a 15 min period. With ingestion of a carbohydrate solution (45 grams of carbohydrate) just prior to the exercise bout, subjects completed 10% more work compared to the placebo trial.

From the present study and the few available studies, it can be concluded that CHO feedings not only improve exercise performance during long lasting submaximal exercise (3, 4, 7-9, 13, 24, 26, 27), but also during 1 h high intensity exercise. With prolonged exercise it has been shown that carbohydrate feedings improve performance by maintaining euglycemia (4, 9, 15) and high rates of carbohydrate oxidation (4, 9). During high intensity exercise, there is no evidence that carbohydrate ingestion exerts its beneficial effect by raising blood glucose concentration and carbohydrate oxidation (2). During high intensity exercise blood glucose levels do not drop, but are often even increased towards the end of exercise (2, 5, 6). In the study of Neufer et al (16) and of Below et al (2) carbohydrate ingestion improved performance despite the fact that no differences in carbohydrate oxidation were observed between the CHO trial and placebo during the initial 45-50 min of steady state exercise. Studies using ^{13}C -labeled carbohydrates to measure exogenous carbohydrate oxidation have shown that with a similar feeding schedule to the one applied in the present study about 10-20% of the ingested carbohydrates is oxidized during the first hour of exercise at 60-70% $\text{VO}_{2\text{max}}$ (20, 23, 25). This means that only a small amount of exogenous carbohydrates (~ 5-20 g) is available for oxidation during the first hour after ingestion (These calculations take into account the underestimation due to the temporary loss of $^{13}\text{CO}_2$ in the bicarbonate pool). This makes it unlikely that carbohydrate ingestion exerts its beneficial effect through a contribution to energy expenditure.

Coggan and Coyle (5) suggested that the key to the improved performance at these high intensities may be the 'relative' CHO availability, i.e., that although CHO availability may not appear limiting in an absolute sense, increasing CHO oxidation may provide the ability to increase the power output (or to tolerate high intensities longer). This effect may be mediated by CHOs promoting the synthesis of TCA-cycle intermediates via the alanine aminotransferase reaction, thereby increasing the capacity of aerobic ATP production.

Although it seems most likely that CHO are responsible for the improved performance after ingestion of the CE solution, with the present study design it cannot be excluded that the electrolytes in the drink may be partially responsible for the observed improvements.

Electrolyte ingestion, especially sodium and potassium, has been associated with increases in plasma volume. This effect has primarily been observed post-exercise (14, 17) and to our knowledge no data are available to support the idea that increases in plasma volume would also occur when electrolytes are ingested during exercise. Moreover, if indeed sodium and/or potassium would have caused a better maintenance of plasma volume in the present study, this could theoretically lead to increased performance at similar heart rates because of a larger cardiac output. However, Powers et al (19) did not observe changes in plasma sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and chloride (Cl^-) when electrolytes were ingested in amounts similar to the amounts ingested in the present study. Although plasma electrolyte concentrations increased during exercise, the increase was the same when water was ingested compared to ingestion of a CE solution. In addition no

effect on plasma volume or exercise performance was observed when only electrolytes were ingested.

It was concluded that carbohydrate feedings ingested during exercise improve time trial cycling performance of about 1 h duration (high intensity exercise; $\sim 80\% \text{VO}_{2\text{max}}$). However, the explanation for this increased performance remains to be established.

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Chapter 12

Glucose kinetics during prolonged exercise following glucose ingestion: a comparison of tracers

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Abstract

This study determined the extent to which glucose disappearing from the plasma (Rd glucose) is oxidized. After an overnight fast, six well-trained cyclists exercised on three occasions for 120 min on a bicycle ergometer at 50% VO_2max and ingested either water (FAST), a 4% glucose solution (LO-GLU) or a 22% glucose solution (HI-GLU) during exercise. A primed continuous intravenous infusion of [^{13}C]-glucose and [6,6- $^2\text{H}_2$]-glucose tracer was started after a $\text{NaH}^{13}\text{CO}_3$ prime. Glucose ingestion markedly increased total Ra and this increase was larger with HI-GLU compared to LO-GLU. After 120 min Rd glucose was $52 \pm 3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ during FAST, $74 \pm 3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ during LO-GLU and $117 \pm 9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ during HI-GLU. In all trials the Rd was closely matched to plasma glucose oxidation rates ($50 \pm 2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ FAST, $72 \pm 4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ LO-GLU and $113 \pm 9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ HI-GLU). The percent of Rd oxidized was between 96 and 100% in all trials. The results of the present study indicate that during exercise glucose disappearing from the plasma is oxidized when substantial amounts of CHO are ingested and Rd glucose is relatively high.

Introduction

During the past 14 years many studies have shown that carbohydrate (CHO) feedings during prolonged exercise can delay the onset of fatigue and enhance exercise performance (4, 5, 7, 8, 14, 17, 24). This effect has been attributed to a maintenance of plasma glucose availability and high rates of carbohydrate oxidation during the later stages of exercise, when muscle glycogen is nearly depleted (4, 5). Although some studies suggested that carbohydrate ingestion may improve endurance performance by slowing the rate of muscle glycogen degradation (1, 8, 14), it is generally believed that muscle glycogen breakdown during continuous submaximal exercise with and without CHO feedings is an ongoing process that cannot be influenced by CHO ingestion (7). So, especially late in exercise, plasma glucose is an important substrate for energy metabolism in the muscle (28). Only a few attempts have been made to study the fate of plasma glucose or to quantify its contribution to energy expenditure during exercise with or without CHO ingestion. Recently, using tracer methodology, Bosch et al (2) and McConnell et al (22) reported that a high rate of appearance of ingested glucose from the gut (Ra gut) reduced the hepatic glucose production and increased glucose uptake (Rd glucose) in the muscle during exercise at 70% $\text{VO}_{2\text{max}}$. McConnell et al (22) have not measured the oxidation of plasma glucose, whereas Bosch et al (2) found oxidation rates of plasma glucose which exceeded the rate of disappearance of glucose (Rd glucose) by 10-20% which is theoretically impossible. Coggan et al (6) reported data of one subject showing that 93% of glucose disappearing from the plasma was oxidized when glucose was ingested during the second hour of exercise at 70% $\text{VO}_{2\text{max}}$. Thus, although it is often assumed that all glucose taken up by the muscle is oxidized, there is only little evidence to support this hypothesis. Kuipers et al (20) suggested that during low-intensity exercise some of the ingested glucose may be used for glycogen synthesis in the muscle illustrating that not necessarily all glucose disappearing from the plasma is oxidized.

The Ra and Rd glucose are usually determined using a continuous infusion of a $[6,6\text{-}^2\text{H}_2]$ -glucose tracer as this tracer does not recycle in the process of gluconeogenesis. However with a ^2H -glucose tracer, oxidation cannot be measured and for this purpose a ^{13}C -glucose tracer has to be used. The use of a ^{13}C -glucose tracer to simultaneously estimate Ra, Rd and the rate of plasma glucose oxidation (Rox) has been limited by the high cost of the tracer (6). One way to reduce the costs is to give smaller amounts of tracer and use more sensitive equipment to measure the plasma glucose enrichment. In the present study we therefore gave small amounts of $[\text{U-}^{13}\text{C}]$ -glucose tracer and measured plasma glucose enrichment by gas chromatography - isotope ratio mass spectrometry (GC-IRMS). Although very low enrichments can be measured with this technique there are also some theoretical complications. When measured by GC-IRMS the ^{13}C -glucose carbon may recycle in the process of gluconeogenesis (32, 34). Therefore the value obtained for Ra may be underestimated. We therefore compared Ra glucose calculated from a $[6,6\text{-}^2\text{H}_2]$ -glucose tracer (GC-MS) and a $[\text{U-}^{13}\text{C}]$ -glucose tracer (GC-IRMS) during exercise

with or without glucose ingestion. With glucose ingestion, gluconeogenesis may be suppressed and we hypothesized that if measurement of total enrichment from $[U-^{13}C]$ -glucose tracer underestimates R_a glucose, this difference would be smaller with glucose ingestion, since the rate of gluconeogenesis will be lower. The primary purpose of the study, however, was to determine the rates of R_a and R_d glucose and the R_{ox} glucose during exercise at 50% $\dot{V}O_{2max}$ with or without CHO feeding.

Material and methods

Subjects

Six highly trained cyclists participated in this study. Their mean age and weight were 24 ± 2 years and 68 ± 1 kg. After explaining the nature and the risks of the experimental procedures to the subjects, their written informed consent was obtained. The study was approved by the local Medical Ethical Committee.

Pretesting

Subjects' $\dot{V}O_{2max}$ was measured on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen The Netherlands) during an incremental exhaustive exercise test (21) one week before the first experimental trial and was found to be 76 ± 1 $ml \cdot kg^{-1} \cdot min^{-1}$. Maximal work rate during the incremental exercise test was 397 ± 10 W. The results of this test were used to determine the 50% $\dot{V}O_{2max}$ workload which was later employed in the experimental trials.

Experimental trials

Each subject performed six exercise trials, each separated by at least seven days. The order of the trials was determined by a latin square design. Each trial consisted of 120 min cycling at ~50% $\dot{V}O_{2max}$. Subjects ingested drinks containing no glucose (FAST), a 4.4% glucose solution (LO-GLU) or a 22.0% (HI-GLU) glucose solution. Each trial with one of these drinks was repeated, once with isotope infusion and once without infusion in order to allow correction for changes in breath $^{13}CO_2$ background enrichment during exercise.

Protocol

Subjects reported to the laboratory at 8:00 AM after an overnight fast. A Teflon catheter (Baxter Quick Cath Dupont, Uden, The Netherlands) was inserted into an antecubital vein of one arm and at 8:30 AM a resting blood sample of 10 ml was drawn. In the contralateral antecubital vein a second teflon catheter was inserted for isotope infusion. Resting breath gases were collected over 5 min periods (Oxycon β , Mijnhardt, Mannheim, Germany) and vacutainer tubes were filled directly from the mixing chamber to determine the $^{13}C/^{12}C$ ratio in expired CO_2 . At 8:55 AM subjects started a warming-up of 5 min at 100 W. At 8:55 AM a sodium bicarbonate prime was given ($5.5 \mu mol \cdot kg^{-1}$ $NaH^{13}CO_3$; Cambridge Isotope Laboratories, USA) followed by a $[6,6-^2H_2]$ -glucose (Cambridge Isotope Laboratories,

USA) and a [U-¹³C]-glucose (Cambridge Isotope Laboratories, USA) prime (dose equal to one hour infusion). Thereafter a continuous infusion of sterile pyrogen free [6,6-²H₂]-glucose and [U-¹³C]-glucose was started via a calibrated IVAC 560 pump (San Diego, CA). The concentration of isotopes in the infusate were measured for each experiment so that the exact infusion rate could be determined. Infusion rates were $0.234 \pm 0.01 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and $0.032 \pm 0.002 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for [6,6-²H₂]-glucose and [U-¹³C]-glucose respectively.

At 9:00 AM the workload was increased to 50% $\text{VO}_{2\text{max}}$ for 120 min. During the first min subjects drank an initial bolus ($8 \text{ ml} \cdot \text{kg}^{-1}$) of one of the glucose solutions (24 g glucose during LO-GLU and 120 g glucose during HI-GLU). Thereafter every 15 min a beverage volume of $2 \text{ ml} \cdot \text{kg}^{-1}$ (6 g glucose during LO-GLU; 30 g glucose during HI-GLU) was provided. This feeding schedule has been shown to result in high rates of gastric emptying (26). The average amount of glucose provided during the 120 min of exercise was $72 \pm 1 \text{ g}$ in the LO-GLU trial and $360 \pm 7 \text{ g}$ in the HI-GLU trial. Blood samples were drawn at 15 min intervals until the end of exercise. Expiratory gases were collected every 15 min.

Glucose solutions

Subjects ingested glucose solutions prepared from potato derived glucose (AVEBE, The Netherlands) containing either $44 \text{ g} \cdot \text{l}^{-1}$ or $220 \text{ g} \cdot \text{l}^{-1}$ glucose. This glucose had a ¹³C-enrichment of $-26.1 \delta \text{ pm}$ vs PDB (determined by on line combustion-IRMS, Carlo-Erba-Finnigan MAT 252, Bremen, Germany) which is similar to the ¹³C-enrichment of expired air of Europeans (33). To minimize shifts in background enrichment as a result of changes in endogenous substrate utilization, standard procedures were followed (18, 19). Subjects were instructed not to consume any products with a high natural abundance of ¹³C during the entire experimental period. Furthermore subjects were instructed to keep their diet as constant as possible the days before the trials.

Analysis

Blood (10 ml) was collected into tubes containing EDTA and was centrifuged for 4 min at 4°C. Aliquots of plasma were frozen immediately in liquid nitrogen and stored at -40°C until analysis of glucose (Roche, Uni Kit III, 0710970), lactate (12) and free fatty acids (FFA) (Wako NEFA-C test kit, Wako Chemicals, Neuss, Germany) which were performed with the COBAS BIO semi automatic analyzer. Insulin was analyzed by radio immuno assay (Nuclilab Ultrasensitive Human Insulin RIA kit).

Breath samples were analyzed for ¹³C/¹²C ratio by gas chromatography continuous flow isotope ratio mass spectrometry (GC-IRMS) (Finnigan MAT 252, Bremen, Germany).

For determination of ¹³C/¹²C ratios of plasma glucose, glucose was derivatized to its trimethylsilyl derivative using TriSil Z® (Pierce). Thereafter the derivative was measured by GC-IRMS (Finnigan MAT 252, Bremen, Germany). By establishing the relationship between the enrichment of a series of glucose standards of variable

enrichment (by combustion-IRMS; Carlo Erba-Finnigan MAT 252, Bremen, Germany) and the enrichment of the glucose trimethylsilyl derivative of these standards, the enrichment of plasma glucose samples was determined.

Plasma ^2H -glucose enrichment was determined by gas chromatography-mass spectrometry (GC-MS) analysis of the glucose pentaacetate derivatives on a Finnigan INCOS-XL (Bremen, Germany). For ^2H glucose enrichment, ion masses of 200 and 202 were selectively monitored.

From indirect calorimetry (RER, VO_2) and stable isotope measurements ($^{13}\text{CO}_2$ / $^{12}\text{CO}_2$) (IRMS, Finnigan MAT 252), total energy expenditure and oxidation rates of total fat, total carbohydrates and exogenous glucose were calculated.

Calculations

From VCO_2 and VO_2 total carbohydrate and fat oxidation rates were calculated using the non-protein respiratory quotient (25).

$$\text{glucose oxidation} = 4.585 \text{ VCO}_2 - 3.226 \text{ VO}_2$$

$$\text{fat oxidation} = 1.695 \text{ VO}_2 - 1.701 \text{ VCO}_2$$

The isotopic enrichment was expressed as the delta per mil difference between the ^{13}C and ^{12}C ratio of the sample and a known laboratory reference standard according to the formula of Craig (9):

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

The $\delta^{13}\text{C}$ will then be related to an international standard Pee Dee Bellemnitella (PDB).

The amount of glucose oxidized will be calculated according to the formula:

$$\text{exogenous glucose oxidation} = \text{VCO}_2 \cdot \left(\frac{\delta \text{ Exp} - \delta \text{ C}}{\delta \text{ Ing} - \delta \text{ C}} \right) \left(\frac{1}{k} \right)$$

In which $\delta \text{ C}$ is the ^{13}C enrichment of expired air in the control test C (background), $\delta \text{ Exp}$ is the ^{13}C enrichment of expired air during exercise at different time points, $\delta \text{ Ing}$ is the ^{13}C enrichment of the ingested glucose and k is the conversion factor (amount of CO_2 (in liters) produced by the oxidation of 1 gram glucose; $k = 0.7467$ liter CO_2 per gram glucose).

