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Fat Metabolism During Exercise: A Review

Part I: Fatty Acid Mobilization and Muscle Metabolism

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████ This is the first part in a series of three articles about fat metabolism during exercise. In this part the mobilization of fatty acids and their metabolism will be discussed as well as the possible limiting steps of fat oxidation. It is known for a long time that fatty acids are an important fuel for contracting muscle. After lipolysis, fatty acids from adipose tissue have to be transported through the blood to the muscle. Fatty acids derived from circulating TG may also be used as a fuel but are believed to be less important during exercise. In the muscle the IMTG stores may also provide fatty acids for oxidation after stimulation of hormone sensitive lipase. In the muscle cell, fatty acids will be transported by carrier proteins (FABP), and after activation, fatty acyl CoA have to cross the mitochondrial membrane through the carnitine palmytoyl transferase system, after which the acyl CoA will be degraded to acetyl CoA for oxidation. The two steps that are most likely to limit fat oxidation are fatty acid mobilization from adipose tissue and transport of fatty acids into the mitochondria along with mitochondrial density and the muscles capacity to oxidize fatty acids.

■ Key words: Fat metabolism, fatty acids, exercise, FABP, intramuscular triacylglycerols, lipolysis, carnitine, VLDL.

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. In the past decade 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. The review of fat metabolism during exercise will be divided into three separate papers in three subsequent editions of the International Journal of Sports Medicine:

Part I   Fatty acid mobilization and muscle metabolism
Part II Regulation of metabolism and the effects of training
Part III The effects of nutritional interventions on fat metabolism

This part discusses processes of lipolysis, and mobilization of fatty acids, the transport of fatty acids and the uptake and oxidation by the muscle. Reviews addressing these issues in the past include Refs. (55) and (108) and a very recent publication by van der Vusse and Reneman (126).

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 triacylglycerols or other particles, and fatty acids that are not incorporated into triacylglycerols. The fatty acids that are not esterified to form a mono-, di- or triacylglycerol are so called nonesterified 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 fatty acid pool) that is really "free" and not bound to any other compound (non-protein bound fatty acids) (44,101,102). Therefore we will use the term fatty acid (and not free fatty acid) in this review to designate fatty acids that are not esterified to mono-, di-, or triacylglycerols, but might be bound to albumin or fatty acid-binding proteins (FABP). The basic chemical structure of fatty acids is depicted in Fig. 1.

In humans, the chain length of fatty acids typically varies from C 14 to C 24, although fatty acids with shorter or longer chain length may occur (Table 1). Fatty acids with a chain length of

Table 1 Nomenclature and formulas of fatty acids (FA)

FA	Double Bonds	Common Name	Chemical formula
2:0	–	acetic	CH_3COO-
4:0	–	butyric	$CH_3(CH_2)_2COO-$
6:0	–	capronic	$CH_3(CH_2)_4COO-$
8:0	–	caprylic	$CH_3(CH_2)_6COO-$
10:0	–	caprynic	$CH_3(CH_2)_8COO-$
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	$CH_3(CH_2)_5CH=CH(CH_2)_7COO-$
18:0	–	stearic	$CH_3(CH_2)_{16}COO-$
18:1	n-9	oleic	$CH_3(CH_2)_7CH=CH(CH_2)_7COO-$
18:2	n-6	linoleic	$CH_3(CH_2)_4(CH=CHCH_2)_2(CH_2)_6COO-$
18:3	n-6	γ-linolenic	$CH_3(CH_2)4(CH=CHCH_2)3(CH_2)_3COO-$
18:3	n-3	α-linolenic	$CH_3(CH_2)(CH=CHCH_2)_3(CH_2)_6COO-$
20:0	–	arachidonic	$CH_3(CH_2)_{18}COO-$
20:2	n-6	eicosadinoic	$CH_3(CH_2)_4(CH=CHCH_2)_2(CH_2)_8COO-$
20:3	n-6	eicosatrinoic	$CH_3(CH_2)_4(CH=CHCH_2)_3(CH_2)_5COO-$
20:4	n-6	arachidonic	$CH_3(CH_2)_4(CH=CHCH_2)_4(CH_2)_2COO-$
20:5	n-3	eicosapentaenoic (EPA)	$CH_3(CH_2)(CH=CHCH_2)_5(CH_2)_2COO-$
22:0	–	behenic	$CH_3(CH_2)_{20}COO-$
22:5	n-3	docosapentaenoic	$CH_3(CH_2)(CH=CHCH_2)_5(CH_2)_4COO-$
22:6	n-3	docosahexaenoic (DHA)	$CH_3(CH_2)(CH=CHCH_2)_6(CH_2)COO-$
24:0	–	lignoceratic	$CH_3(CH_2)_{22}COO-$

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) (67). 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 third carbon counting from the terminal methyl group (Fig. 1). Another way of indicating the fatty acid and the position of the double bond is C20:4 ω3.

Oxidizable lipid fuels include fatty acids, intramuscular triacylglycerols (IMTG) and circulating plasma triacylglycerols (TG; chylomicrons and very low density lipoproteins (VLDL)). These plasma TG are usually incorporated in lipoproteins like chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), intermediate density lipoproteins (IDL) or 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 triacylglycerols 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
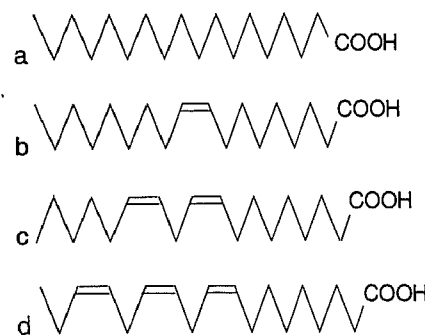
Fig. 1 Chemical structure of some common fatty acids. a stearic acid, 18:0; b oleic acid, 18:1 (n-9); c linoleic acid 18:2 (n-6); d α-linolenic acid 18:3 (n-3)

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 as glucose.

