

Review article

Utilization of lipids during exercise in human subjects:
metabolic and dietary constraintsFred Brouns^{1*} and Ger J. van der Vusse²¹Novartis Nutrition Research Unit, PO Box 1350, NL-6201 BJ Maastricht and Department of Human Biology, Nutrition Toxicology and Environment Research Institute, Maastricht, The Netherlands²Department of Physiology, Cardiovascular Research Institute, Maastricht, Maastricht University, Maastricht, The Netherlands

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During endurance exercise, skeletal muscle relies mainly on both carbohydrate (CHO) and fat oxidation to cover energy needs. Numerous scientific studies have shown that increasing the exercise intensity leads to a progressive utilization of CHO. The latter will induce a state of glycogen depletion which is generally recognized as being a limiting factor for the continuation of strenuous exercise. Different dietary interventions have been proposed to overcome this limitation. A high-CHO diet during periods of intense training and competition, as well as CHO intake during exercise, are known to maintain a high rate of CHO oxidation and to delay fatigue. However, it has been recognized also that enhancing fatty acid (FA) oxidation during exercise induces a reduced rate of glycogen degradation, resulting in an improved endurance capacity. This is most strikingly observed as a result of frequent endurance exercise which improves a number of factors known to govern the FA flux and the oxidative capacity of skeletal muscle. Such factors are: (1) blood flow and capillarization; (2) lipolysis of triacylglycerol (TAG) in adipose tissue and circulating TAG and transport of FA from blood plasma to the sarcoplasm; (3) availability and rate of hydrolysis of intramuscular TAG; (4) activation of the FA and transport across the mitochondrial membrane; (5) the activity of enzymes in the oxidative pathway; (6) hormonal adaptations, i.e. sensitivity to catecholamines and insulin. The observation that the plasma FA concentration is an important factor in determining the rate of FA oxidation, and that some dietary factors may influence the rate of FA supply to muscle as well as to the mitochondria, has led to a number of dietary interventions with the ultimate goal to enhance FA oxidation and endurance performance. It appears that experimental data are not unequivocal that dietary interventions, such as a high-fat diet, medium-chain TAG-fat emulsions and caffeine intake during exercise, as well as L-carnitine supplementation, do significantly enhance FA oxidation during exercise. So far, only regular endurance exercise can be classified as successful in achieving adaptations which enhance FA mobilization and oxidation.

Fat intake: Exercise: Medium-chain triacylglycerols: Caffeine: L-Carnitine

During physical exercise, skeletal muscle can rely on both fat and carbohydrate (CHO) oxidation to fulfil the need for chemical energy. However, fat as an energy source has advantages over CHO in that the energy density is higher (37.5 kJ/g v. 16.9 kJ/g), causing the relative weight of an amount of energy at storage to be lower. CHO stored as glycogen binds approximately 2 g water/g glycogen stored (Holloszy, 1990). This means that changes in muscle glycogen content cause substantial volume effects. As a result,

the storage capacity of glycogen in muscle and liver is limited, being approximately 450 g glycogen in a healthy, untrained male.

Fat can be stored in much higher amounts; in a healthy, untrained male up to approximately 10 kg fat is stored in adipose tissue, whereas intramuscular fat storage is relatively small. Muscle may contain approximately 400 g fat, of which the major part is stored as lipid droplets in the myocytes (Hoppeler *et al.* 1973; Björkman, 1986).

Abbreviations: CHO, carbohydrate; FA, fatty acids; FABP, fatty acid-binding protein; LPL, lipoprotein lipase; MCT, medium-chain triacylglycerols; TAG, triacylglycerol; $V_{O_2,max}$, maximum O_2 uptake.