The total rate of appearance (i.e. total Ra) and rates of disappearance (i.e. total Rd) of glucose was calculated using the single-pool non-steady state equations of Steele (31) as described elsewhere (34). Total Ra represents the splanchnic Ra from ingested CHO, hepatic glycogenolysis and gluconeogenesis.

$$\text{total Ra} = \frac{F - V[(C_2 + C_1)/2] [(E_2 - E_1)/(t_2 - t_1)]}{(E_2 + E_1)/2}$$

$$\text{total Rd} = \text{Ra} \cdot V \cdot \left(\frac{C_2 - C_1}{t_2 - t_1} \right)$$

where F is the infusion rate; V , the plasma volume of distribution = $160 \text{ ml} \cdot \text{kg}^{-1}$, C_2 and C_1 are the glucose concentrations at time 2 and 1 (i.e.; t_2 , t_1), respectively; and E_2 and E_1 are the plasma glucose enrichments at time 2 and 1 (i.e.; t_2 , t_1), respectively.

The $V^{13}\text{CO}_2$ production from the tracer infusion was calculated as:

$$V^{13}\text{CO}_2 (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = (E_{\text{CO}_2} - E_{\text{bkg}}) \cdot V\text{CO}_2$$

where E_{CO_2} is the breath $^{13}\text{C}/^{12}\text{C}$ ratio at a given time and E_{bkg} is the breath $^{13}\text{C}/^{12}\text{C}$ ratio at the same time during the trial with no tracer infusion for correction of background (i.e.; bkg) shifts. The conversion factor is $1 \text{ mol CO}_2 = 22.4 \text{ L}$. The percent of the infused $[\text{U-}^{13}\text{C}]$ tracer oxidized was calculated as:

$$\% \text{ infused tracer oxidized} = (V^{13}\text{CO}_2 / k) / [\text{U-}^{13}\text{C}]\text{-tracer infusion rate} \cdot 100$$

Plasma glucose oxidation was calculated as

$$\text{Plasma glucose oxidation} = \text{Rd glucose} \cdot \% \text{ infused glucose tracer oxidized}$$

The muscle glycogen oxidation in $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ was calculated as:

$$\text{Muscle glycogen oxidation} = \text{Total CHO oxidation} - \text{Plasma glucose oxidation}$$

Statistics

Analysis of variance for repeated measures was performed to study differences between the three conditions. A Scheffé post hoc test was applied in case of a significant ($P < 0.05$) F-ratio to locate the differences. Comparisons between results from two different tracer methods were compared by a paired t-test. All results are expressed as means $\pm$ SEM.

Results

Plasma glucose, insulin, free fatty acids and glycerol

In the FAST trial plasma glucose concentrations were in the range of 4.2–4.6 mM at rest and throughout the exercise bout (Figure 12.1). With glucose ingestion in the HI-GLU and LO-GLU trial plasma glucose concentrations peaked at 10–20 min at values of 5.5–5.8 mM. Plasma glucose concentrations were higher throughout exercise with glucose ingestion compared to FAST, although only HI-GLU versus FAST reached statistical significance at all time points ($P < 0.05$).

Figure 12.1: (Values are mean $\pm$ SEM) Plasma glucose, insulin FFA and glycerol concentrations during 120 min of exercise at 50% VO_2max with water ingestion (FAST; white circles), a low dose of glucose (LO-GLU; grey circles) or a high dose of glucose (HI-GLU; black circles).

* denotes a significant difference between FAST and HI-GLU, $P < 0.05$.

§ denotes a significant difference between FAST and LO-GLU; $P < 0.05$.

Plasma insulin was low at rest and during exercise when fasted (i.e.; $5\text{--}7 \mu\text{U.ml}^{-1}$) (Figure 12.1), but was significantly elevated by glucose ingestion during exercise. The highest insulin values were observed after 30 min ($9\pm 2 \mu\text{U.ml}^{-1}$ with LO-GLU and $17\pm 4 \mu\text{U.ml}^{-1}$ with HI-GLU). Plasma insulin decreased in all trials but remained elevated in the GLU trials in comparison with FAST. In all conditions plasma insulin decreased to or below the resting fasting level at the end of the trials. During the FAST trial, plasma FFA concentration initially decreased during the first 10 min and thereafter gradually increased during exercise to about three times basal level ($914\pm 99 \mu\text{M}$ at 120 min) (Figure 12.1). Plasma FFA in both GLU conditions followed a similar pattern but was significantly suppressed during LO-GLU (to $522\pm 79 \mu\text{M}$ at 120 min; $P < 0.05$) compared with FAST and even more when HI-GLU was ingested (to $301\pm 51 \mu\text{M}$ at 120 min; $P < 0.05$). Plasma glycerol concentrations were increased during exercise from resting values

in the range of 55-97 μM to 406 $\pm$ 39 μM at 120 min during FAST, 238 $\pm$ 35 μM during LO-GLU and 152 $\pm$ 38 μM during HI-GLU (Figure 12.1; $P<0.05$).

Whole body fat and carbohydrate oxidation

VO_2 was similar during each exercise session (38-39 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and elicited approximately 51 $\pm$ 2 % $\text{VO}_{2\text{max}}$ (Table 12.1). R decreased in the FAST trial ($P<0.05$) and remained stable with GLU ingestion. R was slightly higher with the HI-GLU feeding (Table 12.1). Carbohydrate oxidation decreased during FAST, decreased less with LO-GLU and remained stable with HI-GLU (Figure 12.2). After 120 min of exercise total CHO oxidation rates were 130 $\pm$ 11 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, 154 $\pm$ 10 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and 179 $\pm$ 6 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for FAST, LO-GLU and HI-GLU respectively (Figure 12.2). Total fat oxidation was markedly suppressed by the GLU feedings (Figure 12.2; $P<0.05$). After 120 min of exercise total fat oxidation rates were 40 $\pm$ 2 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, 33 $\pm$ 3 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and 26 $\pm$ 2 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for FAST, LO-GLU and HI-GLU respectively ($P<0.05$).

Breath ratio of $^{13}\text{CO}_2/^{12}\text{CO}_2$

In the exercise trials without tracer infusion, used to measure shifts in background or endogenous $^{13}\text{CO}_2$ production, the subjects displayed only a very slight non-significant elevation in $^{13}\text{CO}_2/^{12}\text{CO}_2$ breath ratio both when fasted and receiving glucose (Figure 12.3).

The glucose ingestion and oxidation did not alter $^{13}\text{CO}_2/^{12}\text{CO}_2$ breath ratio during the background trial (i.e.; without tracer infusion). The breath ratio's during the experimental trials with [U- ^{13}C]-glucose tracer infusion are shown in Figure 12.3.

Table 12.1. Whole body oxygen consumption (VO_2), respiratory exchange ratio, total carbohydrate oxidation, V^{13}CO_2 production and oxidation rates of the intravenously infused [U- ^{13}C]-glucose tracer during the 90-120 min period of exercise

Trial	Time (min)	VO_2 ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	Respiratory Exchange Ratio	Carbohydrate Oxidation ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	V^{13}CO_2 Production ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	% of Infused Tracer Oxidized
FAST	90-105 min	38.9 $\pm$ 0.7	0.83 $\pm$ 0.01	130 $\pm$ 13	0.209 $\pm$ 0.024	98.6 $\pm$ 3.4
	105-120 min	39.3 $\pm$ 0.7	0.83 $\pm$ 0.01	130 $\pm$ 11	0.210 $\pm$ 0.026	98.6 $\pm$ 3.4
LO-GLU	90-105 min	38.2 $\pm$ 0.3	0.86 $\pm$ 0.01*	156 $\pm$ 10*	0.194 $\pm$ 0.007	98.7 $\pm$ 1.4
	105-120 min	38.5 $\pm$ 0.3	0.85 $\pm$ 0.01*	155 $\pm$ 10*	0.192 $\pm$ 0.006	97.9 $\pm$ 1.0
HI-GLU	90-105 min	37.7 $\pm$ 0.5	0.89 $\pm$ 0.01*†	182 $\pm$ 5*†	0.187 $\pm$ 0.003	98.3 $\pm$ 1.0
	105-120 min	37.9 $\pm$ 0.5	0.89 $\pm$ 0.01*†	179 $\pm$ 6*†	0.183 $\pm$ 0.004	96.1 $\pm$ 1.3

(Values are mean $\pm$ SEM) V^{13}CO_2 production is the product of breath $^{13}\text{CO}_2$ enrichment above baseline and VCO_2 production.

% of infused tracer oxidized is calculated as (V^{13}CO_2 production / intravenous tracer infusion rate)

* indicates that GLU trials are significantly different from FAST at that time; $P<0.05$.

† indicates that HI-GLU trial is significantly different from the LO-GLU trial at that time; $P<0.05$.

Figure 12.2: (Values are mean $\pm$ SEM) The rates of total CHO and fat oxidation during 120 min of exercise at 50% $\text{VO}_{2\text{max}}$ with water ingestion (FAST; white circles), a low dose of glucose (LO-GLU; grey circles) or a high dose of glucose (HI-GLU; black circles).

* denotes a significant difference between FAST and HI-GLU, $P < 0.05$.

§ denotes a significant difference between FAST and LO-GLU; $P < 0.05$.

The $\text{NaH}^{13}\text{CO}_3$ prime, given just prior to beginning exercise during each trial, as expected, markedly raised breath $^{13}\text{CO}_2/^{12}\text{CO}_2$ during the first ten min. However, $^{13}\text{CO}_2/^{12}\text{CO}_2$ breath ratios reached a plateau after ~ 60 min (Figure 12.3).

[U- ^{13}C]-tracer oxidation

No differences were observed between the $^{13}\text{CO}_2$ production rates of the three trials (Table 12.1). During the 60–120 min period of exercise, the mean % oxidation of the infused tracer was $99 \pm 4\%$ for FAST, $99 \pm 1\%$ for LO-GLU and $100 \pm 2\%$ for HI-GLU. The values for the % oxidation of the infused tracer for the 90–105 and 105–120 min period are displayed in Table 12.1.

Rate of appearance and disappearance of plasma glucose

The increase in plasma $^{13}\text{C}/^{12}\text{C}$ -glucose ratio and ^2H -enrichment as a result of the tracer infusion was stable during the 60–120 min period in all trials (Figure 12.3).

Figure 12.3: (Values are mean $\pm$ SEM) Plateau in the breath $^{13}\text{CO}_2/^{12}\text{CO}_2$ enrichment, the plasma glucose ^{13}C -enrichment and the plasma $^2\text{H}_2$ -glucose enrichment during exercise at 50% VO_2max with water ingestion (FAST; white circles), a low dose of glucose (LO-GLU; grey circles) or a high dose of glucose (HI-GLU; black circles). Breath $^{13}\text{CO}_2/^{12}\text{CO}_2$ enrichment is also displayed for the background (BKG) correction trials (Squares).

Ra and Rd glucose were calculated from both tracers ([6,6- $^2\text{H}_2$]-glucose and [U- ^{13}C]-glucose) and were slightly lower when using the plasma ^{13}C -glucose enrichment (Table 12.3, Figure 12.4). Both Ra and Rd glucose were markedly elevated with glucose ingestion (44% with LO-GLU and 125% with HI-GLU; $P < 0.05$; Table 12.2).

Figure 12.4: (Values are mean $\pm$ SEM) a) (upper panel) Comparison of the rate of disappearance of glucose (Rd) measured by a $^2\text{H}_2$ -glucose tracer and the rate of plasma glucose oxidation (Rox) and b) (lower panel) comparison of two different tracers to measure the rate of appearance of glucose (Ra). The line represents the 1:1 relationship.

Figure 12.5: (Values are mean±SEM) The rate of disappearance of glucose (Rd) and the rate of plasma glucose oxidation (Rox) during the 60-120 min period with ingestion of water (FAST; white circles and squares), a low dose of glucose (LO-GLU; grey circles and squares) or a high dose of glucose (HI-GLU; black circles and squares).

Oxidation of plasma glucose

Plasma glucose oxidation showed an identical pattern as the Ra and Rd glucose. LO-GLU caused a 44% increase in plasma glucose oxidation compared to FAST and HI-GLU a 126% increase. The rate of disappearance ($[6,6-^2\text{H}_2]$ -glucose tracer) and the rate of oxidation ($[U-^{13}\text{C}]$ -glucose tracer) during the second hour of exercise are displayed in Figure 12.5. Plasma oxidation was not significantly different from Rd glucose.

Figure 12.6: (Values are mean±SEM) The energetic contribution of substrates during the 60-120 min period of exercise at 50% VO_2max with water ingestion (FAST), a low dose of glucose (LO-GLU) or a high dose of glucose (HI-GLU).

Table 12.2. Effect ingestion of a low dose of glucose (LO-GLU) or a high dose (HI-GLU) during exercise compared to fasting (FAST) on various aspects of CHO metabolism during exercise including total CHO oxidation, the rate of appearance of plasma glucose (Ra glucose), the rate of disappearance of plasma glucose (Rd glucose), plasma glucose oxidation and glycogen oxidation and/or lactate oxidation.

Trial	Time (min)	Total CHO Oxidation ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	Ra glucose ¹ ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	Rd glucose ¹ ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	Plasma glucose oxidation ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	Percent of Rd oxidized (%)	Muscle glycogen and/or lactate oxidation ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)
FAST	90-105 min	130 $\pm$ 13	51 $\pm$ 3	53 $\pm$ 3	52 $\pm$ 3	99 $\pm$ 3	78 $\pm$ 12
	105-120 min	130 $\pm$ 11	51 $\pm$ 3	52 $\pm$ 3	50 $\pm$ 2	99 $\pm$ 3	79 $\pm$ 11
LO-GLU	90-105 min	156 $\pm$ 10	71 $\pm$ 1*	73 $\pm$ 1*	72 $\pm$ 2	99 $\pm$ 1%	84 $\pm$ 11
	105-120 min	155 $\pm$ 10	73 $\pm$ 3*	74 $\pm$ 3*	72 $\pm$ 4	98 $\pm$ 1%	82 $\pm$ 13
HI-GLU	90-105 min	182 $\pm$ 5	109 $\pm$ 8*†	111 $\pm$ 8*†	109 $\pm$ 8*†	98 $\pm$ 1%	73 $\pm$ 9
	105-120 min	179 $\pm$ 6	119 $\pm$ 10*†	117 $\pm$ 9*†	113 $\pm$ 9*†	97 $\pm$ 1%	66 $\pm$ 6

(Values are mean $\pm$ SEM for 6 subjects). Plasma glucose oxidation is calculated as the product of Rd glucose and the % of infused [U-¹³C]-glucose tracer oxidized. Glycogen and direct or indirect oxidation via lactate is calculated as the difference between total CHO oxidation and plasma glucose oxidation.

¹ Ra glucose and Rd glucose calculated using the [6,6-²H₂]-glucose tracer.

* indicates that GLU-trials are significantly different from the FAST trial; P<0.05.

† indicates that the HI-GLU trial is significantly different from the LO-GLU trial; P<0.05.

Table 12.3. Comparison of tracers. The rate of appearance of plasma glucose (Ra glucose) and the rate of disappearance of plasma glucose (Rd glucose) calculated from the [6,6-²H₂]-glucose tracer compared to the [U-¹³C]-glucose tracer.