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 carbohydrates, 38 kJ · g^{-1} (9 kcal · g^{-1}) for fats versus 18 kJ · g^{-1} (4 kcal · g^{-1}) for carbohydrates. Furthermore, carbohydrates are stored in the presence of water, whereas fat is stored almost anhydrous (1 g of glycogen binds about 2 g of

water [63]). This makes fat a far more efficient fuel per unit of weight. If all the fat in our body would be replaced by an equi-energetic 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 (43).

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 circulation. The carbohydrate stores are small. The total amount of muscle glycogen of an 80 kg man is about 400 grams (Table 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. It has been estimated that running a marathon which requires about 83 kJ/min (21 kcal/min) for elite marathon runners while non-elite runners expend a similar amount of energy over the entire distance (42.2 km; about 12000 kJ) (92). So it can be calculated that 500 grams of glycogen can fuel about 95 min of running at marathon pace to elite runners. Calculating the amount of energy stored as fat for an 80 kg man and 60 kg woman (average body composition) this would provide respectively 450000 kJ (110000 kcal) and 550000 kJ (135000 kcal). Energy stored in the form of carbohydrates can vary in a range from 6000 kJ (1500 kcal) to 12000 kJ (3000 kcal). In other words, if only fat or only carbohydrates could be utilized as a fuel, the carbohydrate stores from exercising muscles would deliver energy for no more than 95 min of marathon running while energy derived from fat stores

would be satisfactory for 119 hours of continuous marathon running (92).

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 triacylglycerols 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 is estimated to be between 7 and 40 mmol · kg ww^{-1} (14,35,40,70,109). The total amount of fat stored in all muscle cells has been estimated to be approximately 300 grams (10), but assuming a muscle mass of 18 kg in a 80 kg man, and TG concentrations in the muscle in the range of 7 to 40 mmol · kg ww^{-1} this may vary from 100 – 600 grams. 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 (35,39). 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 fatty acid, bound to albumin.

Fatty acids provide more ATP per molecule than glucose does. A glucose molecule can produce 38 ATP whereas a molecule of stearic acid can produce 147 ATP. However, to produce the same amount of ATP, oxidation of fatty acids requires more oxygen than the oxidation of carbohydrates (88). 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 (88). 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} (126). 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 tricarboxylic 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.

Table 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 (approximately)	0.52	8000
fat	plasma fatty acid	0.0004	16
	plasma triacylglycerols	0.004	160
	adipose tissue	12	404000
	intramuscular triacylglycerols	0.3	10800
	total (approximately)	12.3	440000

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 (160000 kJ) mainly located in the muscle.

Fat as a Fuel During Exercise

In the early years, Zuntz (145,146), Krogh and Lindhardt (80) and later Christensen and Hansen (24) 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 (24,32,80). In general over 50% of the energy requirements at rest is derived from fatty acid oxidation (50). Fatty acids remain a very important substrate during exercise, as long as the exercise intensity is below 80 – 90% VO₂max (47,50,105). Above this exercise intensity carbohydrates are the predominant substrate (1,47,50, 105).

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 (97). Very important was also the reintroduction of the muscle biopsy technique by Bergström and Hultman (8,9,69) in the late sixties. These techniques made it possible to quantify substrate fluxes and measure tissue substrate concentrations.

Processes that Potentially Limit Fat Oxidation

Since body glycogen stores are relatively small and it has been shown that depletion of these stores results in fatigue, it would be beneficial for performance if at the same exercise intensity more fatty acids could be oxidized and less carbohydrates. However, 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 for 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 fatty acids 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 fatty acids
   b. The uptake of fatty acids from circulating plasma lipo-protein-TG
4. Mobilization of fatty acids from IMTG pools
5. The transport of fatty acids into the mitochondria
6. The oxidation of fatty acids in the mitochondria

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 small in comparison to the fatty acid turnover during exercise. 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 fatty acids from adipose tissue is dependent on 1. the rate of lipolysis, 2. the rate of reesterification of fatty acids within the adipocyte and 3. the transport rate of fatty acids from adipose tissue to the blood.

Lipolysis in adipose tissue

In adipose tissue, fatty acids are constantly mobilized by lipolysis (hydrolysis of triacylglycerols), 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 trans-
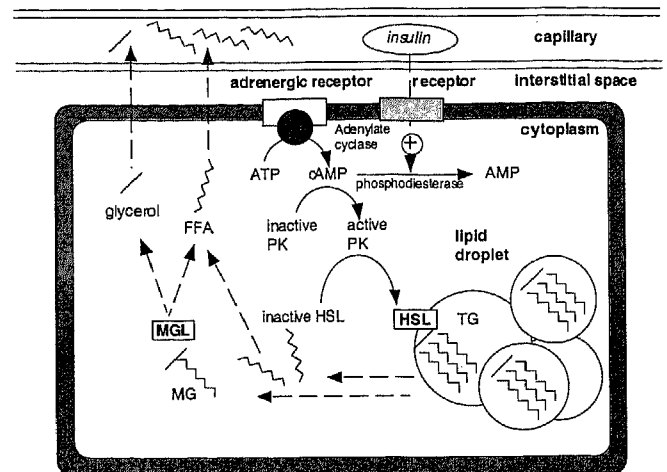