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Under resting conditions and during low-intensive exercise, fatty acid (FA) oxidation contributes considerably to total energy provision. With increasing exercise intensity, however, there is a shift to more pronounced CHO utilization. The relatively low amount of CHO stored in the body poses a limitation to the ability to maintain a high power output during prolonged endurance exercise. Thus, athletes seek measures which will induce a greater utilization of fat as a fuel during exercise, in favour of reducing CHO utilization and, hence, improving endurance capacity. The present review will describe the mechanisms and regulatory factors involved in the utilization of fat as an energy source during physical activity, as well as the adaptations that occur as a result of training and dietary intervention.

Fatty acid supply to muscle

Both FA stored in adipose tissue and fat entering the circulation after a meal can serve as potential energy sources for the muscle cell. Moreover, small but physiologically important amounts of FA are stored as triacylglycerols (TAG) inside the muscle cells.

FA liberated from TAG stored in adipocytes are released into the blood, where they are bound to albumin. Each albumin particle has eight binding sites for FA (Spector *et al.* 1971). The human blood albumin concentration is approximately 6 mmol/l, while the FA concentration is approximately 0.2–1.0 mmol/l. This shows that the albumin transport capacity is in excess of the FA actually bound under physiological circumstances and as such will not be a limiting factor for FA oxidation by muscle.

FA can also be derived from the TAG core of circulating chylomicrons and VLDL, both of which are formed from dietary fat in the post-absorptive state. Chylomicrons are formed in the epithelial wall of the intestine. They reach the bloodstream after passage through the lymphatic system. VLDL are synthesized in the liver after which they are released directly into the bloodstream.

During perfusion of the muscle capillaries, FA bound to albumin or stored in the core of chylomicrons and VLDL have to be released before transport across the vascular membrane. In the case of VLDL and chylomicrons this is achieved by the action of the enzyme lipoprotein lipase (*EC* 3.1.1.34; LPL). LPL is synthesized within the muscle cell. After activation by metabolic stimuli, the enzymes are translocated from an intracellular pool to the vascular endothelial cell membrane where they exert their enzymic action on TAG in the core of circulating lipoproteins (Camps *et al.* 1990). LPL activity is up-regulated by catecholamines and adrenocorticotrophic hormone, and down-regulated by insulin (Lithell & Broberg, 1978; Górski & Stankiewicz-Choroszuca, 1982; Kiens & Lithell, 1989; Kiens *et al.* 1989). Both heparin (often used in clinical studies to enhance lipolytic activity in blood plasma) and caffeine stimulate LPL activity (Heaf *et al.* 1977; Braun *et al.* 1992).

LPL additionally expresses phospholipase A_2 (*EC* 3.1.1.4) activity (Groot *et al.* 1979) needed for the degradation of phospholipids, which make up the surface

lipid layer of chylomicrons and VLDL. After TAG hydrolysis, most of the FA will be taken up by muscle, whereas glycerol will be taken away with the bloodstream to pass on to the liver where it may serve as a gluconeogenic substrate. Slow-twitch muscle fibres are particularly rich in LPL, in contrast to fast-twitch fibres, which have a minor content of LPL (Linder *et al.* 1976; Oscai *et al.* 1982; Oscai, 1983).

During the post-absorptive state, the concentration of circulating TAG in plasma is usually higher than that of FA, in contrast to the fasting state when chylomicrons are practically absent in the circulation (Terjung *et al.* 1983). Nevertheless, the quantitative contribution of circulating TAG to FA oxidation by the exercising muscle cells in human subjects is uncertain. Due to technical limitations, no realistic information is available to indicate whether FA derived from the TAG core of VLDL or chylomicrons substantially contribute to overall FA utilization. It should be kept in mind, however, that even a small extraction (of the order of 2–3%) of FA from TAG can account for > 50% of total exogenous FA uptake and subsequent oxidation (Havel *et al.* 1967; van der Vusse *et al.* 1992).