Trial	Time (min)	Ra glucose ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) [6,6- ² H ₂]-glucose	Ra glucose ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) [U- ¹³ C]-glucose	Rd glucose ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) [6,6- ² H ₂]-glucose	Rd glucose ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) [U- ¹³ C]-glucose
FAST	90-105 min	51 $\pm$ 3	42 $\pm$ 4	53 $\pm$ 3	44 $\pm$ 4
	105-120 min	51 $\pm$ 3	46 $\pm$ 5	52 $\pm$ 3	46 $\pm$ 5
LO-GLU	90-105 min	71 $\pm$ 1*	65 $\pm$ 5*	73 $\pm$ 1*	67 $\pm$ 5*
	105-120 min	73 $\pm$ 3*	67 $\pm$ 5*	74 $\pm$ 3*	68 $\pm$ 6*
HI-GLU	90-105 min	109 $\pm$ 8*†	105 $\pm$ 5*†	111 $\pm$ 8*†	107 $\pm$ 5*†
	105-120 min	119 $\pm$ 10*†	113 $\pm$ 6*†	117 $\pm$ 9*†	111 $\pm$ 6*†

(Values are mean $\pm$ SEM for 6 subjects).

* indicates that GLU-trials are significantly different from the fast trial; P<0.05.

† indicates that the HI-GLU trial is significantly different from the LO-GLU trial; P<0.05.

Furthermore, calculated glycogen oxidation (total CHO oxidation minus plasma glucose oxidation) did not change as a function of CHO ingestion. Figure 12.6 describes the shifts in substrate utilization as a result of glucose ingestion. It should be noted that glucose feedings, markedly reduced fat oxidation and increased plasma glucose oxidation but did not alter the rate of muscle glycogen (and lactate) oxidation.

Discussion

Effect of glucose ingestion

To our knowledge this is the first study that systematically compared the rate of disappearance and the oxidation of plasma glucose during exercise by using infusions of two different tracers. Glucose appearance in the plasma increased with increasing rates of ingestion while the Rd glucose was closely matched by plasma glucose oxidation (Figure 12.4 and 12.5). So at least in the experimental conditions of the present study no plasma glucose was stored as glycogen. This is in agreement with several direct measurements of muscle glycogen concentrations before and after continuous exercise with or without carbohydrate ingestion (7, 10, 11, 13).

The present study investigated the rate of glucose appearance in the plasma during moderate intensity exercise in the fasted state (FAST) or with glucose ingestion. Subjects received either a low dose of glucose (LO-GLU) or a high dose of glucose (HI-GLU). The LO-GLU ingestion increased Ra glucose by 44% and HI-GLU ingestion increased Ra glucose by 126%. However, whereas glucose ingestion during HI-GLU was five times larger than during LO-GLU, the Ra glucose increased less than twofold ($72 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ during LO-GLU and $109\text{--}113 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ during HI-GLU). Average ingestion during the second hour of exercise was $0.3 \text{ g}\cdot\text{min}^{-1}$ in the LO-GLU and $1.5 \text{ g}\cdot\text{min}^{-1}$ in the HI-GLU trials. The rate of appearance of glucose was $0.84 \text{ g}\cdot\text{min}^{-1}$ and $1.36 \text{ g}\cdot\text{min}^{-1}$ respectively, indicating that with the LO-GLU the liver is still producing glucose whereas with the HI-GLU feeding it is theoretically possible (in case absorption is assumed not to be limiting) that hepatic glucose production was completely suppressed. However, dual tracer studies with infusion of one glucose tracer and ingestion of another are needed to distinguish between gut derived Ra and hepatic glucose output. Without glucose ingestion the hepatic glucose production was approximately $0.5 \text{ g}\cdot\text{min}^{-1}$ indicating that the liver during the 2 hour period of exercise may have produced about 60 g glucose. This is a large amount since it is believed that the liver contains approximately 80 grams of glycogen. Glucose ingestion during exercise may reduce hepatic glucose production (i.e. spare liver glycogen) at $70\% \text{VO}_{2\text{max}}$ as shown by Bosch et al (2) and McConnell et al. (22).

These results also indicate that with large doses of ingested glucose (360 g), not all of the glucose appears in the plasma. This is in agreement with previous suggestions that the rate of exogenous glucose oxidation may be limited by the rate of digestion, absorption, maximal hepatic glucose output and subsequent transport of

glucose into the systemic blood supply rather than glucose uptake and oxidation by the muscle (15). Several studies showed that oral carbohydrate oxidation is limited to about $1 \text{ g} \cdot \text{min}^{-1}$ (16). Even when large amounts of carbohydrate were ingested oxidation rates did not exceed $1 \text{ g} \cdot \text{min}^{-1}$ (27, 33).

Glucose ingestion also markedly suppressed fat oxidation compared with fasting and this effect was dose dependent; HI-GLU suppressed fat oxidation more than LO-GLU. The lower rates of fat oxidation may be at least in part insulin mediated (Figure 12.1). Insulin has been shown to be a potent inhibitor of lipolysis and Ra FFA (3, 23). Here, higher insulin levels after glucose ingestion reduced whole body lipolysis as indirectly indicated by lower plasma glycerol and FFA concentrations (Figure 12.1). Decreased FFA availability, higher plasma insulin concentrations along with an increased glycolytic flux have been shown to reduce FFA oxidation at rest (30). Although in this study we observed that glucose ingestion during exercise increased total CHO oxidation, McConnell et al (22) and Bosch et al (2) did not observe increased CHO oxidation rates with CHO ingestion during exercise. This may in part be explained by the differences in the exercise intensity and possibly the corresponding difference in plasma insulin concentration. Whereas in their studies the exercise intensities were $70\% \text{VO}_{2\text{max}}$, we employed an exercise intensity of $50\% \text{VO}_{2\text{max}}$. At low to moderate exercise intensities ($50\% \text{VO}_{2\text{max}}$) glucose utilization may be enhanced by CHO ingestion whereas during more intense exercise ($>70\% \text{VO}_{2\text{max}}$) exogenous CHO simply seems to substitute for endogenously produced glucose, with no overall increase in CHO oxidation (2, 22).

Ra calculated from [U- ^{13}C]-glucose or [6,6- $^2\text{H}_2$]-glucose

When the plasma glucose ^{13}C -enrichment is measured by GC-IRMS the [U- ^{13}C]-glucose carbon may recycle during the process of gluconeogenesis and Ra glucose may be underestimated. The reason for this is the following: One molecule of uniformly labeled glucose produces two molecules of uniformly labeled pyruvate. This pyruvate may be converted into lactate or alanine which are again precursors for gluconeogenesis and this may lead to the incorporation of label into newly synthesized glucose. In GC-IRMS analysis the whole molecule is oxidized and therefore the increase in ratio $^{13}\text{C}/^{12}\text{C}$ of all carbons of the glucose molecule is measured. The Ra glucose calculated from GC-IRMS measurements of this tracer therefore excludes gluconeogenesis and consequently represents a possible underestimation of the true Ra glucose. It is believed that a more appropriate method to measure total Ra glucose is the use of a [6,6- $^2\text{H}_2$]-glucose tracer which measures total glucose production when the enrichment is measured by GC-IRMS as the tracer atoms do not recycle during gluconeogenesis (34). To compare the above mentioned methods to measure Ra glucose we also infused a [6,6- $^2\text{H}_2$]-glucose tracer in this study. The difference between the two methods theoretically gives an estimate of glucose carbon recycling (and a rough estimate of gluconeogenesis). Similar approaches have been used in the past to estimate glucose carbon recycling (32).

It has been reported that using a ^{13}C -tracer for studying fatty acid metabolism part

of the tracer may be fixated in exchange reaction with the TCA-cycle. For example, some ^{13}C -label may be incorporated into the glutamate/glutamine pool via α -ketoglutarate, or into phosphoenolpyruvate via oxaloacetate. This label fixation results in a decreased recovery of label in the expired gases and in order to correct for this loss of label, the acetate correction factor was proposed (29). However, if we would apply the acetate correction factor to the glucose oxidation data of the present study plasma glucose oxidation rates would exceed the rates of disappearance which of course is impossible. Most likely there is a simultaneous recycling of label in gluconeogenesis, which leads to an overestimation of R_a glucose which compensates for the underestimation due to label loss in the TCA-cycle. By comparison of two different tracers however, we showed that there is probably only little recycling due to gluconeogenesis. In this study we found that R_a and R_d glucose were similar, independent of the tracer used (i.e. $[\text{U-}^{13}\text{C}]$ -glucose measured by GC-IRMS or $[6,6\text{-}^2\text{H}_2]$ -glucose measured by GC-MS) (Table 12.3). In Figure 12.4, R_a glucose calculated from the $[6,6\text{-}^2\text{H}_2]$ -glucose tracer is plotted against the $[\text{U-}^{13}\text{C}]$ -glucose tracer (total enrichment). This figure shows that generally R_a glucose calculated from the $[\text{U-}^{13}\text{C}]$ -glucose tracer gave slightly lower numbers, which is in agreement with the expectations. The difference, however, is small ($\sim 4\text{-}10\%$) and statistically not significant and there are no differences between the FAST and the GLU trials (i.e. glucose carbon recycling during exercise is independent of glucose feeding). Therefore we conclude that under the conditions of the present study, the rate of glucose recycling was limited.

In summary the results of this study indicate that carbohydrate feedings during exercise at $50\%\text{VO}_{2\text{max}}$ decreased fat oxidation and increased R_a and R_{ox} glucose. Muscle glycogen breakdown, however, was not affected. All glucose disappearing from the plasma was instantly oxidized. Comparison of a $[6,6\text{-}^2\text{H}_2]$ - and a $[\text{U-}^{13}\text{C}]$ -glucose tracer revealed that only a small amount of glucose carbons is recycling during gluconeogenesis.

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Chapter 13

Fatty acid oxidation is directly regulated by carbohydrate metabolism during exercise

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Abstract

We determined if increased glycolytic flux from hyperglycemia and hyperinsulinemia directly reduces fatty acid oxidation during exercise, possibly by inhibiting fatty acid transport into mitochondria. Fatty acid oxidation rates were measured during constant rate intravenous infusion of trace amounts of a long-chain fatty acid ($1\text{-}^{13}\text{C}$ -palmitate; PAL) *vs.* a medium-chain fatty acid ($1\text{-}^{13}\text{C}$ -octanoate; OCT). Six endurance-trained men cycled for 40 min at 50% $\text{VO}_{2\text{max}}$: 1) after an overnight fast (FAST); and 2) following glucose ingestion ($2.8\text{ g}\cdot\text{kg}^{-1}$ total), 60 and 10 min before exercise (GLU). GLU caused hyperglycemia, hyperinsulinemia and a 34% reduction in total fat oxidation due to an approximately equal reduction in oxidation of plasma FFA and intramuscular triglycerides (all $P<0.05$). Oxidation of PAL was significantly reduced during GLU compared to FAST (i.e.; $70.0\pm 4.1\%$ *vs.* $86.0\pm 1.9\%$ of tracer infusion rate; $P<0.05$). However, GLU had no effect on OCT oxidation, which is apparently not limited by mitochondrial transport. Furthermore, GLU reduced plasma FFA appearance 36% ($P<0.05$), indicating a coordination of effects on adipose tissue and muscle. In summary, substrate oxidation during exercise can be regulated by increased glycolytic flux which is accompanied by a direct inhibition of long-chain fatty acid oxidation, possibly through reductions in fatty acid transport into mitochondria. These observations indicate that carbohydrate availability can directly regulate fat oxidation during exercise.

Introduction

The factors that regulate the interplay between fat and carbohydrate oxidation, especially in skeletal muscle during exercise, are not well understood. The classical concept is that fat metabolism regulates carbohydrate metabolism. The evidence most often cited to support this concept is that reported by Randle and co-workers (10, 19) whereby increases in fatty acids are proposed to increase acetyl-CoA/CoA and citrate levels which then inhibit pyruvate dehydrogenase and phosphofructokinase with glucose-6-phosphate (G6P) increasing to reduce hexokinase and glucose uptake in rat diaphragm, heart and liver. However, there has been little support for this classical concept in exercising skeletal muscle of humans (5, 6, 21). During intense exercise (i.e.; cycling at 85% VO_2max), the elevation of plasma FFA does not increase muscle citrate or G6P and blood glucose uptake is not reduced (5, 6, 21). In exercising people, the major interplay by which plasma FFA alters carbohydrate metabolism appears to be that the lowering of plasma FFA during high intensity exercise deprives the muscle of a plasma substrate and thus increases muscle glycogenolysis as a result of the increased metabolic stress (i.e.; higher AMP levels) (5, 6, 21). Therefore, it generally appears that the ability of fat to regulate carbohydrate metabolism in people during exercise is limited and not in accordance with the classical glucose-fatty acid cycle (10, 19).

The present paper tests the converse of the classical concept by hypothesizing that carbohydrate metabolism largely regulates fat oxidation in man during exercise. More specifically, we tested the hypothesis that increases in glycolytic flux and carbohydrate oxidation in skeletal muscle reduce fat oxidation by inhibiting the oxidation of long-chain fatty acids, possibly by reducing their transport into mitochondria. In the present study, glycolytic flux during exercise (i.e.; 50% maximal oxygen consumption) was increased by making the subjects hyperinsulinemic and hyperglycemic through dietary preexercise glucose ingestion. Fatty acid oxidation during exercise is presently assessed by the extent to which trace amounts of an intravenously infused (exogenous constant rate) long-chain fatty acid (i.e.; 1- ^{13}C -palmitate) are oxidized both when fasted and fed glucose. This is compared to the oxidation rates of a medium-chain fatty acid (i.e.; 1- ^{13}C -octanoate), also administered intravenously and in trace amounts both when fasted and fed glucose. Octanoate oxidation is not as limited by transport into mitochondria as is palmitate oxidation (11, 23). Therefore, we hypothesize that the increased glycolytic flux from glucose ingestion should reduce palmitate oxidation to a greater extent than octanoate oxidation if indeed fatty acid oxidation during exercise is regulated by mitochondrial transport.

Additionally, the hyperinsulinemia from glucose ingestion would obviously be expected to lower endogenous fat oxidation, to some extent, by reducing lipolysis and plasma fatty acid mobilization (3). Therefore, another purpose was to determine the relative extent to which whole body fat oxidation was reduced by

impaired plasma fatty acid mobilization compared to impaired oxidation by muscle. By directly measuring plasma fatty acid oxidation and total fat oxidation, it is also possible to calculate intramuscular triglyceride oxidation and identify factors regulating its metabolism.

Methods

General experimental design

Six endurance trained men were each studied during exercise on six occasions each separated by 4-7 days and randomly ordered. During three trials, they exercised after an overnight fast (FAST), with the goal that their metabolic responses would be similar because the only difference among these three FAST trials was the isotopically labeled fatty acid infusion during exercise, which were only in trace amounts. During one FAST trial, they received intravenous infusion of trace amounts of 1-¹³C-palmitate (i.e.; FAST-Palmitate) to measure long-chain fatty acid oxidation as well as plasma FFA kinetics. During a second FAST trial they received trace intravenous infusion of 1-¹³C-octanoate (i.e.; FAST-Octanoate) to measure medium-chain fatty acid oxidation. A third fasting trial was performed without tracer infusion to measure background shifts in breath ¹³C/¹²C ratio during the 40 min of exercise, to ideally correct the tracer infusion trials for any changes in endogenous ¹³CO₂ production throughout exercise.

Responses during exercise were evaluated during an additional three trials performed after receiving glucose feedings 60 and 10 min before exercise (i.e.; GLU) to raise insulin and alter fat and carbohydrate metabolism. Again, each of these three GLU trials were similar to one and other, the only difference being whether 1-¹³C-palmitate (i.e.; GLU-Palmitate), or 1-¹³C-octanoate (i.e.; GLU-Octanoate) or no tracer were infused intravenously. Tracer infusion was begun 5 min prior to exercise and maintained constant during 40 min of exercise at 50% VO₂max. During the FAST-Palmitate and FAST-Octanoate trials, the subjects continued exercising for an additional 40 min (i.e.; 40-80 min) after cessation of tracer infusion at 40 min so that the decay in breath ¹³C/¹²C ratio could be described.