Fig. 2   Mobilization of fatty acids from adipose tissue. Triacylglycerols are transported from the lipid droplet to the cytoplasm of the adipocyte. This triacylglycerol is subjected to cleavage by the enzyme hormone-sensitive lipase (HSL); a monoacylglycerol is formed and two fatty acids which can diffuse into the circulation. The remaining monoacylglycerol is split by the enzyme monoacylglycerol lipase (MGL) into glycerol and another fatty which can also diffuse into the circulation. The rate limiting step in the mobilization of fatty acids from the lipid droplet is 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 = triacylglycerol, fatty acid = free fatty acids).

fer of triacylglycerols from the main lipid droplet to the site of enzymatic cleavage in the cytoplasm of the adipocyte (Fig. 2). Although there is only little information about this process, it is believed not to be rate-limiting (11). A second step in the mobilization of fatty acids is the enzymatic cleavage of the triacylglycerol in the cytoplasm. The fatty acids in the outer or β-position of the triacylglycerols are hydrolyzed by the action of hormone sensitive lipase (HSL). This enzyme is subjected to hormonal regulation. The remaining monoacylglycerol (glycerol with a fatty acid in the α-(inner) position) is then hydrolyzed by the more active enzyme monoacylglycerol lipase.

Glycerol cannot be reutilized by the adipocyte to form new triacylglycerols since the enzyme glycerol kinase, is only present in very low concentrations or even absent in adipose tissue (and muscle) (104). 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 (29) for heart muscle. Furthermore, Elia et al. (33) raise the possibility that glycerol released from IMTG might be directly oxidized, which may result in an underestimation of tracer determined whole-body lipolysis. Unlike glycerol, fatty acids can be reesterified to form new triacylglycerols, a process called triacylglycerol-fatty acid cycling. Tracer studies generally as-

sume that 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 (41) and insulin is probably the main counterregulatory hormone (58). Studies in isolated human adipocytes have revealed that at physiological concentrations, catecholamines, glucocorticoids, thyroid stimulating hormone and growth hormone are good stimulators of lipolysis (26,58). The relative importance of these stimulating factors on lipolysis is not fully elucidated. On the contrary, lactate (53) ketone bodies (26) and especially insulin (58) have an inhibitory effect on the enzyme.

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 (26,37). Through the adrenergic receptors a cascade of events is triggered: the adenylate cyclase system is activated which activates the protein kinase system. The protein kinase, in its turn, will activate the lipase by phosphorylation. 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 on caffeine, part III, Effects of nutritional interventions).

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 the inhibiting effect of epinephrine and to a lesser extent norepinephrine upon pancreatic insulin release. 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 (133). The combination of these effects results in an increased lipolysis in adipose tissue during low to moderate intensity exercise. At high exercise intensities (> 80% VO_2max), high circulating levels of epinephrine, in combination with increased glycolytic flux, increased plasma lactate concentrations may reduce lipolysis and increase reesterification in adipose tissue resulting in a decreased Ra fatty acids.

Removal rate of fatty acids from adipose tissue

Although the major regulatory factors controlling adipose tissue fatty acid mobilization are through adrenergic stimulation and insulin, the rate at which fatty acids are removed from the adipose tissue appears to be another factor. The removal rate of fatty acids from the adipose tissue is dependent on the plasma albumin concentration, the arterial fatty acid/albumin ratio and the blood flow through adipose tissue (17). The plasma al-

bumin concentration in plasma is fairly constant (about 0.6 mmol $\cdot$ l^{-1}) while in most conditions (except for prolonged fasting and long lasting endurance exercise) the fatty acid concentration varies between 0.2 – 1.0 mmol $\cdot$ l^{-1}. During moderate intensity exercise arterial plasma fatty acid concentrations may increase up to twenty-fold. This leads to changes in the fatty acid/albumin ratio from a resting value of 0.2 to values of 3 – 4 during exercise. Since albumin binds fatty acids with decreasing affinity when more binding sides are occupied (113), increases in the fatty acid/albumin ratio are accompanied by increased concentrations of the non-protein bound fatty acid fraction in plasma. This in turn favours reesterification within the adipocyte since the fatty acid/albumin ratio in plasma is increased even more (15,20). It has also been shown that adipose tissue blood flow markedly increases the removal rate from adipose tissue and thus the mobilization of fatty acids (17,20). Madsen et al. (83) showed that in perfused adipose tissue increases in the fatty acid/albumin ratio as well as decreases in the adipose tissue blood flow decreased fatty acid mobilization. They suggested that the decreased net fatty acid 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 three-fold which may partly compensate for the decreased transport capacity caused by the increased plasma fatty acid/albumin ratio (16,18,19).

Triacylglycerol-fatty acid cycling

As mentioned before, glycerol which is liberated after lipolysis, cannot be incorporated into triacylglycerols since the enzyme glycerol kinase is virtually absent in adipose tissue and muscle (104). In adipose tissue this enzyme is needed to convert glycerol to glycerol-3-phosphate (G-3-P), which is the backbone of a triacylglycerol molecule. Therefore the rate of appearance (Ra) of glycerol is often used as a direct index of lipolysis (141). 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 triacylglycerols. With low blood glucose levels, less G-3-P will be formed and consequently the rate of fatty acid reesterification will decrease (142,143). The excess fatty acids will be released into the circulation. This TG hydrolysis and subsequent reesterification was the first documented substrate cycle and is called the triacylglycerol-fatty acid cycle (Fig. 3). The functioning of this cycle allows the adipocyte to adjust the release of fatty acids rapidly in response to alterations in metabolic demands. For instance, when there is not enough glucose available, reesterification will be decreased and more fatty acids will be mobilized from the adipose tissue (142). Reesterification may occur within the adipocyte (intracellular reesterification) or fatty acids may be released and reesterified in some other tissue (extracellular reesterification).