Fatty acid uptake by muscle

It is generally accepted that the arterial FA concentration strongly affects FA uptake into muscle at rest and during low-intensity exercise (Armstrong *et al.* 1961; Hagenfeldt & Wahren, 1971). This implies a FA gradient from blood to muscle under these conditions (van der Vusse & Roemen, 1995), which is achieved by a relatively rapid conversion of free FA, taken up by the muscle cell, to fatty acyl-CoA. The rate of the latter reaction step is controlled by fatty acyl CoA synthase (*EC* 2.3.1.86; Groot *et al.* 1976).

During transport of FA from blood to muscle several barriers have to be passed. Each of these barriers may theoretically limit FA uptake and subsequent oxidation by muscle. The following barriers have to be considered:

- (1) the membranes of the vascular wall (endothelium);
- (2) the interstitial space between the endothelium and muscle cell;
- (3) the membrane of the muscle cell;
- (4) cytoplasm of the muscle cell;
- (5) mitochondrial membrane.

Uptake by endothelial cells is most probably protein-mediated. Both albumin-binding protein and membrane-associated FA-binding proteins (FABP) may play a role. After uptake, most FA will diffuse from the luminal to the abluminal membrane of the endothelial cells as free molecules (van der Vusse & Reneman, 1996). Model studies (Bassingthwaite *et al.* 1989; van der Vusse & Reneman, 1996) predict that FA have to cross the endothelial cytoplasm to reach the abluminal side of the endothelial cell. Because FABP is present in the endothelial cytoplasmic space only in minor quantities, their role in cytoplasmic FA transport is assumed to be unimportant (Linssen *et al.* 1990).

On entering the interstitial space, the FA will be bound to albumin for transport to the muscle cell membrane

(sarcolemma; van der Vusse *et al.* 1992; van der Vusse & Roemen, 1995). At the sarcolemma the FA are released and taken over by sarcolemmal FA-transporting proteins (Harmon *et al.* 1992), or will cross the membrane by a direct flip-flop exchange mechanism, because of the lipophilic characteristics.

After appearance in the sarcoplasm, FABP is crucial for FA transport to the mitochondria. This FABP-mediated transport is not assumed to be limiting for FA uptake in muscle because of the relatively high FABP content in muscle (Vork *et al.* 1993). Finally, the acyl chain of the FA moiety has to be transported across the mitochondrial inner membrane by a carnitine-mediated mechanism (van der Vusse & Reneman, 1996).

Intramuscular fat store

As indicated earlier, an alternative source of FA are TAG present inside the skeletal-muscle cells. For the storage of FA, glycerol is obtained from glycolysis (as glycerol-3-phosphate) then reacts with fatty acyl-CoA, after which further condensation to and storage as TAG take place in small fat droplets, mainly located in the proximity of the mitochondrial system (Hoppeler *et al.* 1973). It has been suggested that adipocytes, positioned between muscle cells, may also supply FA for oxidation, but the significance of this has never been quantified (van der Vusse & Reneman, 1996).

Release of FA from muscle TAG is achieved by the action of muscle lipase (*EC* 3.1.1.3) which is partly under hormonal control. Noradrenaline infusion has been observed to cause a significant reduction in the muscle content of TAG (Fröberg *et al.* 1975), whereas insulin counteracts this effect (Abumrad *et al.* 1980). Apart from hormonal stimuli there is also a local muscular control, shown by the observation that electrical stimulation of muscle enhances TAG hydrolysis (Fröberg, 1969; Barclay & Stainsby, 1972; Spriet *et al.* 1986; Côté *et al.* 1988).

Slow-twitch muscle fibres have the highest muscle lipase activity (Górski & Stankiewicz-Choroszuca, 1982; Górski, 1992), as well as the highest TAG content, compared with fast twitch fibres (Essén, 1977; Essén *et al.* 1977). Endurance exercise has been shown to deplete muscle TAG significantly (Fröberg & Mossfeldt, 1971; Essén, 1977; Essén *et al.* 1977; Lithell *et al.* 1979; Brouns *et al.* 1989; Staron *et al.* 1989). However, determinations were done using muscle biopsy samples, which generally have the disadvantage of a large sample variation. More recently, the use of stable-isotope techniques or direct measurement by NMR (Boesch *et al.* 1997) has been proposed to overcome these problems. The use of stable-isotopes allows for the calculation of the contribution of blood-borne FA to total fat oxidation. Assuming that all FA taken up by muscle are oxidized and that the remainder comes from fat stored within the muscle cells, it is possible to calculate intramuscular TAG utilization (Romijn *et al.* 1993).