Subjects, preliminary testing and diet

Six well trained endurance cyclists volunteered for this study that was approved by the local Ethical Committee. Their mean age and weight were 22±2 years and 69±2 kg. Approximately one week before the experimental trials, maximal oxygen uptake (VO₂max) was determined during incremental cycling (Lode Ergometer, Groningen, The Netherlands) and found to average 72±2 ml·kg⁻¹·min⁻¹. Subjects consumed the same foods during the three days before each trial, making sure that carbohydrate from C4 plants was low, so as to maintain a generally similar ¹³C enrichment in their glycogen and triglyceride and thus minimize background shifts from endogenous ¹³CO₂ production during exercise. They also refrained from exercise for 46 hours prior to each trial.

Experimental procedures, glucose feeding and indirect calorimetry

Upon arrival at the laboratory, Teflon catheters were placed percutaneously in an antecubital vein of one arm for isotope infusion and in a contralateral antecubital vein for blood sampling. After catheter placement, subjects remained seated for approximately 60 min. During the GLU trials, subjects ingested $1.4 \text{ g}\cdot\text{kg}^{-1}$ of glucose (dissolved in water to a 40% solution) both at 60 min and 10 min before exercise (a total of approximately 200 grams). This glucose was derived from potato with $^{13}\text{C}/^{12}\text{C}$ ratio of 0.0109437, which is identical to the enrichment of CO_2 produced from their endogenous carbohydrate and glycogen stores. During the entire 40 min of exercise (cycling a Lode Ergometer at 80-90 RPM with fan cooling), the subjects breathed through a mouthpiece and expired gases were directed to a 10 L mixing chamber. Oxygen consumption and carbon dioxide production were measured using open circuit spirometry (i.e.; Oxycon- Mijnhard, The Netherlands) and averaged during the 25-30 min and 35-40 min period of exercise, with total carbohydrate and fat oxidation calculated as described below.

Isotope infusion and blood sampling

Blood samples were obtained ten minutes before exercise to determine plasma substrate and insulin concentrations and background isotope enrichment. Additionally, a resting breath sample for $^{13}\text{C}/^{12}\text{C}$ ratio was collected directly from the mixing chamber into a 20 ml vacutainer tube (Becton Dickinson, Meylan Cedex, France). Immediately thereafter, subjects received an intravenous priming dose of $18 \text{ }\mu\text{mol}\cdot\text{kg}^{-1}$ of $\text{NaH}^{13}\text{CO}_3$. Three minutes before subjects started exercising, a continuous constant rate intravenous infusion of either $1\text{-}^{13}\text{C}$ -palmitate ($0.167\pm 0.005 \text{ }\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) or $1\text{-}^{13}\text{C}$ -octanoate ($0.238\pm 0.001 \text{ }\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was started and then continued during the 40 min of exercise via a calibrated IVAC 560 pump (San Diego, CA). The concentration of isotopes in the infusate was determined for each experiment so that the exact infusion rate could be determined.

The palmitate tracer ($1\text{-}^{13}\text{C}$ -potassium palmitate; 99% enriched; Cambridge Isotope Laboratories; Andover, MA) was dissolved in heated sterile water and passed through a $0.2 \text{ }\mu\text{m}$ filter into warm 5% human serum albumin (Central Blood Bank, The Netherlands) to make a $2.3\pm 0.1 \text{ mM}$ solution. The octanoate tracer ($1\text{-}^{13}\text{C}$ -octanoic acid; 99% enriched; Isotech, Miamisburg, OH) was dissolved in 0.9% saline by heating and adjusting pH and made to a concentration of 1.9 mM . Blood samples were collected at 10, 20, 30 and 40 min of exercise to measure plasma palmitate kinetics. Blood samples for analysis of substrate concentrations and isotope enrichment were collected in 10 ml EDTA tubes. For plasma insulin analysis, 4 ml of blood were placed in tubes containing 0.2 U of aprotinin and 9.6 mg of EDTA. All blood samples were placed on ice then centrifuged at 4°C and aliquots of plasma were immediately frozen in liquid nitrogen and stored at -80°C until analysis. Breath samples of mixed expired air were collected at 10, 20, 30 and 40 min for determination of $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio and at 50, 60, 70, and 80 min of continued exercise during FAST following cessation of tracer infusion.

Sample analysis

Breath samples were analyzed for $^{13}\text{C}/^{12}\text{C}$ ratio by GC continuous flow IRMS (Finnigan MAT 252, Bremen, FRG). For determination of plasma palmitate and FFA kinetics, FFAs were extracted from plasma, isolated by thin-layer chromatography, and derivatized to their methyl esters. Isotope enrichment of palmitate was determined by gas chromatography-mass spectrometry (GC-MS) analysis of the methyl ester derivatives on an INCOS (Finnigan INCOS-XL, Bremen, FRG). For palmitate $^{13}\text{C}/^{12}\text{C}$ ratios, ion masses of 270 and 271 were selectively monitored. Palmitate concentration was determined using heptadecanoic acid as internal standard and on average it comprised $21.1 \pm 1.1\%$ of total FFA. Total plasma free fatty acid (FFA) concentration was determined with a Wako NEFA-C test kit (Wako Chemicals, Neuss, Germany) and glucose was analyzed with the glucose oxidase method (Roche, Uni Kit III). Both analyses were performed on a COBAS BIO centrifugal analyzer (Roche diagnostics). Plasma insulin concentration was determined using radioimmunoassay (Linco Inc.; St Louis).

Calculations

Carbohydrate and fat oxidation were calculated using stoichiometric equations (9). Fatty acid oxidation was determined by converting the rate of triglyceride oxidation to its molar equivalent, with the assumption that the average molecular weight of triglyceride is $860 \text{ g}\cdot\text{mol}^{-1}$, and multiplying the molar rate of triglyceride oxidation by three because each molecule contains three moles of fatty acids. The rate of appearance (i.e. R_a) and rates of disappearance (i.e.: R_d) of palmitate were calculated using the non-steady state equations of Steele (29) as previously described (32) with:

$$\text{total } R_a = \frac{F - V[(C_2 + C_1)/2] \cdot [(E_2 - E_1)/(t_2 - t_1)]}{(E_2 + E_1)/2}$$

$$\text{total } R_d = R_a \cdot pV \cdot \left(\frac{C_2 - C_1}{t_2 - t_1} \right)$$

where F is the infusion rate; V , the plasma volume of distribution was $40 \text{ ml}\cdot\text{kg}^{-1}$, C_2 and C_1 the palmitate concentration at time 2 and 1 (i.e.; t_2 , t_1), respectively. The R_a of FFA was calculated by dividing palmitate R_a by the fractional contribution of palmitate to the total FFA concentration. The $V^{13}\text{CO}_2$ production from the tracer infusion was calculated as:

$$V^{13}\text{CO}_2 (\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}) = (E_{\text{CO}_2} - E_{\text{bkg}}) \cdot V\text{CO}_2$$

where E_{CO_2} is the breath $^{13}\text{C}/^{12}\text{C}$ ratio at a given time and E_{bkg} is the breath $^{13}\text{C}/^{12}\text{C}$ ratio at the same time during the trial with no tracer infusion for correction of background (i.e.; bkg) shifts. The conversion factor is $1 \text{ mol } \text{CO}_2 = 22.4 \text{ L}$.

The percent of the infused $1\text{-}^{13}\text{C}$ tracer (e.g. palmitate or octanoate) oxidized was calculated as:

$$\% \text{ infused tracer oxidized} = (V^{13}\text{CO}_2 / k) / 1\text{-}^{13}\text{C-tracer infusion rate} * 100.$$

To account for the loss of $^{13}\text{CO}_2$ label in breath due to fixation at any step between the entrance of labeled acetyl-CoA into the TCA cycle until recovery in breath CO_2 , including bicarbonate loss, the $V^{13}\text{CO}_2$ oxidation values were corrected by dividing it by the recently reported acetate correction factor (27). During exercise at $35\text{-}36 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in the present study, the correction factor (i.e.; k) is relatively small and equal to 0.9. Plasma FFA oxidation was calculated as:

$$\text{Plasma FFA oxidation} = \text{Rd FFA} * \% \text{ infused palmitate tracer oxidized}$$

The FFA oxidation from intramuscular triglycerides was expressed in $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of FFA and calculated as:

$$\text{FFA oxidation from IMTG} = \text{Total fat oxidation} - \text{Plasma FFA oxidation}$$

Statistical Analysis

Significant differences between the FAST and GLU trials were identified using a two-way (treatment X time) analysis of variance. Differences at a given time were identified using a paired Student's t -test with a Bonferoni correction. Statistical significance was set at $P < 0.05$. The results are presented as means $\pm$ SEM.

Results

Plasma glucose, free fatty acid and insulin

The FAST trials were characterized by plasma glucose in the range of $4.4\text{-}4.8 \text{ mM}$ at rest and throughout the exercise bout (Figure 13.1). Additionally, plasma insulin was low at rest and during exercise when fasted (i.e.; $3\text{-}6 \mu\text{U}\cdot\text{ml}^{-1}$) (Figure 13.1).

Glucose ingestion 60 min and 10 min before exercise significantly raised preexercise plasma glucose (i.e.; $5.6\text{-}6.0 \text{ mM}$; $P < 0.05$) and insulin ($42 \mu\text{U}\cdot\text{ml}^{-1}$) during the GLU trials. Furthermore, plasma insulin during exercise was significantly elevated during GLU compared to FAST (16 ± 2 vs. $4 \pm 1 \mu\text{U}\cdot\text{ml}^{-1}$; $P < 0.05$). During both FAST trials, plasma FFA concentration was maintained at moderate levels during the 0-40 min period of exercise (317 ± 19 to $405 \pm 40 \mu\text{M}$; $P < 0.05$; Figure 13.1). However, the GLUCOSE trials were characterized by low plasma FFA in the range of 154 ± 3 to $186 \pm 15 \mu\text{M}$ throughout the 0-40 min period, due to suppressed Ra FFA, as discussed below.

Figure 13.1 Responses before (0 min) and during 40 min of exercise at 50% VO_2max when fasted (FAST) compared to when fed glucose before exercise (GLUCOSE). Under both conditions, subjects received intravenous tracer infusion of palmitate (FAST-Palmitate and GLUCOSE-Palmitate) or octanoate (FAST-Octanoate and GLUCOSE-Octanoate). A) Plasma Insulin; B) Plasma Glucose; C) Plasma Free Fatty Acids. Values are means $\pm$ SE for 6 subjects. * GLUCOSE trials significantly different from FAST trials; $P < 0.05$.

Whole body fat and carbohydrate oxidation

VO_2 was similar during all four experimental trials and was quite stable during the 25-40 min period of exercise (i.e.; 35.5 to 36.5 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and elicited approximately 50 ± 2 % VO_2max (Table 13.1). RER was also stable during exercise but was significantly increased ($P < 0.05$) during GLU compared to FAST and therefore carbohydrate oxidation was increased 24-28% ($P < 0.05$; Table 13.1, Figure 13.2).

Table 13.1 Oxidation during exercise of the intravenously infused 1-¹³C fatty acid tracer (either palmitate or octanoate) when fasted (i.e.; FAST) or fed glucose (GLU) before exercise. Also reported are whole body oxygen consumption (VO₂), respiratory exchange ratio, carbohydrate oxidation and oxidation rates of the intravenously infused tracers.

Trial	Time (min)	VO ₂ (ml·kg ⁻¹ ·min ⁻¹)	Resp. Exchange Ratio	CHO Oxidation (μmol·kg ⁻¹ ·min ⁻¹)	V ¹³ CO ₂ Production (μmol·kg ⁻¹ ·min ⁻¹)	% Infused Tracer Oxidized
FAST	25-30	36.3±1.0	0.86±0.01	144±4	0.131±0.004	87.7±2.0
-Palmitate	35-40	36.4±0.9	0.86±0.01	141±5	0.129±0.003	86.0±1.9
GLU	25-30	35.9±0.9	0.91±0.01*	187±15*	0.106±0.006*	70.8±4.1*
-Palmitate	35-40	35.8±0.9	0.90±0.01*	181±15*	0.105±0.006*	70.0±4.1*
FAST-	25-30	35.5±0.8	0.87±0.01	152±4	0.202±0.005	94.6±2.2†
Octanoate	35-40	36.0±0.9	0.86±0.01	148±5	0.211±0.005	98.7±2.5†
GLU	25-30	36.1±0.8	0.91±0.05*	185±4*	0.202±0.005	95.5±2.2†
-Octanoate	35-40	36.0±0.9	0.90±0.04*	183±5*	0.210±0.005	98.1±2.1†

Values are means±SE. V¹³CO₂ production is the product of breath ¹³CO₂ enrichment above baseline and VCO₂ production. % of infused tracer oxidized is calculated as ((V¹³CO₂ production/ 0.9) / intravenous tracer infusion rate); with 0.9 representing the acetate correction factor for this VO₂ according to reference 19. * indicates that GLU trial is significantly different from FAST at that time; P<0.05. † indicates that the % oxidation of the octanoate tracer is significantly higher than the palmitate tracer; P<0.05

Conversely, total fat oxidation was markedly suppressed throughout the GLU trials (Figure 13.2). After 40 min of exercise, total fat oxidation was 21.1±1.6 μmol·kg⁻¹·min⁻¹ during both GLU trials which represents a 29-34% reduction compared to the FAST trials.

Breath ratio of ¹³CO₂/¹²CO₂

During the 0-40 min period of exercise without tracer infusion, used to measure shifts in background or endogenous ¹³CO₂ production, the subjects displayed only a very slight reduction in ¹³CO₂/¹²CO₂ breath ratio both when fasted and receiving pre-exercise glucose (Figure 13.3). The glucose ingestion and oxidation did not alter ¹³CO₂/¹²CO₂ breath ratio during the background trial (i.e.; without tracer infusion) due to the fact that its ¹³C enrichment was identical to the subjects' breath ratio. The breath ratios during the other four experimental trials with 1-¹³C fatty acid tracer infusion (i.e.; either palmitate or octanoate) are shown in Figure 13.3. The NaH¹³CO₃ prime, given just prior to beginning exercise during each trial, as expected, markedly raised breath ¹³CO₂/¹²CO₂ during the first ten min. However, ¹³CO₂/¹²CO₂ breath ratios stabilized after 20 min in accordance with the

Figure 13.2 Total fat oxidation (A) and total carbohydrate oxidation (B) during 40 min of exercise at 50% VO_2max when fasted (FAST) compared to when fed glucose before exercise (GLUCOSE). Under both conditions, subjects received intravenous tracer infusion of palmitate (FAST-Palmitate and GLUCOSE-Palmitate) or octanoate (FAST-Octanoate and GLUCOSE-Octanoate). Values are means $\pm$ SE for 6 subjects.

* GLUCOSE trials significantly different from FAST trials; $P < 0.05$

rates of tracer infusion and oxidation and were constant during the 30 to 40 min period (Figure 13.3).

During both fasting trials (i.e.; FAST-Palmitate and FAST-Octanoate) the subjects continued exercising after the ^{13}C fatty acid tracer infusion was stopped at 40 min so that the decay of breath $^{13}\text{CO}_2/^{12}\text{CO}_2$ could be determined during the 40–80 min period. As displayed in Figure 13.3, both trials displayed a mono-exponential decay of breath $^{13}\text{CO}_2/^{12}\text{CO}_2$, with a similar one-half time of decay (i.e.; 13.1 ± 4.1 min for FAST-Palmitate and 19.3 ± 5.2 min for FAST-Octanoate), indicating that the palmitate tracer was not incorporated into intracellular fatty acid pools but was immediately oxidized during exercise, as recently reported (26, 27).