Assuming complete hydrolysis of each TG molecule, the release of 1 molecule glycerol should be accompanied by the release of 3 molecules fatty acids, if no reesterification occurs after hydrolysis of triacylglycerols. However, the release ratio between glycerol (Ra glycerol) and fatty acids (Ra FA) is usually found to be 1 – 2 (142). This means that part of the fatty acids derived from lipolysis will not enter the blood stream but will
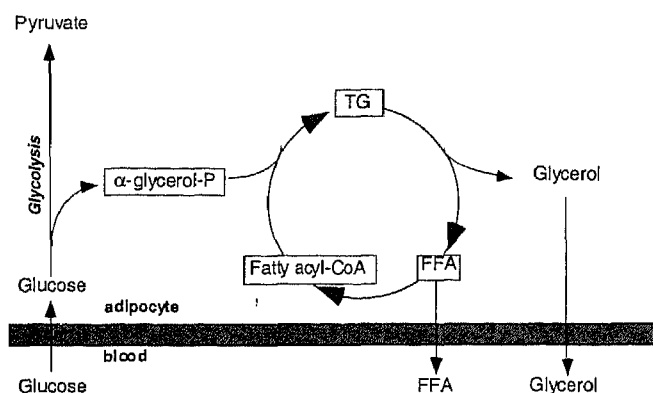
Fig. 3 The triacylglycerol-fatty acid cycle. Fatty acids (FA) mobilized after hydrolysis of triacylglycerols (TG) can either be released into the circulation or reesterified with a molecule of glycerol-3-phosphate (G-3-P). This TG hydrolysis and subsequent reesterification is called the triacylglycerol-fatty acid cycle.

be used for reesterification. 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. During the first 30 min of exercise at 40% VO_2max, the reesterification of fatty acids was markedly decreased. Whereas at rest approximately 70% of all released fatty acids were reesterified, during exercise only 25% was reesterified (142). Similar findings were obtained by Hodgetts et al. (62) during exercise at 50–70% VO_2max. 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 (16). This results in a massive increase in rate of appearance and availability of fatty acids in the plasma. The rate of reesterification is also dependent on the ability of plasma to carry away released fatty acids (i.e. the number of free albumin binding sites for fatty acids 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 fatty acid reesterification (73,98,111). Shaw et al. (111) stated that a lactate concentration of 2 mmol · l^{-1} reduces fatty acid output by 35–40% in the whole animal.

Transport of lipids by the blood

When fatty acids have passed the cell membrane of the adipocyte either passively or mediated by membrane associated proteins such as fatty acid translocase (FAT) or fatty acid-transport protein (FATP) (60,110,126), 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 (113), under physiological circumstances a minority of all binding sites are occupied. The largest fraction of fatty acids in the plasma (more than 99.9%) are carried bound to albumin (101,102). Prior to extraction by skeletal muscle, fatty acids have to be released from albumin since the permeability of endothelial cells, lining the wall of muscle capillaries, is very low for the albumin-fatty acid complex (7). As discussed above, blood flow and the saturation of the albumin-fatty acid complex appear to be important factors in the mobilization of fatty

acids from adipose tissue (21). In the liver fatty acids can be incorporated in 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]).

Mechanism of uptake of fatty acids

Havel et al. (61) showed in 1967 that during exercise plasma fatty acid concentrations increased along with the fatty acid uptake although the fractional extraction rate decreased. Studies in men and dogs have suggested that a linear relationship exists between fatty acid concentration in the blood and the utilization of fatty acids, suggesting that the uptake of fatty acids by the muscle is a passive process that occurs by passive diffusion (4,71). However, this traditional view has recently been challenged (7,125). Evidence is accumulating indicating the existence of a "carrier system" for fatty acids across the cell membrane. Although the details of the transport of fatty acid into the muscle cell are largely unknown, it is generally accepted that one or more membrane-associated proteins are involved (22,99,129). Carrier proteins such as FAT and FATP with fatty acid-transport stimulating properties have been identified in adipocytes (60). Recently, FABPpm (22), FAT (2,45), and FATP (2,45) 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 (45,126).

The initial step in the uptake of fatty acids 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 albuminal membrane of the endothelial cell (Fig. 4). Next, the fatty acids have to be transported through the interstitial space most probably bound to albumin (125). Then the fatty acids will be transported through the sarcolemma, either by passive diffusion or facilitated by plasma membrane bound FABP (FABPpm) (125) or as recently suggested by proteins in the membrane: FAT and FATP (45,129). In the cytoplasm, fatty acids are bound to cytoplasmic FABP (FABPc) and transport of the fatty acids within the cytoplasm occurs bound to this protein (45,46,129). Vork et al. (130) 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 fatty acid oxidation.