Interestingly, the content of TAG stored within the myocyte is increased by regular endurance training (Morgan *et al.* 1969; Hoppeler *et al.* 1973; Howald *et al.*

1985). These and other training adaptations are described later in the present review in more detail (see pp. 120–121).

Fatty acid oxidation by muscle and possible limitations

In the resting muscle cell a relatively high percentage of the overall energy production stems from FA oxidation (Bülow, 1988; Gollnick & Saltin, 1988). This high contribution is either maintained or becomes slightly reduced during light aerobic exercise (Saltin *et al.* 1986; Gollnick & Saltin, 1988). However, with high exercise intensities there will be a more pronounced shift from fat as the energy source to CHO, particularly at intensities above 70–80% maximum O₂ uptake ($V_{O_{2,max}}$; Gollnick, 1985; Terjung & Kaciuba-Uscilko, 1986; Abernethy *et al.* 1990). This points to the fact that there are limitations to the increase in FA oxidation rate in order to replenish sufficient ATP to meet requirements. Several theoretical explanations have been given for this exercise-induced shift from fat to CHO:

- (1) an increase in circulating catecholamines stimulates both glycogen breakdown (primarily in the liver (Wendling *et al.* 1996)) and lipolysis. However, an increased rate of glycogen degradation and glycolysis also enhances lactate formation, which will counter-effect catecholamine-induced lipolysis (Ahlborg & Felig, 1982; Bonen *et al.* 1985; Ahlborg *et al.* 1986; McDermott *et al.* 1987, 1991; Mazzeo & Marshall, 1989). The net result will be a decrease in plasma FA concentration and, hence, the supply of FA to muscle cells. As a consequence, enhanced CHO oxidation will most probably compensate for the reduced FA oxidation;
- (2) the lower ATP production rate per unit time from fat compared with CHO, as well as the fact that more O₂ is needed for the production of a particular amount of ATP from fat compared with CHO (McGilvery *et al.* 1975; Hultman & Harris, 1988);
- (3) limitations in the FA flux from blood to mitochondria. As indicated earlier, this flux is the final result of blood FA concentration, capillary density, transport capacity across vascular membranes and muscle cell membranes, mitochondrial density and mitochondrial capacity to take up and oxidize FA. Mitochondrial FA oxidation rate depends on the actual capacity of the carnitine transport system. The capacity of this system to transport long-chain fatty acids has recently been described to be regulated by malonyl-CoA (McGarry *et al.* 1983; Saggerson *et al.* 1992). This substance is a potent inhibitor of carnitine palmitoyltransferase I (*EC* 2.3.1.21), an enzyme catalyzing the first committed step in mitochondrial FA uptake. During exercise, malonyl-CoA formation is reduced and, therefore, the capacity to transport FA across the mitochondrial inner membrane is enhanced (Winder *et al.* 1989). Theoretically, there may be a mechanism by which CHO and fat metabolism interact via the carnitine transport system during exercise. As workload is increased, indicated by lactate accumulation, the percentage of carnitine in the acetylated form appears to increase

from approximately 9 to 60–67 (Hiatt *et al.* 1989). This is possibly a result of an imbalance in net activities of pyruvate dehydrogenase (*EC* 1.2.4.1) and citrate synthase (*EC* 4.1.3.28). As such, it is theoretically possible that the decrease in the percentage of free carnitine, from 77–90 at rest to 30–37 during exercise (Harris *et al.* 1987; Hiatt *et al.* 1989), negatively influences the carnitine palmitoyltransferase reaction, and, hence, the transport of fatty acyl moieties across the mitochondrial inner membrane and subsequent β -oxidation.