Octanoate tracer oxidation

The large shifts in whole body carbohydrate and fat oxidation elicited by the pre-exercise glucose feeding had absolutely no influence on ^{13}C -octanoate oxidation. V^{13}CO_2 production from ^{13}C -octanoate infusion remained remarkably constant during the 35–40 min period of exercise and were identical during FAST-Octanoate compared to GLU-Octanoate (Table 13.1).

The rate of ^{13}C -octanoate oxidation was calculated to be 98–99% of the rate of tracer infusion, after correcting (i.e.; 10%) for loss of label in bicarbonate and fixa-

Figure 13.3 Breath ratio of $^{13}\text{CO}_2 / ^{12}\text{CO}_2$ before and during 40-80 min of exercise at 50% VO_2max when fasted (FAST) compared to when fed glucose before exercise (GLUCOSE). Under both conditions, measurements were made a) without tracer infusion (background); or while being infused with b) 1- ^{13}C -palmitate at $0.167 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ or c) 1- ^{13}C -octanoate at $0.238 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. A bicarbonate priming dose of $18 \mu\text{mol}\cdot\text{kg}^{-1}$ of $\text{NaH}^{13}\text{CO}_3$ was administered just prior to exercise. Tracer infusion was stopped after 40 min but subjects continued to exercise until 80 min during the fasting trials so that the decay of breath $^{13}\text{CO}_2 / ^{12}\text{CO}_2$ ratio could be compared following cessation of 1- ^{13}C -palmitate vs. 1- ^{13}C -octanoate infusion.

tion reactions (27). The relative oxidation of the octanoate tracer was almost complete and it was significantly higher than the palmitate tracer (i.e.; $98.1 \pm 2.1\%$ vs. $86.0 \pm 1.9\%$ when fasted; $P < 0.05$; Table 13.1).

Palmitate tracer oxidation

V^{13}CO_2 production from the 1- ^{13}C -palmitate infusion was constant during the 30-40 min period of exercise. It was suppressed during GLU-Palmitate compared to FAST-Palmitate trial (Table 13.1). After 40 min of exercise, the % oxidation of the infused tracer was $70.0 \pm 4.1\%$ during GLU-Palmitate compared to $86.0 \pm 1.9\%$ during FAST-Palmitate; which represents a 19% reduction ($P < 0.05$; Table 13.1).

Rate of appearance and disappearance of plasma FFA

The increase in plasma $^{13}\text{C}/^{12}\text{C}$ -palmitate ratio (i.e.; enrichment) above background levels as a result of the 1- ^{13}C -palmitate infusion was stable during the 20, 30 and 40 min period of both the FAST-Palmitate (i.e.; 0.0659 ± 0.0064 , 0.0612 ± 0.0068 and 0.0583 ± 0.0066 , respectively) and GLU-Palmitate (i.e.; 0.1114 ± 0.0063 , 0.1088 ± 0.0066 and 0.1029 ± 0.0035 , respectively). Both R_a and R_d FFA were markedly

reduced (i.e.; 36%) during GLU-Palmitate compared to FAST-Palmitate (i.e.; Rd 8.2 ± 0.6 vs. 12.7 ± 1.0 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P < 0.05$; Table 13.2).

Table 13.2 Effect of glucose ingestion (GLU) compared to fasting (FAST) on various aspects of fat metabolism during exercise including total fat oxidation, the rate of appearance of plasma FFA (Ra FFA), the rate of disappearance of plasma FFA (Rd FFA), plasma FFA oxidation and the fatty acid oxidation from intramuscular triglycerides. $1\text{-}^{13}\text{C}$ -Palmitate was intravenously infused during both trial.

Trial	Time (min)	Total Fat Oxidation ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	Ra FFA ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	Rd FFA ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	Plasma FFA oxidation ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	Fatty Acid oxidation from IMTG ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)
FAST-Palmitate	20-30	30.9 ± 2.1	12.2 ± 1.0	12.0 ± 1.0	10.5 ± 0.8	20.4 ± 2.1
	30-40	32.1 ± 1.6	12.8 ± 1.0	12.7 ± 1.0	10.9 ± 0.8	21.2 ± 1.9
GLU-Palmitate	20-30	$19.7 \pm 1.5^*$	$8.3 \pm 0.8^*$	$8.2 \pm 0.8^*$	$5.7 \pm 0.4^*$	$14.0 \pm 1.8^*$
	30-40	$21.1 \pm 1.6^*$	$8.2 \pm 0.6^*$	$8.1 \pm 0.5^*$	$5.7 \pm 0.4^*$	$15.4 \pm 1.8^*$

Values are means $\pm$ SEM for 6 subjects. Plasma FFA oxidation is calculated as the product of Rd FFA and the % of infused palmitate tracer oxidized. Fatty acid oxidation from intramuscular triglyceride is calculated as the difference between total fat oxidation and plasma FFA oxidation.

* indicates that GLU-Palmitate trial is significantly lower than FAST-Palmitate at that time; $P < 0.05$.

Oxidation of plasma FFA and intramuscular triglyceride

Plasma FFA oxidation was markedly reduced (i.e.; 48%) during GLU-Palmitate compared to FAST-Palmitate (i.e.; 5.7 ± 0.4 vs. 10.9 ± 0.8 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at 40 min; $P < 0.05$; Table 13.2). Furthermore, calculated FFA oxidation from intramuscular triglyceride was also reduced (i.e.; 27%) during GLU-Palmitate compared to FAST-Palmitate (15.4 ± 1.8 vs. 21.2 ± 1.9 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P < 0.05$, Table 13.2). Figure 13.4 describes the extent to which the reduction in total fat oxidation as a result of the preexercise glucose feeding was due to inhibition of FFA oxidation from both plasma FFA and fatty acids from intramuscular triglycerides.

It should be recognized that in these endurance trained subjects, intramuscular triglyceride provided the majority of FFA for oxidation both during FAST (i.e.; $61 \pm 6\%$) and GLU (i.e.; $70 \pm 4\%$). The 11 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ reduction in total fat oxidation (34%; $P < 0.05$) from the glucose feeding was derived from roughly an equal (i.e.; $5\text{--}6$ $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) reduction in oxidation of each plasma FFA and fatty acids from intramuscular triglycerides. However, this represented a greater relative inhibition of plasma FFA oxidation compared to intramuscular triglyceride oxidation (i.e.; 48% vs. 27%; $P < 0.05$).

Figure 13.4 Comparisons after 40 min of exercise when fasted (FASTED) compared to when fed glucose before exercise (GLUCOSE FEEDING) total fat oxidation, plasma free fatty acid oxidation and calculated oxidation of FFA from intramuscular triglyceride (IMTG).

Values are means $\pm$ SEM for 6 subjects. *Significant reduction during GLUCOSE FEEDING compared to when FASTED; $P < 0.05$.

Discussion

A major finding of the present study was that increases in glycolytic flux and carbohydrate oxidation during exercise (GLU) resulted in a large reduction below FAST (i.e.; 19%; $P < 0.05$) in the oxidation of intravenously infused palmitate (i.e.; long-chain fatty acid) whereas it had absolutely no effect on oxidation of octanoate (i.e.; medium-chain fatty acid). Glycolytic flux and carbohydrate oxidation during exercise (i.e.; 50% VO_2max) were increased by making the subjects hyperglycemic and hyperinsulinemic through preexercise glucose ingestion (GLU). These observations agree with the hypothesis that glycolytic flux regulates long-chain fatty acid oxidation in skeletal muscle, possibly by inhibiting its transport into the mitochondria. This is because palmitate oxidation appears limited by transport into skeletal muscle mitochondria via carnitine palmitoyltransferase (i.e.; CPT) (11, 23). However, octanoate oxidation is not limited by mitochondrial transport and it is not inhibited to a great extent by malonyl-CoA (11, 23). This is supported by our present observation that in the fasted state, $98.7 \pm 2.5\%$ of infused $1\text{-}^{13}\text{C}$ -octanoate was oxidized compared to $86.0 \pm 1.9\%$ of the infused $1\text{-}^{13}\text{C}$ -palmitate ($P < 0.05$, Table 13.1).

The present study also demonstrated that the effect of glucose ingestion on reducing total fat oxidation during exercise is also a function of reduced mobilization of plasma FFA and oxidation of fatty acids from intramuscular triglyceride. Therefore, the effects of carbohydrate ingestion on regulating fat metabolism and oxidation appear to involve several different systems (i.e.; lipolysis, mobilization, and oxidation) that are well coordinated and seem to minimize the appearance of FFA that can not be oxidized by muscle during exercise, possibly due to inhibition of long-chain fatty acid transport into the mitochondria.

The present study has no information regarding the direct mechanism by which increased glycolytic flux specifically reduces long-chain fatty acid oxidation, but these observations point to an active process within skeletal muscle. By intravenously infusing trace amounts (i.e.; 1-¹³C-labeled) of palmitate and octanoate at a constant rate throughout exercise, during both FAST and GLU, the availability of these tracers for oxidation was not reduced during GLU compared to FAST, and in fact the plasma 1-¹³C-palmitate enrichment actually increased during GLU. During the 30-40 min period of exercise, the ¹³CO₂ enrichment in breath was quite constant as was plasma palmitate enrichment, indicating that steady-state equilibrium had been established in all conditions. Therefore, the reduction in 1-¹³C palmitate oxidation during GLU compared to FAST (i.e.; 70% vs. 86% of the infusion rate; $P < 0.05$) most likely reflects a muscle phenomenon during exercise whereby less of the palmitate tracer disappearing from the circulation is oxidized and presumably more is reesterified. This is separate from the effects of GLU on other aspects of fatty acid mobilization, as discussed below. The rates of 1-¹³C fatty acid infusion were in only tracer amounts that did not add appreciable substrate or influence metabolism. Furthermore, the metabolic responses during GLU-palmitate and GLU-octanoate were identical. Therefore, the reduction in 1-¹³C palmitate oxidation and not 1-¹³C octanoate oxidation during GLU compared to FAST can be ascribed to a differential effect of GLU on muscle fatty acid oxidation rather than some other aspect of metabolism.

For example, given the potent effect of hyperinsulinemia on lipolysis (3), it was conceivable that all of the reduction in fat oxidation during GLU could have resulted from the passive withdrawal of fatty acid availability (i.e.; solely from reduced lipolysis), without the additional imposition of impaired fatty acid oxidation by muscle; although this did not occur. It was also conceivable that the increased glycolytic flux and acetyl-CoA production during GLU might have served to dilute acetyl-CoA from fat, thus reducing fat oxidation by simple mass action with more acetyl-CoA being derived from carbohydrate. This also did not occur, however, as it would have resulted in an equal dilution and equally reduced oxidation of the octanoate and palmitate label by the increased glycolytic flux generated by GLU compared to FAST. Besides, these means for reducing fat oxidation whereby the muscle is passive and not actively or directly reducing fatty acid oxidation when glycolytic flux is increased would produce even more excessive substrate flux. It therefore appears that the preferred oxidation of carbohydrate when both carbohydrate and fat are made available to muscle is mediated, in part, by the active inhibition of fat oxidation due to impaired fatty acid transport into mitochondria.

A possible cellular mechanism by which glycolytic flux regulates fat oxidation within skeletal muscle could involve the formation of malonyl-CoA, which was not determined in the present study due to the difficulty of measurement from muscle biopsy samples in people (18). Malonyl-CoA is formed from acetyl-CoA through the activity of acetyl-CoA carboxylase and it is a potent inhibitor of CPT (23, 25) with much less of an effect on COT (23). It is hypothesized that malonyl-

CoA concentration can reflect the availability of carbohydrate as a substrate (i.e.; fuel sensing) with increased glycolytic flux producing more pyruvate and thus increasing acetyl-CoA/CoA and malonyl-CoA, which then reduces fatty acid oxidation by reducing transport of long-chain fatty acids into mitochondria via inhibition of CPT (25). Malonyl-CoA has been found to increase in resting rat skeletal muscle incubated in glucose and insulin (25) and to be higher in exercising skeletal muscle of rats infused with glucose at sufficient rates to elevate insulin (7). However, of these recent animal studies that have clearly demonstrated that hyperinsulinemia induced increases in glycolytic flux in skeletal muscle increase malonyl-CoA, no direct measures were made of fatty acid oxidation or any aspect of fat metabolism (7, 24, 25, 30). The first attempt to measure malonyl-CoA in human skeletal muscle, relying upon relatively small biopsy samples, has recently reported low levels at rest (i.e.; $1.5 \mu\text{mol}\cdot\text{kg}^{-1}$) and a 20% decline during exercise that failed to be statistically significant (18). However, these authors did detect a significant decline in malonyl-CoA in perfused rat muscle during electrical stimulation (18). Therefore, it is not clear if malonyl-CoA declines with exercise in humans. The present observation that the increased glycolytic flux generated by preexercise glucose ingestion specifically reduced palmitate oxidation without reducing octanoate oxidation, agrees with but provides only indirect support for the malonyl-CoA hypothesis of reduced palmitate transport and oxidation due to CPT inhibition.

The validity of our measurements of plasma fatty acid oxidation has recently been established and it appears that these measures are best made during exercise at high steady-state rates of oxygen consumption (26, 27), as designed in the present study. It had been thought previously that fatty acids taken up by cells mix with a large intracellular pool of fatty acids, thus causing underestimation of their calculated oxidation (13). However, this does not appear to happen as demonstrated previously (26), and verified presently by the observation that the half-time decay of breath enrichment during exercise is similar following cessation of tracer infusion when comparing palmitate with octanoate (Figure 13.3) or acetate (26). There are no pools of octanoate or acetate in the body for tracer dilution and thus the similarity of their half-time decay, compared to palmitate, indicates that palmitate entering cells destined for oxidation is not mixed with a fatty acid pool but instead is oxidized directly. The reason that plasma FFA oxidation is often calculated to be unreasonably low, especially at rest, has recently been shown to be due to loss of the tracer's label to bicarbonate and fixation reactions with carboxylase and in the tricarboxylic acid cycle (26). This relative label loss has been shown to decrease as absolute oxygen consumption increases and, as in the present study, exercise at $36 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ has been shown to result in only a 10% loss of label, which was corrected using the acetate correction factor (27). Because loss of label is small and comparable under all the present experimental conditions of a high absolute oxygen consumption, in no way do the major findings or their interpretation hinge upon the exactness of correction for label loss.

The lowering of total fat oxidation during GLU appeared due to concerted reductions in lipolysis, fatty acid mobilization (Ra FFA) as well as fatty acid oxidation

by muscle, probably mediated by an insulin effect on the various tissues. Insulin is known to be a potent inhibitor of lipolysis (3) and a profound effect of GLU was the increase in plasma insulin both preexercise (i.e.; 42 vs. 5 $\mu\text{U}\cdot\text{ml}^{-1}$ for GLU and FAST, respectively; $P<0.05$) and throughout the 40 min of exercise (i.e.; 16 vs. 4 $\mu\text{U}\cdot\text{ml}^{-1}$ for GLU and FAST, respectively; $P<0.05$). Figure 13.4 indicates that the 11 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ reduction in total fat oxidation during GLU compared to FAST was due to an approximately equal absolute (i.e.; 5–6 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) reduction in oxidation of plasma FFA and intramuscular triglyceride. However, this represented a greater relative decline in plasma FFA oxidation (i.e.; 48%) compared to intramuscular triglyceride oxidation (i.e.; 27%). Our direct measures indicate that the decline in plasma FFA oxidation resulted from both a reduction in R_a and R_d FFA (i.e.; 4.6 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; 36%) combined with the 19% reduction in the oxidation of the plasma FFA disappearing from the circulation, resulting in the 5.2 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ reduction in plasma FFA oxidation. The total fat oxidation not met by plasma FFA oxidation is assumed to be derived predominantly from oxidation of intramuscular triglyceride, which in these endurance trained athletes appears to be the source for most (i.e.; 66–73%) of their fat oxidation, as previously shown (14, 17). It should be realized that plasma triglyceride could also contribute to the total fat oxidation not met by plasma FFA, and presumed to be intramuscular triglyceride, yet plasma triglyceride turnover during exercise is not thought to add significantly to total fat oxidation (8, 14, 17). It is quite likely that lipolysis of intramuscular triglyceride was reduced by the hyperinsulinemia of GLU and that this, combined with impaired muscle fatty acid oxidation, accounted largely for the reduced intramuscular triglyceride oxidation.