Although the mechanism of fatty acid transport into the muscle cell is largely unknown, the existence of a carrier mediated transport might explain the findings of Turcotte et al. (124) and Kiens et al. (77). In isolated perfused rat skeletal muscle (124) and in man (77), it was observed that the uptake of non-protein bound fatty acids by the muscle followed saturation kinetics and did not increase linearly with the increasing fatty acid concentration in the blood as was generally believed. Above a certain fatty acid concentration, saturation of the transport process occurred, which could indicate a limitation in the transport over the cell membrane although limitations distal from the fatty acid uptake cannot be excluded (i.e. oxidation). The data implying saturation kinetics of fatty acid uptake are derived from plots of fatty acid uptake against the delivered non-protein bound fatty acid fraction (51,77,112,118,
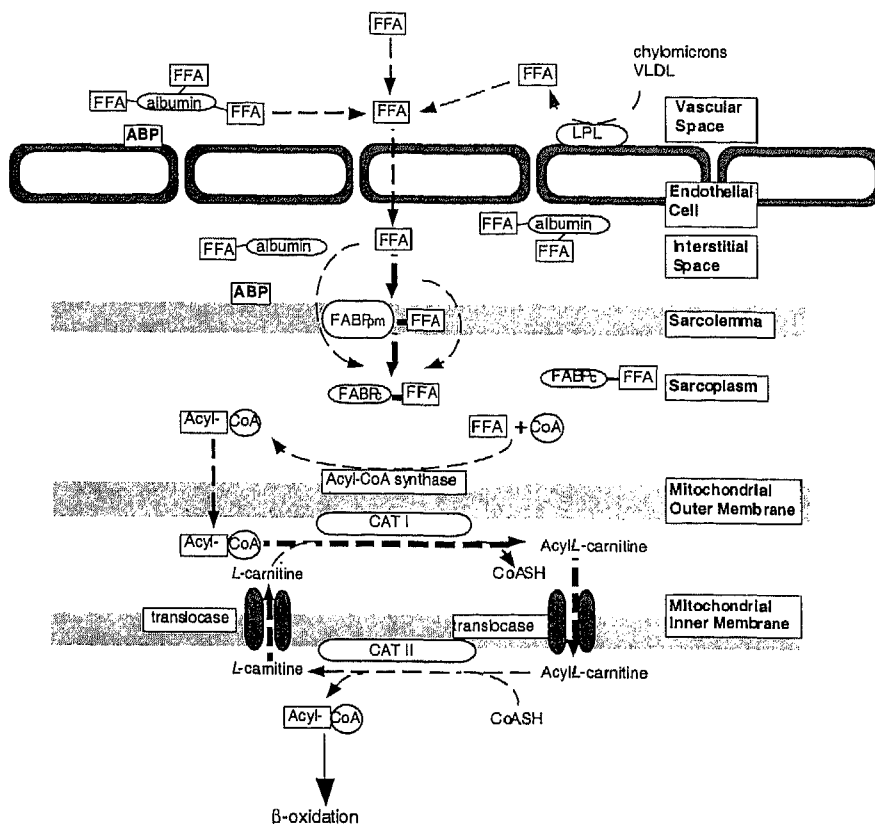
FFA

FFA

FFA—albumin   FFA — — — ▶ FFA ◀ — — — chylomicrons VLDL

ABP

FFA

Vascular Space

LPL

Endothelial Cell

FFA—albumin   FFA

FFA—albumin   FFA

FFA

Interstitial Space

ABP

FABPpm — FFA

Sarcolemma

FABPc — FFA

CFABR — FFA

Sarcoplasm

Acyl-CoA

FFA + CoA

Acyl-CoA synthase

Mitochondrial Outer Membrane

CAT I

Acyl-CoA   Acyl L-carnitine

L-carnitine   CoASH

translocase   translocase

Mitochondrial Inner Membrane

CAT II

L-carnitine ◀ — — — — — — Acyl L-carnitine

Acyl-CoA   CoASH

β-oxidation

Fig. 4 Transport of fatty acids from the vascular space into the mitochondria of the muscle cell. Fatty acids in plasma are bound to albumin and after transport into the interstitial space again bound to albumin. Fatty acid cross the sarcolemma by a plasma membrane bound "fatty acid-binding protein (FABP$_{pm}$) and in the sarcoplasm they will be bound to a cytoplasmic fatty acid-binding protein (FABP$_c$). Subsequently fatty acid will be activated by acyl-CoA synthetase to form a acyl-CoA which can be transported via carnitine palmytol transferase I (CPT I), a translocase and carnitine palmytoyl transferase II (CPT 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.

124). Recently it has been suggested that although the largest fraction of fatty acids in plasma (99.9%) are carried bound to albumin, fatty acid uptake may be dependent on the small non-protein bound fatty acid fraction in the plasma (0.1% of the total fatty acid pool in plasma) (77,124).

Availability of plasma fatty acids

Because fat is stored mainly outside the muscle cell in adipose tissue, availability of fatty acid as a substrate is dependent on lipolysis in adipose tissue, transport through the blood and fatty acid uptake by the muscle. However lipolysis in the muscle may be another very important factor determining fatty acid availability especially in endurance trained humans. During the first minutes of exercise plasma fatty acid concentration decreases as a result of a delay between the increased uptake of fatty acid directly after the onset of exercise and the stimulation of lipolysis (23). Once lipolysis is fully activated and re-esterification is suppressed, the plasma fatty acid concentration will rise (142). Plasma fatty acid concentration increases progressively with increasing submaximal exercise intensity (48,49,92). At high exercise intensities (> 80% VO$_2$max), however, fatty acid availability may be limited as a result of decreased fatty acid (Ra fatty acid) release from adipose tissue (105).