These findings support the concept that carbohydrate metabolism regulates its own oxidation and by doing so, also directly regulates fat oxidation. The two primary sources of carbohydrate for muscle oxidation are blood glucose and muscle glycogen (8, 20). Clearly, when blood glucose is elevated sufficiently to cause hyperinsulinemia, the large increase in blood glucose uptake by muscle during contraction produces a large increase in glycolytic flux and carbohydrate oxidation (1). The present observation that preexercise hyperglycemia increased its own glycolytic flux and oxidation, presumably via insulin stimulated blood glucose uptake (1), while also directly inhibiting long-chain fatty acid oxidation in muscle, provides strong evidence in support of the concept that carbohydrate availability dictates its own flux while also directly influencing fatty acid oxidation, presumably by regulating fatty acid transport into the mitochondria. Furthermore, increases in muscle glycogen concentration have been found to increase its own oxidation during exercise (12) and it remains to be determined if this increased glycogen flux from high muscle glycogen concentration also directly inhibits fatty acid oxidation in muscle. It should also be remembered that the hyperinsulinemia in response to hyperglycemia during GLU reduced $R_a\text{FFA}$ substantially, indicating that carbohydrate availability (via insulin) also regulates metabolism in adipose tissue independently from muscle, but apparently to the same end of reducing fat oxidation. Our present concept that carbohydrate metabolism generally regulates fat metabo-

lism and thus determines the relative contribution of these substrates to oxidative metabolism is opposite the classical concept proposed by Randle and co-workers (10, 19). Their concept proposes that increases in fatty acids reduce pyruvate dehydrogenase activity (PDH) and increase citrate, which lowers phosphofructokinase activity, thus increasing glucose-6-phosphate (G6P) which reduces hexokinase activity and thus glucose uptake and oxidation. However, the classical concept does not appear to operate in skeletal muscle of humans because an increase in plasma FFA concentration during intense exercise does not increase citrate or G6P (5, 6) and it does not reduce blood glucose uptake (21). This is not to say there is no effect of plasma FFA on carbohydrate metabolism because the lowering of plasma FFA during high intensity exercise deprives the muscle of a plasma substrate and thus increases muscle glycogenolysis as a result of the increased metabolic stress (i.e.; higher AMP levels) (5, 6, 21). However, this is different from the classical "glucose-fatty acid cycle" proposed by Randle et al. (10, 19) in that the absence of FFA passively stimulates glycogenolysis rather than the presence of FFA actively reducing glycolysis. Therefore, it appears that the ability of fat to regulate carbohydrate metabolism in people during exercise is limited. The operation of the classic glucose-fatty acid cycle in resting humans has also been challenged recently, using an approach similar to the present study (28). They reported that 50% of long-chain FFA (i.e.; oleate) that disappear from the circulation under basal conditions is oxidized and that this value is reduced to 25% when glycolytic flux is increased at rest during a hyperinsulinemic, hyperglycemic clamp (28).

Understanding the factors that regulate fatty acid uptake and fat oxidation in skeletal muscle during physical activity is clinically important as it is becoming more apparent that obesity is related to impaired fat oxidation primarily in skeletal muscle. Along these lines, Blaak et al. (2) recently found that obese men, compared to lean men, displayed an impaired plasma FFA uptake in forearm muscle when energy expenditure was increased via beta-adrenergic stimulation. We are unaware of studies that have directly measured fatty acid oxidation during the increased energy expenditure of exercise in relation to obesity. Bodily movements when performing daily activities are, of course, made possible by contraction of skeletal muscle and oxidation fat and carbohydrate (i.e.; aerobic exercise). Therefore, skeletal muscle seems to be the primary tissue dictating total daily fat oxidation and it is most appropriate to study it during exercise. Colberg et al. (4) recently reported that FFA uptake by skeletal muscle of fasted resting women is positively correlated with CPT activity, which in turn is negatively correlated with visceral obesity. The important implication of these observations is that obesity may stem, partly, from impaired CPT activity in skeletal muscle which reduces FFA oxidation by muscle and increases reesterification to triglyceride. The present findings also suggest that fat oxidation is regulated by mitochondrial transport (possibly CPT activity) in skeletal muscle. More importantly, the present study clearly indicates that the hyperglycemia and hyperinsulinemia from dietary carbohydrate ingestion (i.e.; 200 grams) equivalent to a meal, profoundly reduces fat oxidation during activity by both reducing long-chain fatty acid oxidation in skeletal muscle and FFA mobiliza-

tion. Interestingly, the hyperglycemia and hyperinsulinemia that is characteristic of muscle insulin resistance in NIDDM is also associated with an impaired free fatty acid utilization by muscle at rest and during exercise (15, 16, 25) that seems to be linked to increased malonyl CoA levels (24). Therefore, our concept that carbohydrate metabolism and glycolytic flux directly regulate fatty acid oxidation by muscle during exercise appears relevant to obesity and insulin resistance.

In summary, the reduction in fat oxidation during exercise elicited by preexercise hyperglycemia and hyperinsulinemia from glucose ingestion is partly due to a specific inhibition of long-chain fatty acid oxidation (i.e.; palmitate) by skeletal muscle in association with increased glycolytic flux. This appears to be due to the active inhibition of mitochondrial long-chain fatty acid transport based upon the observation that increased glycolytic flux did not alter octanoate oxidation. Carbohydrate ingestion further reduced fat oxidation by markedly reducing the mobilization of plasma FFA (Ra FFA) as well as the oxidation of intramuscular triglyceride. Apparently, carbohydrate ingestion exerted well coordinated effects on muscle and adipose tissue to minimize the appearance of FFA that can not be oxidized by muscle during exercise due to inhibition of long-chain fatty acid oxidation, possibly from reduced transport into the mitochondria.

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Chapter 14

General discussion

Parts of this general discussion will be published in:

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14.1 Aspects of carbohydrate and fat metabolism

Carbohydrates (CHO) and fat are the most important fuels during exercise (For review see **Chapter 2**). Since especially CHO are required to maintain high exercise intensities it is very important to guarantee sufficient CHO availability during exercise and to be economical with the scarce CHO reserves in the body. Fat supplementation has been suggested to be a way to spare endogenous glycogen stores whereas CHO supplementation may directly increase (i.e. maintain) CHO availability during exercise. The purpose of this general discussion is to summarize the findings in this thesis and put them in the context of the available literature. In addition attention will be paid to the important, yet unsolved questions in this area and directions for future research will be formulated. Generally the discussion below will be divided in three sections: 1. fat supplementation during exercise, 2. carbohydrate supplementation during exercise and 3. the interaction between carbohydrate and fat metabolism.

14.2 Fat supplementation during exercise

Although theoretically fat supplementation could be a way to conserve endogenous glycogen (and/or IMTG) stores, fat supplementation during exercise is usually regarded as undesirable since endogenous fat stores are very large and there may not be a need to supplement additional fat. In addition long chain fatty acids (or long chain triglycerides) have a slow gastric emptying rate (43) and appear only slowly in the systemic circulation (27, 30). They enter the systemic circulation in chylomicrons which are not believed to be a major fuel during exercise (96).

Although fat in general is slowly hydrolyzed and absorbed this also depends on the chain length of the fatty acids. In contrast to the long chain triglycerides, short- and medium-chain triglycerides are more rapidly absorbed and they are not transported by chylomicrons but directly enter the systemic circulation through the portal vein (3). Consequently they will enter the systemic circulation more rapidly (3, 4, 31). Therefore, it has been suggested that MCT may be a valuable exogenous energy source during exercise in addition to carbohydrates (53) (**Chapter 4**). Also, it has been suggested that MCT ingestion may improve exercise performance by elevating plasma FFA levels and sparing muscle glycogen (100) since it has been observed that increased availability of plasma FFA reduced the rate of muscle glycogen breakdown and delayed the onset of exhaustion (18, 39, 44, 87, 101).

MCT added to CHO drinks did not inhibit gastric emptying (**Chapter 3**). In fact, drinks up to 30% MCT, emptied faster from the stomach than an isoenergetic CHO drink (5). In a subsequent study the oxidation rate of ingested MCT was investigated (53) (**Chapter 4**). During the second hour (60–120 min period) of exercise, the amount of MCT oxidized was 72% of the amount ingested with CHO+MCT whereas during the MCT trial only 33% was oxidized. It was concluded that more MCT is oxidized when ingested in combination with CHO.

There may be several explanations for this phenomenon. It is possible that there are differences in gastric emptying between MCT and CHO+MCT (In **Chapter 3** gastric emptying of different CHO+MCT suspensions was investigated but not of MCT in the absence of CHO). It may be that MCT is better soluble in the presence of CHO. CHO may keep the MCT in suspension whereas in the absence of CHO, MCT may float on top of the suspension. In the stomach the lower part will empty from the stomach whereas the upper part (with MCT) may be emptied somewhat later. This may explain the observed delayed entrance of MCT into the systemic circulation. Theoretically it could also be possible that MCFA are elongated and incorporated into triglycerides or other lipid pools and that this incorporation may be influenced by glucose ingestion. However, we observed that only very small amounts of MCFA were elongated to LCFA (predominantly palmitate; unpublished findings) so that this cannot explain the large differences in MCT oxidation between the MCT and CHO+MCT group. Therefore, the mechanisms underlying the observed lower MCT oxidation rates during MCT compared to CHO+MCT remain speculative.

Table 14.1 Effects of MCT feedings during exercise from various studies

Amount ingested	Oxidation (%)	Contribution to EE	Glycogen sparing	Ergogenic effect	Time (min)	Exercise intensity %VO ₂ max	Ref.
25 g	54%	7%	—	—	120	65	(66)
25 g	30%	11%	no	—	120	60	(23)
45 g	45%	14%	—	no	180	60	(94)
30 g	—	—	—	—	60	70	(45)
25 g	—	—	no	—	60	60	(42)
86 g	—	—	yes	yes	120	60	(100)
30 g	30%*	3-7%*	—	—	180	57	(53)
27 g	14%*	6-7%*	—	—	90	57	(54)
30 g	—	—	no	—	180	57	(56)
86 g	—	—	—	no	120	60	(55)

Data in **Chapter 4** confirmed the hypothesis that oral MCT serves as an energy source during exercise in addition to glucose since the metabolic availability of MCT was high during the last hour of exercise. However, the maximal amount of oral MCT (trioctanoate) that can be tolerated in the gastrointestinal tract is small (about 30 g) and this limited the contribution of oral MCT to total energy expenditure to values between 3 and 7% (**Chapter 4**). We also investigated MCT oxidation in CHO+MCT supplements under conditions where the reliance on blood substrates is maximal such as in a glycogen depleted state (54) (**Chapter 6**). Although total fat oxidation was markedly increased compared to the glycogen loaded state, MCT oxidation increased only marginally. The contribution of MCT to total energy expenditure was still small (about 6-8%) (54) (**Chapter 6**). The contribution of exogenous CHO at a similar exercise intensity is larger: in **Chapter 5** the contribution of exogenous CHO was ~20%, in **Chapter 9** and **10** the contribution was even higher (25-28%). This is mainly because more CHO can be ingested. When equicaloric amounts of MCT and CHO were ingested the contribution to total energy expenditure was only slightly lower for MCT compared to glucose (5.9 kJ·min⁻¹ versus 6.7 kJ·min⁻¹) (66). The effects of MCT feedings during exercise in various studies are depicted in Table 14.1.

It has also been suggested that elevating plasma FFA levels by infusing Intralipid and heparin will spare muscle glycogen and improve exercise performance (18, 101). In theory, MCT ingestion could also be a way to elevate plasma FFA levels. However, well-trained athletes who ingested CHO+MCT or CHO displayed no significant differences in plasma FFA levels nor in muscle glycogen breakdown or respiratory exchange ratio during exercise (56) (**Chapter 5**). Again, this may be attributed to the relative small amount of MCT (about 30 grams) that can be tolerated in the gastro-intestinal tract.

14.2.1 Effect of MCT ingestion on exercise performance

Van Zeyl et al (100) recently argued that in some of the studies the amount of MCT ingested was too small to exert a positive effect on performance. Therefore they fed subjects a large amount of MCT (86 g) during 120 min of exercise at 60% VO_2max and found elevated plasma FFA levels, decreased glycogen breakdown and increased performance in a subsequent 40 km time trial.

In order to investigate the effect of larger quantities of MCT on endurance exercise performance we fed subjects 85 grams of MCT during 2 hours of exercise at moderate intensity (60% VO_2max) (Chapter 8). After those 120 min of exercise a time trial (described and validated in Chapter 7) was applied. Although plasma FFA were somewhat elevated, we did not find the positive effects on performance reported by van Zeyl et al. (100). We even observed a negative effect on performance when only MCT was ingested and we attributed this to the gastro-intestinal discomfort reported by the subjects.

The different effects of MCT ingestion on performance may be explained by the fact that in the study of van Zeyl et al (100) subjects did not experience major GI-complaints whereas in Chapter 8 most subjects suffered from GI-discomfort. The reason, why the subjects in our study had more problems with MCT ingestion than those in the study of van Zeyl (100) is not clear. We provided MCT which consisted of 100% trioctanoate (C8) while van Zeyl et al (100) gave their subjects a mixture of trioctanoate (C8) and tridecanoate (C10). However, Horowitz and Coyle observed a high incidence of GI-problems when more than 30 g 100% tridecanoate (C10) was ingested (personal communication). So the problems do not appear to be specific to trioctanoate. Although unlikely, a mixture of C8 and C10 may be better tolerated than a similar amount of the separate triglycerides.

Another difference between our study and the study of van Zeyl et al (100) is the duration of the time trial. Our time trial lasted only ~15 min (time to complete an amount of work equal to 15 min 75% Wmax), whereas van Zeyl et al (100) applied a 40 km time trial (~1h). In pilot studies in 2 subjects, we also applied a time trial of approximately 1h. However, both subjects had to abort the test preliminary because of severe gastro-intestinal distress. Therefore we decided to shorten the test to about 15 min duration. It is unlikely that the duration of the time trial can explain the different findings.

14.2.2 Conclusions

In conclusion: 1. MCT is rapidly emptied from the stomach, absorbed and oxidized, and 2. the oxidation of exogenous MCT was enhanced when co-ingested with CHO. 3. Ingestion of 30g MCT did not affect muscle glycogen breakdown and 4. contributed only 5-8% to energy expenditure. 5. Ingestion of larger amounts of MCT resulted in gastrointestinal distress. Therefore we could not confirm the positive effects on performance that have been claimed by van Zeyl et al (100).

14.3 Carbohydrate supplementation during exercise

Carbohydrate supplementation during exercise has become a common practice among athletes and ingestion of carbohydrate drinks is often recommended to prevent dehydration and hypoglycemia. Carbohydrate feedings during exercise have been shown to improve endurance capacity in events lasting 2h or longer. Since this has been shown convincingly, research has focused on ways to maximize exogenous CHO oxidation.

14.3.1 Effect of CHO ingestion on exercise performance

Multiple studies have demonstrated the ergogenic effect of carbohydrate ingestion during prolonged exercise (12, 14, 21, 33, 46, 68, 73, 75, 76, 108). However, there are also reports that did not find increased time to exhaustion after CHO ingestion (1, 7, 28, 34, 88). The reason that most, but not all studies were able to demonstrate the positive effects of CHO ingestion on endurance exercise performance may be explained by differences in preexercise glycogen levels (28) but also by the type of test used to evaluate endurance performance.

In **Chapter 7**, we have shown that exercise time to exhaustion is not always a good way to measure performance because this test is poorly reproducible (52) (at least at exercise intensities of 75%W_{max}). However, when a time trial was applied, as described in **Chapter 7**, reproducibility was very good (CV of 3.4% compared to 26.6% of the exercise time to exhaustion test). Similar reproducibility was later reported by Palmer et al (78) who used a comparable time trial protocol. So for the first time a validated time trial laboratory performance test was available which is a fairly sensitive tool to measure performance because day to day variation is minimal.

Although several studies have shown that exercise capacity is increased after carbohydrate ingestion during moderate intensity exercise (50-70%VO_{2max}) of long duration (>2h), little information is available on the effect of carbohydrates on high intensity exercise performance of about 1 h duration. The findings in **Chapter 11** demonstrate that CHO feedings not only improve exercise performance during long lasting submaximal exercise (11, 12, 19-21, 68, 97, 103, 108), but also during 1 h high intensity exercise (50). Results are in agreement with results obtained by other performance tests at comparable exercise intensities (2, 6, 83).

The reason for this improved performance during high-intensity exercise is not clear since only very small amounts of the ingested CHO (~5-20 g) may have entered the systemic circulation during 1 h exercise. This makes it unlikely that carbohydrate ingestion exerts its beneficial effect through a contribution to energy expenditure.

Coggan and Coyle (13) suggested that the key to the improved performance at these high intensities may be the 'relative' CHO availability, i.e., that although CHO availability may not appear limiting in an absolute sense, increasing CHO oxidation may provide the ability to increase the power output (or to tolerate high intensities longer). This effect may be mediated by CHO promoting the synthesis

of TCA-cycle intermediates via the alanine aminotransferase reaction, thereby increasing the capacity of aerobic ATP production. Pyruvate may react with glutamate under formation of α -ketoglutarate, a TCA-cycle intermediate, and alanine (98, 99). Theoretically pyruvate carboxylation could also be a pathway to provide TCA-cycle intermediates. However, the importance of this pathway has recently been questioned (98, 99).

Glucose may also have central effects and neural signals or hormonal signals may influence certain areas of the brain as soon as glucose enters the stomach (or maybe even before that). However, very little is known about possible central effects of glucose. Another practical example, however, may illustrate that these effects may not be excluded. When an athlete is hypoglycemic and feels weak and dizzy, few minutes after he has eaten CHO he is already feeling better. The athlete feels better long before the bulk of ingested CHO enters systemic circulation. This example illustrates that there must be neural or hormonal signals from the GI-tract directly to the brain that change the feelings of weakness and dizziness caused by hypoglycemia. These signals could also be responsible for the improved time trial performance with CHO ingestion.

14.3.2 Oral CHO oxidation

As discussed in the ~~Chapter 1~~ ^{Chapter 1} Costill et al (17) were among the first to investigate the oxidation of CHO ingested during exercise. However, these early studies using radioactive isotopes suggested a minor contribution of ingested CHO to total CHO oxidation. In 1977, using stable isotopes, Pirnay et al (80, 81) concluded that significant amounts of ingested glucose were oxidized during exercise. Subjects ingested 100 g naturally ^{13}C labeled glucose before they walked on a treadmill up to 225 min. Maximal exogenous glucose oxidation rates were observed after 75-90 min and mounted up to $0.65 \text{ g} \cdot \text{min}^{-1}$. After 225 min of exercise 95% of all ingested glucose had been oxidized. Similar results were later found by others (47, 48, 58, 59).

Several factors that influence the exogenous CHO oxidation rates have been recognized. For instance the oxidation of ingested CHO increases with increasing exercise intensity (62, 67, 79, 82). The amount ingested is also of importance because the oxidation increases when more CHO is ingested. However several studies showed that ingesting larger amounts of CHO will not necessarily result in linearly increased exogenous CHO oxidation rates (102). In a review Hawley et al (37) concluded that the maximal exogenous CHO oxidation rate is about $1 \text{ g} \cdot \text{min}^{-1}$. Another important determinant of exogenous CHO oxidation may be the type of CHO ingested. Generally it has been shown that all carbohydrates are oxidized at similar rates (glucose, maltose, sucrose, glucose polymers, soluble starch). Exceptions, however, are fructose, galactose and insoluble starch which are oxidized at slightly lower rates. Although many factors that influence oral CHO oxidation have been identified, the effect of glycogen availability on exogenous CHO oxidation is still unclear. Also the effect of training is still largely unknown. Both training and glycogen availability cause large shifts in carbohydrate and fat oxidation

during exercise and it therefore may also affect the oxidation of ingested CHO. Therefore in **Chapter 9** and **10** the effects of respectively glycogen availability and training were investigated.

Table 2 Factors (possibly) influencing exogenous CHO oxidation (ex CHO ox)

Factors influencing exCHO ox	References
Amount CHO	(74, 86, 102)
Type of CHO	(29, 38, 60, 63, 64, 74, 86, 93)
Exercise intensity	(62, 67, 79, 82)
Feeding schedule	(86)
Availability of glycogen	(49, 65, 85)
Training	(51, 59)
Type of exercise	(24)

14.3.2.1 Effects of endogenous glycogen availability

In **Chapter 9** we studied the effects of endogenous glycogen availability on exogenous CHO oxidation. Most remarkably we found that exogenous glucose oxidation was lower when the glycogen stores were reduced by strenuous exercise the day before the exercise trial and kept low by ingestion of a CHO-restricted diet (49). Exogenous glucose oxidation was 28% lower in the low glycogen trial (LG) compared to the normal to high glycogen trial (HG). Peak CHO oxidation rates were $0.64 \text{ g} \cdot \text{min}^{-1}$ and $0.88 \text{ g} \cdot \text{min}^{-1}$ during LG and HG respectively. Ravussin et al (85) studied oxidation rates of exogenous carbohydrates in a group of glycogen depleted subjects and a control group and found no significant differences. Unfortunately, the study had no cross-over design and the glycogen depletion protocol may have been less exhaustive than the one applied here. Muscle glycogen concentrations were not measured in this study but plasma insulin and FFA concentrations were comparable. Massicotte et al (65) studied exogenous carbohydrate oxidation during 2 hours of exercise in fed and fasted conditions and could not find differences in the exogenous oxidation rates of ingested glucose. This may be due to the fact that carbohydrate stores may not have been depleted. Fasting will predominantly empty the liver glycogen stores, while muscle glycogen stores change little. These studies suggest that exogenous CHO oxidation may be limited by factors like absorption and entrance of glucose into the systemic circulation rather than demand for carbohydrate as substrate.

14.3.2.2 Effects of training

The effect of physical training on exogenous glucose oxidation is largely unknown. Krzentowski et al (59) measured the oxidation of orally ingested glucose during

exercise at the same absolute intensity (40% of the pretraining $\text{VO}_{2\text{max}}$), before and after a 6 week training program. Although endurance training is known to cause a large shift from predominantly carbohydrate oxidation towards fat oxidation (15, 40, 41), Krzentowski et al (59) observed no changes in total carbohydrate or fat oxidation. Yet, exogenous glucose oxidation was increased by 17% by the training. Since these workers found a unphysiologically large increase in $\text{VO}_{2\text{max}}$ (29%!), the results of this study may be questioned. In the study described in **Chapter 10**, we observed similar contributions of exogenous glucose in a group of trained and untrained subjects (25.0% versus 27.5%) during exercise at the same *relative* intensity (51). For both groups in our study peak oxidation rates of the ingested glucose were 0.95–0.96 $\text{g}\cdot\text{min}^{-1}$. Therefore, we conclude that ingested CHO are oxidized at similar rates in trained and untrained subjects at the same relative exercise intensity. The mechanisms underlying the large shifts in substrate utilization after training are not completely understood. An increased oxidative capacity of the muscle cell as observed after training may improve the energy status of the muscle at a certain work rate. This in turn may reduce allosteric stimulation of glycolysis as described in **Chapter 2**. However, many other regulatory factors may be involved (malonyl-CoA, pyruvate dehydrogenase, hormones etc.) and additional research is needed to obtain information about the complex mechanisms that regulate the fuel mix during exercise before and after training.

14.3.3 Limits of exogenous CHO oxidation

As discussed above, oxidation rates of ingested CHO peak at about 1 $\text{g}\cdot\text{min}^{-1}$. It is not known what factors limit the oxidation of ingested carbohydrates. This question can also be rephrased as: if only part of the ingested CHO is oxidized, what is the fate of the remaining carbohydrates? Theoretically the factors limiting exogenous CHO oxidation during exercise may be: gastric emptying, intestinal absorption, glucose uptake and release by the liver, glucose transport across the cell membrane or intracellular muscle glucose metabolism. Several studies have shown that most of the ingested fluid is emptied from the stomach and gastric emptying is not the rate limiting step for exogenous carbohydrate oxidation (38, 74, 86). These studies showed that the amount of CHO leaving the stomach after repeated ingestion of a CHO solution is more than double the amount oxidized (38, 74, 86). Data of Rehrer et al (86) suggests that also intestinal absorption is not limiting because the absorption of CHO was more than 300% faster with 17% glucose solution compared to a similar amount of a 4.5% glucose solution, while exogenous CHO oxidation increased only 33%. However, these data were obtained in resting conditions and with the triple-lumen-technique, which reflects a small section of the total intestinal tract. Therefore it is not known how representative these data are for whole body glucose absorption and we cannot exclude the possibility that part of the ingested carbohydrates still remains in the intestine.

Another possibility, however, is that the limitation is located at the muscular level. In **Chapter 12**, we tested this by infusing two different tracers, a $[6,6\text{-}^2\text{H}_2]$ -glucose tracer to measure the rate of appearance (R_a) of glucose in plasma (glucose derived

from liver and intestine) and a [U- ^{13}C]-glucose tracer to measure plasma glucose oxidation. To our knowledge, this is the first study that systematically compared the R_a glucose and rate of plasma glucose oxidation. We found that all glucose appearing in the plasma was oxidized, even when large amounts of CHO were ingested (~ 360 g in 3 h) indicating that it is unlikely that there is any limitation for exogenous glucose oxidation at the muscular level.

This is in agreement with findings of Hawley et al (35) who bypassed both intestinal absorption and liver glucose uptake by employing intravenous glucose infusion. They observed that a higher delivery of glucose into the systemic circulation increases the rates of plasma glucose oxidation by the working muscles and concluded that well trained subjects have an exceptional ability to dispose large amounts of infused glucose. The glucose infusion caused a hyperglycemia (10 mM) and hyperinsulinaemia ($24.5 \mu\text{U}\cdot\text{ml}^{-1}$) which completely suppressed hepatic glucose production and increased both R_d and plasma glucose oxidation during the final 30 min of exercise to $1.8 \text{ g}\cdot\text{min}^{-1}$. These results provide convincing evidence that neither glucose transport into the muscle nor glucose oxidation are the rate limiting steps for exogenous CHO oxidation.

More likely may be the possibility of glucose being trapped in the liver. The liver is the primary regulator of blood glucose concentration and an abundance of CHO delivered by the intestine may be trapped in the liver to prevent elevated blood glucose levels and elevated plasma glucose oxidation rates. The findings in **Chapter 9** and **10** may indicate that the liver indeed is the critical step. In **Chapter 10** no difference was found in exogenous glucose oxidation between trained and untrained whereas in **Chapter 9** exogenous glucose oxidation was lower in the glycogen depleted state. In both studies no differences in the absorption of glucose are to be expected and the lower exogenous glucose oxidation rates in the low-glycogen trial may be the result of glucose being trapped in the liver as a result of the low circulating insulin concentration. Furthermore, it has been shown that hepatic glucose production is suppressed when exogenous CHO are ingested (10, 36, 69), while total R_a barely increased. This also indicates that the liver strongly regulates the glucose entrance into the systemic circulation. Although the liver may be the most likely organ responsible for the limitations in exogenous CHO oxidation, the intestine cannot be excluded. During exercise there will be a redistribution of the blood and blood flow to the intestine will be reduced. This may result in an inhibited transport of nutrients from the intestine to the liver.

At present it is not entirely clear whether the rate limiting factor for exogenous CHO oxidation is in the intestine or in the liver. We can however, conclude that gastric emptying and muscle metabolism are not rate limiting factors.

14.4 The interaction between fat and carbohydrate metabolism

As described in **Chapter 12** and **13**, the factors that regulate the interplay between fat and carbohydrate oxidation, especially in skeletal muscle during exercise, are

not well understood. One of the possible mechanisms could be regulation through the glucose-fatty acid cycle as first introduced by Randle et al (84). This concept describes that an increase in fatty acid oxidation increases the mitochondrial ratio of acetyl-CoA to CoA (acetyl-CoA/CoA), which suppresses pyruvate dehydrogenase (PDH) activity directly and phosphofructokinase (PFK) and hexokinase activity indirectly via citrate and glucose-6-phosphate (G-6-P) accumulation respectively (Figure 2.5). The results is inhibition of glycolysis and glucose transport. So the cornerstone of this traditional glucose-fatty acid cycle is that the availability of FFA determines the mix of CHO and fat oxidized in a certain situation. Or in other words: fat regulates CHO metabolism. However, there is little evidence that the glucose fatty acid cycle is operative in humans (9, 25, 95).

In **Chapter 13** we tested the converse of the classical concept by hypothesizing that CHO metabolism largely regulates fat oxidation in man during exercise (22). The mechanism by which accelerated glucose metabolism decreases FFA oxidation is not known but work from animal studies suggests that carnitine acyl transferase (CAT) may play an important role (26, 70-72, 92, 104-107). When long chain fatty acids (LCFA) enter the mitochondria they have to be bound to carnitine, a reaction catalyzed by carnitine acyl transferase I (CAT I). In rat liver increased pyruvate availability results in increased intracellular malonyl-CoA concentrations (26), which in turn is a potent inhibitor of CAT I (92). Thus, accelerated glycolysis may inhibit FFA oxidation by inhibiting LCFA transport into the mitochondria. The findings in **Chapter 13** support this theoretical view since LCFA oxidation was markedly impaired when subjects were hyperglycemic and hyperinsulinemic, whereas MCFA oxidation was not impaired. MCFA have separate chain length specific enzymes for transport into the mitochondria which are less sensitive to malonyl-CoA inhibition (32, 89, 90).

The observations in **Chapter 4, 5, 6, 8** and **12** may be supportive to the concept that glucose regulates fat oxidation during exercise rather than the other way around. In **Chapter 4, 5, 6** and **8** we observed that MCT ingestion did not alter the rates of total fat and CHO oxidation whereas in **Chapter 8** and **12** ingestion of glucose increased the rates of CHO oxidation and inhibited fat oxidation. In chapter 8 an isoenergetic amount of MCT and CHO were ingested and while MCT ingestion did not influence R, CHO ingestion markedly increased R.

Although **Chapter 13** provides evidence for the concept that CHO regulates fat metabolism, it does not provide direct evidence regarding the exact mechanism since we did not measure tissue concentrations of malonyl-CoA. Others attempted to measure malonyl-CoA concentrations in the muscle but did not measure plasma FFA oxidation (77). So, additional studies are needed to show simultaneously the changes in muscle malonyl-CoA concentrations and changes in LCFA oxidation to provide evidence in humans that malonyl-CoA is a major regulator of the fuel selection. This may be a one of the key questions for future research in this field since understanding the factors that regulate fatty acid uptake and fat oxidation in skeletal muscle during physical activity is clinically important as it is becoming more apparent that obesity (8, 16) and insulin resistance (57, 61, 91) are related to

impaired fat oxidation primarily in skeletal muscle.