At cessation of exercise the utilization of fatty acid is drastically reduced whereas the lipolytic activity will be maintained because of the metabolic activation. As a result fatty acid levels in the blood will increase in the postexercise period (92). After 10 - 15 min a peak fatty acid concentration is reached, and concentrations will decline again to resting levels (0.2 - 0.5 mmol ·

l^{-1}). After exercise, and sometimes even during exercise, fatty acid concentrations may increase up to 2 mmol · l^{-1}. It is generally assumed that fatty acid concentrations above 2 mmol · l^{-1} are toxic in humans (74,92). High post-exercise fatty acid concentrations can also be regarded in a positive way, since they replenish muscle TG stores (134). Hagenfeldt et al. (57) showed that fatty acid oxidation is in large part dependent on the plasma fatty acid concentration. However, the rate of fatty acid oxidation in the working muscle is not only determined by the plasma fatty acid concentration, but factors like the number and the size of mitochondria (see part II, the effects of training on plasma fatty acid utilization), and probably also the presence of other substrates such as glucose determines the rate at which fatty acids can be oxidized. Coyle et al. (27) showed that the reduced oxidation of long chain fatty acids during exercise when hyperglycemic and hyperinsulinemic was partly due to a reduction in the availability of plasma fatty acids and partly due to other factors located inside the muscle cell. Horowitz et al. (68) showed that pre-exercise glucose ingestion resulted in decreased fatty acid oxidation. This effect was partly caused by an insulin induced reduction in lipolysis and a reduction in plasma fatty acid concentration. However, when plasma fatty acid levels were restored to the same level as during the control trial (no pre-exercise glucose feeding) by infusing a triacylglycerol emulsion plus heparin, fat oxidation was only partially restored. These findings indicate that fatty acid concentration may be an important factor but it is not the only factor determining plasma fatty acid oxidation.

In other studies it has been shown that higher rates of glycogenolysis and subsequent glycolysis which lead to higher muscle pyruvate concentrations at high exercise intensities (107,

114) are accompanied by lower rates of fat oxidation. Apart from a reduction of the rate of fatty acid oxidation by high muscle pyruvate concentrations (either through activation of PDH (glucose-fatty acid cycle) or through malonyl-CoA; see discussion in part II), there are also suggestions that high rates of fatty acid oxidation can only be maintained in the presence of a minimal amount of TCA cycle intermediates (TCAI) (107, 127,128). In the absence of carbohydrates, the fatty acid oxidation is inhibited in isolated perfused hindlimb from the rat (103,123). It has been shown clearly that the concentration of TCAI in skeletal muscle increases severalfold, rapidly after the start of exercise (107). The alanine aminotransferase reaction in muscle (pyruvate + glutamate ↔ alanine + α-ketoglutarate) seems to function to achieve this increase and maintain high concentrations of TCAI during prolonged exercise (107,127, 128). The increase in muscle pyruvate thereby is the driving force to shift the alanine aminotransferase reaction (equilibrium reaction) to the right towards synthesis of α-ketoglutarate and the other TCAI. However, an adequate anaplerotic rate cannot be maintained when muscle glycogen becomes depleted during prolonged exercise and muscle pyruvate concentrations start to fall. This implies that the maximal TCA cycle flux will be reduced once muscle glycogen has been depleted and fatty acids have become the main substrate. Decreased fatty acid oxidation in the absence of carbohydrates (103,123) 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 (107,127). This may explain why endurance athletes during competition have to reduce the exercise intensity to about 50% VO₂max when glycogen stores have become depleted and fatty acids have become the main fuel.

Uptake of fatty acids from circulating lipoproteins

Another potential source of fatty acids is triacylglycerols bound to lipoproteins (VLDL and chylomicrons) (61). Muscular endothelium is virtually impermeable for circulating lipoproteins. Before fatty acid can be taken up by the muscle, they have to be released from the triacylglycerols forming the core of the lipoproteins (VLDL and chylomicrons). Only after hydrolysis of the lipoprotein-TG by lipoprotein lipase (LPL), can fatty acid be transported into the muscle cell. LPL is located on the luminal surface of the vascular wall (13) 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 (81). 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 (81).

Attempts have been made, using arteriovenous balances and radioactive labelled TG, to quantify the plasma TG oxidation. From most of these studies, contribution of fatty acids derived from plasma TG to the total fat metabolism during exercise appears to be very small (59,61,95,96). Attempts to quantify VLDL-TG utilization during exercise have revealed that the fatty acid uptake from plasma lipoprotein triacylglycerols occurs slowly (144) and accounts for less than 5% of the fatty-acid derived CO₂ during prolonged exercise (61,72). Therefore it is often believed that plasma TG contribute only minimally to en-

ergy production during exercise (61,72,144). However, there are some interesting observations that need further investigation. For instance LPL activity is significantly increased after training (79) and after a high-fat diet (78), both situations in which fat oxidation is markedly increased. In addition, acute exercise also stimulates muscle LPL activity (121). Furthermore, studies in exercising dogs showed that the contribution of VLDL-TG is not negligible (122). These findings together suggest that TG from plasma lipoproteins may be an important fuel during exercise even though 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.

Muscle triacylglycerols

Muscle triacylglycerols are stored in depots, mostly adjacent to the mitochondria (66) (Fig. 5).

Skeletal muscle contains on average about 12 mmol·kg⁻¹ ww TG (14,35,52,70,109) but this can vary markedly because of 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 (35). Glycolytic fibers rely less on TG as a source of energy and as a consequence TG pools can be smaller in these fibers. As will be discussed below, it is sometimes hard to distinguish between intramuscular triacylglycerol and intermuscular triacylglycerol (i.e. triacylglycerol from adipocytes which are interlaced between muscle fibers). Little is known about the role of inter- versus intramuscular triacylglycerol stores. However, the fact that trained subjects have more intramuscular and less intermuscular triacylglycerols (66) while they oxidize more muscle triacylglycerols (84) suggests that the intramuscular triacylglycerol stores are the most important in terms of energy provision.
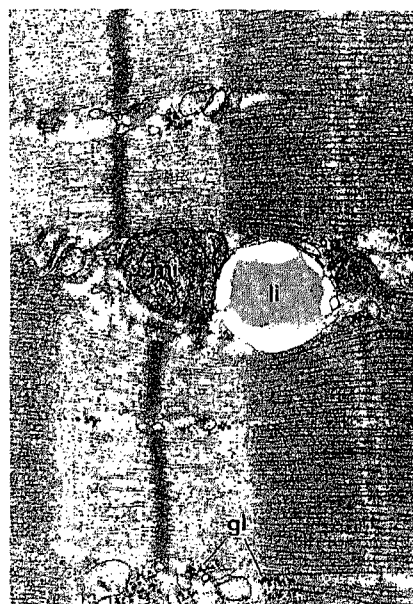

Fig. 5 Electron microscopy image of a lipid droplet (li) in close proximity to the mitochondria (mi) of skeletal muscle. Also visible are the intramuscular glycogen (Hgl) granules. Micrograph Courtesy: H. Claassen, Institute of Anatomy, University of Bern, Switzerland.

Although triacylglycerol metabolism in adipose tissue has been thoroughly described over the years, our knowledge about the regulation of muscle TG metabolism is very limited. Surprisingly, not even the enzyme responsible for the hydrolysis of TG has been identified. In rat skeletal muscle three different triacylglycerol lipases (TGL) have been found, each with distinct activities at a pH of 5.0, 7.0 or 8.5. These lipases are known as acid, neutral and alkaline lipase, respectively. The alkaline lipase was identified as lipoprotein lipase (119). It has been suggested that this alkaline TGL (LPL) in muscle serves mainly to catalyze the cleavage of fatty acids from circulating TG. The neutral TGL, which is most likely under hormonal control, and influenced by the adrenergic system, may be the enzyme responsible tor the intramuscular lipolysis (52). However, the information on this enzyme in skeletal muscle is scarce. The regulation of the intramuscular lipases has been studied indirectly. When norepinephrine was infused, a significant reduction in the IMTG content was observed in humans (40). Also, stimulation of β-adrenergic receptors in rat diaphragm with isoproterenol increased in vitro lipolysis and this increase was partially prevented by the addition of insulin to the incubation medium (3). Furthermore, propanolol administration to swimming rats was found to prevent a decline in muscle triacylglycerol, indicating that β-adrenoceptor blockade effectively inhibits intramuscular neutral lipid degradation during exercise (116). In isolated perfused heart it was shown that with epinephrine, the glycerol release was increased (137). Furthermore, by perfusion with dibutyryl adenosine 3',5'-cyclic monophosphate (cAMP), TG decreased by 50% in perfused rat hearts (42). This may indicate that, as in adipose tissue, cardiac lipolysis is mediated at least in part, through the classical cAMP cascade. Cleroux et al. (25) showed that muscle triacylglycerol lipolysis is mediated by β2-adrenoceptors. In subjects performing prolonged exercise until exhaustion, muscle triacylglycerol utilization was completely blocked by nadolol, a non-selective β-blocker, whereas it was unchanged with atenolol, a selective β1-adrenoceptor blocker. Indirect support for these observations in skeletal muscle comes from studies showing that muscle triacylglycerol lipolysis occurs mainly in slow-twitch fibers (115,117) which also have a higher density of β2-adrenoceptors (136). Because results indicated that extramuscular lipolysis decreased to the same extent with β1-adrenoceptoror β1 + β2-adrenoceptor-blockade it was concluded that adipose tissue lipolysis is only partially controlled by the adrenergic system and mainly through β1-adrenoceptors, whereas skeletal muscle triacylglycerol breakdown appears to be controlled by the adrenergic system through β2-adrenoceptors (25). However, at present it is still not completely clear to what extent β1, β2 and β3-adrenoceptors are involved in skeletal muscle lipolysis. There is also evidence that lipolysis in muscle and IMTG degradation can be influenced by direct stimulation of the sciatic nerve (115) or after direct stimulation of a muscle with electrodes (65), indicating that other factors than adrenergic stimulation (i.e., local mechanisms) may play a role in regulating intramuscular lipolysis. Gorski et al. (52) hypothesized that Ca^{2+} may be a likely candidate for exerting a local stimulating effect on endogenous TG lipases.

Early studies using ^{14}C-labelled fatty acids to quantify fatty acid turnover indicated that muscle TG were used during exercise (56,61). These studies revealed that during submaximal exercise (60–120 min), plasma fatty acid contributed only 50% to total fat oxidation, implicating that the residue must have come from other fat sources, most likely intramuscular triacylglycerols. With electron microscopy, fat vacuoles in the muscle were studied before and after exercise (94). The size of the vacuoles was smaller after exercise suggesting that indeed fatty acids were mobilized from these vacuoles and subsequently oxidized (94). Studies using muscle biopsies indicated that muscle TG content decreases during exercise (14,36,39, 100). However, other studies were not able to find differences between pre- and post-exercise TG content in muscle biopsies (48,75). 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 smal 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 (109). Especially in human muscle biopsies this may be a problem since pre- and post-exercise muscle biopsies usually have a slightly different fiber type composition. Wendling et al. (135) 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 triacylglycerol utilization, it is of utmost importance that all surrounding fat is carefully dissected from the muscle fibers before the triacylglycerol content is analyzed. If this dissection is not performed, the measured triacylglycerol content will be the sum of intramuscular triacylglycerols and adipocytes located between muscle fibers. Recently MR spectroscopy techniques were used to quantify intramuscular triacylglycerol concentrations in vivo (12). These techniques may enable more precise measurements of intramuscular triacylglycerol concentrations in the future. Boesch et al. (12) reported a 6% error in their method. However, they also reported considerable differences between individuals when the same muscle was investigated.