For example, Blaak et al. (8) observed that obese men, compared to lean men, displayed an impaired plasma FFA uptake in forearm muscle when energy expenditure was increased via β -adrenergic stimulation. Colberg et al. (16) recently reported that FFA uptake by skeletal muscle of fasted resting women is positively correlated with CPT activity, which in turn is negatively correlated with visceral obesity. The important implication of these observations is that obesity may stem, partly, from impaired CPT activity in skeletal muscle which reduces FFA oxidation by muscle and increases reesterification to triglyceride.

The notion that the availability of glucose rather than FFA, predominantly controls substrate oxidation fits with several physiological and pathophysiological observations that cannot be explained by the glucose-fatty acid cycle. For example the observation that FFA oxidation decreases when the exercise intensity increases may be partly explained by the increased glycolytic flux which may impair LCFA transport into the mitochondria by elevating malonyl-CoA. Conversely, the increased FFA oxidation during fasting may be explained by a lower glucose availability, lower malonyl-CoA levels and decreased inhibition of LCFA transport into the mitochondria.

Furthermore, **Chapter 13** indicates that the hyperglycemia and hyperinsulinemia from dietary CHO ingestion, markedly reduces fat oxidation during exercise by both reducing LCFA oxidation in skeletal muscle and FFA mobilization. Interestingly, the hyperglycemia and hyperinsulinemia and insulin resistance as observed in NIDDM is also associated with an impaired free fatty acid utilization by muscle at rest and during exercise (57, 61, 92) that seems to be linked to increased malonyl CoA levels (91, 92). Thus the concept that intracellular availability of glucose, rather than FFA, determines the mix of fat and CHO oxidized is not only supported by the findings of **Chapter 13**, but can also help explain several physiological and pathophysiological responses of energy substrate metabolism. Future research should elucidate the exact mechanisms and determine the role of malonyl-CoA in this regulation.

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Summary

Although it has been known for a long time that carbohydrate (CHO) and fat are the most important fuels during exercise, many aspects of CHO and fat metabolism are still incompletely understood. For example we know that CHO intake during exercise can improve endurance in exercise lasting 2 hours or longer but the effect of CHO intake during short term (1 h) high-intensity exercise is relatively unknown. The study described in **chapter 11** provides evidence that carbohydrate-electrolyte drinks (sports drinks) can improve performance also during high intensity time trials which last approximately 1h. The underlying mechanism remains to be elucidated. In order to measure the contribution of ingested CHO to total energy expenditure we used stable isotope techniques. In **chapter 9** the effect of variation in the size of glycogen stores on exogenous glucose oxidation during exercise was investigated. Remarkably, we found that exogenous CHO oxidation was lower when glycogen stores were lowered by a glycogen depletion trial the evening preceding the trial. The lower plasma insulin and elevated plasma FFA levels following glycogen depleting exercise may have contributed to this difference. Training results in a decreased reliance on carbohydrates as an energy source during exercise and decreases plasma glucose turnover. However, training status appeared to have no effect on exogenous carbohydrate oxidation or other aspects of CHO metabolism during exercise at the same relative exercise intensity (**chapter 10**). Although trained subjects oxidized relatively more fat during exercise at 60%VO₂max no differences were observed in exogenous glucose oxidation.

As in previous studies the maximal exogenous CHO oxidation rates did not exceed 1 g·min⁻¹. This appears to be an absolute maximum, although the factors responsible for this limitation are still largely unknown. In **chapter 12** we showed that it is unlikely that the limitation is located at the muscular level because the rate of glucose appearance in the plasma was similar to the rate of plasma glucose oxidation even if large quantities of glucose were ingested and the rate of appearance increased threefold. Carbohydrates inhibited fat oxidation and this inhibition was dependent on the amount of CHO ingested. In **chapter 13** we studied the interaction between fat and carbohydrate metabolism in more detail by infusion of ¹³C-labeled palmitate or octanoate (a long chain or a short chain fatty acid). Palmitate transport across the mitochondrial membrane is dependent on the carnitine acyl transferase transport system in contrast to transport of octanoate. An increased glycolytic flux provoked by glucose ingestion prior to exercise resulted in hyperglycemia and hyperinsulinaemia and caused a 19% reduction in palmitate oxidation while octanoate oxidation was unaffected. This may indicate that there is active inhibition of fatty acid transport across the mitochondrial membrane, possibly by malonyl-CoA inhibition of carnitine acyl transferase. CHO intake had an inhibiting effect on fatty acid mobilization from adipose tissue and the oxidation of intramuscular triglycerides. These results provide evidence that glucose is an

important regulator of fat metabolism.

Although the oxidation of exogenous CHO is limited to about 1 g·min⁻¹ it is theoretically possible to provide extra exogenous energy by ingesting another substrate in addition to carbohydrates. Medium chain triglycerides (MCT) are such an alternative substrate. MCT are more soluble in water than long chain triglycerides (LCT) and may be more rapidly absorbed and digested compared to LCT. For these reasons MCT appears to be a substrate which is rapidly available for metabolism. In **chapter 3** it was shown that equi-energetic CHO+MCT drinks emptied faster from the stomach when they contained more MCT (and thus less CHO). In **chapter 4** we studied the oxidation rate of MCT in MCT-containing beverages which were ingested during exercise. It appeared that MCT is a substrate which is rapidly (and relatively completely) oxidized and oxidation rates increased even more when co-ingested with CHO. However, the contribution of MCT was relatively small because only small amounts of MCT were ingested (30 g). Ingestion of larger amounts of MCT generally lead to gastro-intestinal distress. MCT ingestion (30 g) had no effect on the oxidation of CHO in that drink nor on muscle glycogen breakdown (**chapter 5**). In addition, the oxidation rates of MCT did not further increase when endogenous glycogen stores were depleted and fat was the major fuel during exercise (**chapter 6**). Because we wanted to study the effect of MCT ingestion on exercise performance we first investigated the reproducibility of different exercise protocols in **chapter 7**. It appeared that a time trial of approximately 1 h duration was more reliable measure of performance than a exercise time to exhaustion at 75%W_{max}. We applied the most reproducible protocol in **chapter 8** to study the effect of ingestion of large amounts of MCT (84 g) on exercise performance. Ingestion of 84 g of MCT in combination with CHO had no positive effect on performance compared to CHO or water placebo ingestion. Ingestion of MCT resulted in a decreased performance most likely as a consequence of gastrointestinal discomfort during exercise.

Samenvatting

Hoewel het reeds lang bekend is dat koolhydraten en vetten de belangrijkste energiebronnen zijn tijdens inspanning, zijn veel aspecten van het koolhydraat- en vetmetabolisme van de spier nog niet volledig of zelfs nog volledig niet begrepen. Zo weten we bijvoorbeeld wel dat koolhydraatinname tijdens langdurige inspanning (> 2 uur) het prestatievermogen kan verbeteren, maar het effect van koolhydraat inname tijdens kortdurende inspanningen van ongeveer een uur (zoals een 40 km tijdrit) is nog onbekend. Het onderzoek beschreven in **hoofdstuk 11** toont aan dat koolhydraat-electrolietdranken (sportdranken) ook het prestatievermogen bij dergelijke intensieve inspanningen kunnen verbeteren, ook al blijven de mechanismen achter deze verbetering voorlopig onopgehelderd. Om de bijdrage van koolhydraten uit een drank aan de totale energievoorziening te bepalen, kunnen we gebruik maken van stabiele isotopen. Alhoewel een aantal factoren die de exogene koolhydraatoxidatie beïnvloeden bekend zijn was er nauwelijks informatie over het effect van de grootte van de lichaamsglycogeenvoorraden en van de mate van getraindheid op de oxidatie van ingenomen koolhydraten. In **hoofdstuk 9** werd het effect van de glycogeenvoorraden bestudeerd door deze te ledigen door middel van een zwaar inspanningsprotocol de avond van te voren. Opmerkelijk was dat de exogene koolhydraatoxidatie lager was wanneer de glycogeenvoorraden lager waren. De lagere plasma insulinespiegels en de hogere plasma FFA spiegels als gevolg van inspanning leidend tot glycogeen-depletie, dragen mogelijk bij aan dit verschil. De mate van getraindheid bleek geen effect te hebben op de exogene koolhydraatoxidatie (**hoofdstuk 10**). Alhoewel getrainden zowel relatief als absoluut meer vet oxideerden tijdens inspanning op 60% VO_2max werden er geen verschillen gevonden in exogene koolhydraatoxidatie. Evenals in voorgaande onderzoeken bleef de maximale exogene koolhydraatoxidatie hier onder 1 $\text{g}\cdot\text{min}^{-1}$. Dit blijkt een absoluut maximum te zijn maar de factoren die deze limitering bepalen zijn nog onbekend. In **hoofdstuk 12** werd aangetoond dat de spier waarschijnlijk geen limiterende factor is omdat glucose welke met een bepaalde snelheid in het plasma verscheen, met ongeveer dezelfde snelheid werd geoxideerd, zelfs wanneer een grote hoeveelheid koolhydraten werd ingenomen tijdens inspanning en de verschijningsnelheid een factor 3 hoger was. Koolhydraatinname tijdens inspanning zorgde voor een remming van het vetmetabolisme die afhankelijk was van de ingenomen hoeveelheid glucose. In **hoofdstuk 13** hebben we deze interactie tussen vet en koolhydraatmetabolisme nader bestudeerd. Door middel van infusie van ^{13}C -gelabeld palmitaat of octanoaat (een lange keten en een middellange keten vet-zuur) werd de oxidatie en de verdwijningssnelheid van vetzuren uit het plasma bestudeerd. Palmitaattransport over het mitochondriële membraan is afhankelijk van het carnitine transportsysteem, terwijl het transport van octanoaat in mindere mate carnitine-afhankelijk is. Een toename van de glycolytische flux door glucose inname voor inspanning leidde tot hyperglycemie en hyperinsulinaemie en

reduceerde de palmitaatoxidatie met 19%, maar had geen effect op de octanoaat-oxidatie. Dit duidt erop dat er een actieve remming is van het transport van lange keten vetzuren over het mitochondriële membraan, mogelijk door remming van carnitine acyl transferase als gevolg van een verhoging van de cytosolische malonyl-CoA concentratie. Koolhydraatinname had verder een remmend effect op de vetzuurmobilisatie uit vetweefsel en op de oxidatie van intramusculaire triglyceriden. Deze resultaten laten zien dat glucose in sterke mate het vetmetabolisme reguleert.

Omdat de oxidatie van exogene CHO beperkt is tot ongeveer $1 \text{ g} \cdot \text{min}^{-1}$ is het theoretisch mogelijk meer exogene energie toe te voegen door een ander substraat te kiezen wat naast de CHO wordt ingenomen. Middellange keten triglyceriden (MCT) vormen mogelijk een alternatief substraat. MCT zijn beter oplosbaar in water dan lange keten vetzuren en mede daardoor sneller worden verteerd en geabsorbeerd in de darmen. In **hoofdstuk 3** werd aangetoond dat inname van MCT de maagleding van koolhydraat (CHO)+MCT dranken niet vertraagt. In tegendeel; dranken met een toenemend MCT gehalte (en afnemend CHO gehalte) werden sneller uit de maag geleid. In **hoofdstuk 4** hebben we de oxidatie van MCT bestudeerd uit MCT-bevattende dranken die tijdens inspanning werden genuttigd. Hieruit bleek dat MCT een substraat is wat snel (en relatief volledig) wordt geoxideerd en dat de oxidatiesnelheid nog toenam wanneer samen ingenomen met CHO. De bijdrage van MCT aan het totale energiemetabolisme was echter maar klein omdat de hoeveelheid MCT die kon worden gegeven relatief klein was (30 g). Bij inname van grotere hoeveelheden ontstaan over het algemeen maag-darmproblemen. MCT inname (30 g) bleek geen invloed te hebben op de oxidatie van de koolhydraten in die drank of op de snelheid van spierglycogeenafbraak tijdens inspanning (**hoofdstuk 5**). De oxidatiesnelheid van MCT nam niet verder toe wanneer de glycogeenvoorraden werden uitgeput en vetten de belangrijkste brandstof waren tijdens inspanning (**hoofdstuk 6**). Omdat we het effect van MCT supplementen op het prestatievermogen wilden meten, hebben we eerste de reproduceerbaarheid van verschillende inspanningsprotocollen vergeleken in **hoofdstuk 7**. Hieruit bleek dat een tijdsrit van ongeveer een uur betrouwbaarder was dan een test op 75% $\dot{W}_{\text{max}}$ tot uitputting. In **hoofdstuk 8** hebben we de meest reproduceerbare test hebben gebruikt om de effecten te bestuderen van inname van grote hoeveelheden MCT (84 g). Inname van 84 g MCT in combinatie met CHO leverde geen prestatieverbetering op ten opzichte van water placebo of een CHO-drink. Inname van alleen MCT leidde tot een prestatiedaling welke waarschijnlijk te wijten is aan het optreden van maag-darmproblemen tijdens inspanning.

Curriculum vitae

Asker Erik Jeukendrup werd op 29 juli 1969 geboren te Roermond, waarna hij opgroeide in Haelen. In 1987 behaalde hij het VWO diploma aan de Scholengemeenschap St Ursula te Horn waarna hij de studie gezondheidswetenschappen aan de Rijksuniversiteit Limburg (nu Universiteit Maastricht) begon. Hier heeft hij zich gespecialiseerd in de richting Bewegingswetenschappen en de studie werd afgesloten met een project uitgevoerd in samenwerking met Matthijs Hesselink naar de effecten van overtraining bij wielrenners. Vervolgens begon hij bij de vakgroep Humane Biologie aan de Universiteit Maastricht als AIO waar hij onder begeleiding van Dr A.J.M. Wagenmakers en Prof W.H.M. Saris onderzoek verrichtte naar verschillende aspecten van het koolhydraat- en vetmetabolisme tijdens inspanning. De theoretische kennis opgebouwd in deze periode werd toegepast in de praktijk door actief te zijn in de voedingsbegeleiding van een aantal topsporters (waaronder de Rabobank wielerploeg) maar ook door deze kennis zelf te toetsen tijdens triathlons. Na het verdedigen van dit proefschrift zal Asker naar de University of Texas in Austin, Texas vertrekken om daar als post-doc de in de AIO periode gestarte samenwerking met Prof Edward F. Coyle voort te zetten.

List of publications

Refereed papers

1. Jeukendrup, A., M. K. C. Hesselink, A. C. Snyder, H. Kuipers and H. A. Keizer. Physiological changes in male competitive cyclists after two weeks of intensified training. *Int J Sports Med* 13: 534-541, 1992.
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14. Jeukendrup, A. E., A. J. M. Wagenmakers, F. Brouns, D. Halliday and W. H. M. Saris.

List of publications

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Book sections

1. **A. E. Jeukendrup.** Duursport en Voeding. In: Zintl, F. Duurtraining: basisprincipes, methoden en trainingsbegeleiding. De Vrieseborch, Haarlem 1995
2. **A. E. Jeukendrup.** Overtraining. In: Zintl, F. Duurtraining: basisprincipes, methoden en trainingsbegeleiding. De Vrieseborch, Haarlem 1995
3. **A. E. Jeukendrup and W. H. M. Saris.** Fat as a fuel during exercise. In: Sport nutrition for the 90s. J. R. Berning and S. N. Steen. In press 1997

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