It was estimated that the contribution of muscle TG to total energy expenditure during exercise (65% VO_2max) was 15–35% (70,75). It must be noted, however, that this contribution was calculated by subtracting plasma fatty acid 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. (105) 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 VO_2max, respectively (Fig. 6). This suggests that there is an optimum in intramuscular TG utilization somewhere between 25% and 85% VO_2max. Endurance trained athletes rely more on their IMTG. Martin et al. (84) showed that training increased the contribution of IMTG utilization to total energy expenditure. This will be discussed in more detail in part II of this review, section "The effect of training on IMTG utilization".

In general it can be concluded that muscle TG are utilized during submaximal exercise, and they are an important substrate for the contracting muscle. Unfortunately, methodological problems make quantification of intramuscular triacylglycerol utilization very difficult.
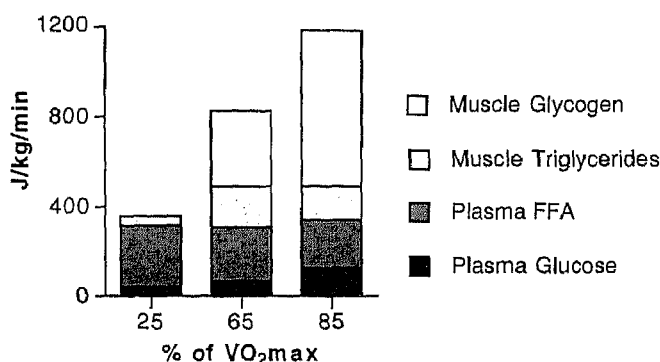
Fig. 6 Substrate utilization at different exercise intensities (25% VO₂max, 65% VO₂max and 80% VO₂max). Data adapted from Romijn et al. (105).

Transport of fatty acids across the mitochondrial membrane

Once fatty acids enter the cytoplasm of the muscle cell they can either be esterified and stored into intracellular TG (28) or the fatty acid 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 synthetase. The activation of the fatty acid is an extramitochondrial process. The mitochondrial inner membrane is impermeable for acyl-CoA (or fatty acid) 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 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 (Fig. 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 (93,126). 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 (54,106). 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 (27,85 – 87,106,138 – 140). The possible role of malonyl-CoA in the regulation of fatty acid oxidation will be discussed in part II of this review, section "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 (120). 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 (27,89). However, at present it is uncertain to what extent the carnitine dependent transport of fatty acid into the mitochondria is rate limiting.

Oxidation of fatty acids

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 (126). 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. (76) studied whole body oxidation rates of fatty acids with a similar chain length (C 18) but different degrees of saturation. Remarkable differences in the oxidation rates were observed: oleic acid (C 18:1 n-9) was more rapidly oxidized than linoleic acid (C 18:2 n-6) which in turn was more rapidly oxidized than stearic acid (C 18:0). In vivo studies in rats (82) showed that the oxidation rates of fatty acids decreased with increasing chain length (C 12:0 > C 14:0 > C 16:0 > C 18:0). There were considerable differences in the oxidation rates of different unsaturated fatty acids (C 18:3 n-3 > C 18:1 n-3 > C 18:3 n-6 > C 22:6 n-3 > C 20:4 n-6). Oleic acid (C 18:1) was oxidized at a remarkably high rate, almost as fast as lauric acid (C 12:0). Of the n-6 fatty acids linoleic acid (C 18:2 n-6) was oxidized at a faster rate than any of its metabolites, with arachidonic acid (C 20:4 n-6) being oxidized at the lowest rate. The rate of oxidation of α-linolenic acid (C 18:3 n-3) was almost as fast as lauric (C 12:0) and oleic acid (C 18:1 n-9). In incubated hepatocyte cultures, rates of fatty acid uptake and ketone body formation were of the order C 16:1 > C 16:0 > C 18:2 > C 18:1 > C 18:0 (34). Hagenfeldt and Wahren (56) also observed a slight preferential extraction of linoleic (C 18:2 n-6) and oleic acid (C 18:1) compared to palmitic acid (C 16:0) across the forearm. From these studies it appears that saturated and unsaturated fatty acids are oxidized at different rates.

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 (90,91).

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 ($50-150\ mmol\cdot l^{-1}$) but may increase markedly after fasting or during prolonged exercise (14,30,31). 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 (38,92).

Summary and Concluding Remarks

Fatty acids are an important fuel for contracting muscle. After lipolysis, fatty acids from adipose tissue have to be transported through the blood to the muscle. Fatty acids derived from circulating TG may also be used as a fuel but are believed to be less important during exercise. In the muscle the IMTG stores may also provide fatty acids for oxidation after stimulation of hormone sensitive lipase. In the muscle cell, fatty acids will be transported by carrier proteins (FABP), and after activation fatty acyl CoA have to cross the mitochondrial membrane through the carnitine palmytoyl transferase system, after which the acyl CoA will be degraded to acetyl CoA for oxidation.

The two steps that are most likely to limit fat oxidation are fatty acid mobilization from adipose tissue and transport of fatty acids into the mitochondria along with mitochondrial density and the muscles capacity to oxidize fatty acids.

In part II of this review we will focus on the interaction between carbohydrate and fat metabolism and the regulation of fatty acid metabolism. Also the effects of training will be discussed.

In part III of this review the effects of various nutritional manipulations will be discussed.

Acknowledgment

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