It follows, therefore, that the oxidation rate of FA is mainly the mutual result of three processes: (1) lipolysis of TAG in adipose tissue and circulating TAG and transport of FA from blood plasma to the sarcoplasm; (2) availability and rate of hydrolysis of intramuscular TAG; (3) activation of the FA and transport capacity across the mitochondrial membrane. Furthermore, processes 1 and 2 may primarily pose the limitations to fat oxidation observed during maximum FA flux. This is most evident during both short-term intense exercise or during the initial phase of long-term exercise. In this situation, lipolysis in adipose tissue and in muscle TAG is insufficiently up-regulated to result in enhanced FA supply. The result will be that the rate of FA oxidation exceeds the rate at which FA are mobilized, leading to a fall in plasma FA and intracellular FA in muscle. As a consequence, the use of CHO from glycogen must be increased to cover the increased energy demand (Lithell *et al.* 1979; for review, see Newsholme, 1988a,b).

The extent to which limitations in FA transport and oxidation must be compensated by an enhanced capacity to utilize CHO also becomes clear when the capacity to oxidize FA is analysed in different muscle fibres. There is a clear functional relationship between fibre type, microstructure, substrate stores and CHO or FA oxidation capacity. Slow-twitch muscle fibres have a relatively high degree of capillarization, a high FABP content, a high mitochondrial density and a high muscle lipase and intracellular TAG content which are associated with a high FA oxidation capacity. Fast-twitch muscle fibres, on the other hand, are low in all these factors, i.e. are extremely limited in their ability to oxidize FA. These fibres, therefore, must rely primarily on CHO as their exercise fuel.

Interventions to enhance fatty acid oxidation

As pointed out earlier there is a progressive shift to the use of CHO oxidation with increasing exercise intensity. This has its origin in stronger metabolic and hormonal responses which induce an enhanced glycogen breakdown and lactate formation, as well as in a progressively increased recruitment of fast-twitch muscle fibres, which generally lack the capacity to oxidize substantial amounts of FA.

Since the storage of CHO in the form of glycogen is limited, the ability to perform high-intensity exercise will be decreased with progressive glycogen depletion (Brouns, 1997). Any adaptation leading to an increased capacity to use FA for ATP resynthesis will lead to a sparing of endogenous CHO, with the consequence that endurance

capacity may be improved. Theoretically, there may be a number of intervention possibilities to increase plasma FA levels and to improve the mechanisms involved in transport and oxidation of FA. Most of these interventions have been studied over the last three decades:

- (1) training;
- (2) medium-chain TAG (MCT) feedings;
- (3) oral fat emulsions and fat infusions;
- (4) caffeine;
- (5) L-carnitine supply;
- (6) high-fat diet.

The second part of the present review will primarily focus on these interventions.

Intervention possibilities to increase fatty acid oxidation

Physical training

Endurance training has been observed to result in a number of structural and metabolic adaptations which will favour FA oxidation. Whereas α -adrenergic mechanisms regulate lipolysis at rest, β -adrenergic activity has been found to determine lipolysis during exercise (Arner *et al.* 1990). The sensitivity of β -adrenoceptors for catecholamines in the adipocyte will increase as a result of exercise (Wahrenberg *et al.* 1987). Sensitivity may be further enhanced as a result of adaptation to regular training. This will theoretically promote the delivery of FA from the fat cells to the blood. However, recently it was shown by Romijn *et al.* (1993) that the rate of appearance of FA from adipose tissue is decreased in the trained individual.

The capillary density of muscle tissue will increase with training, which in itself augments the exchange surface area, promotes blood flow and with it the delivery of O₂ and FA (Gollnick & Saltin, 1982, 1988). Training also induces an increase in sarcolemmal FABP, which contributes to the translocation of FA into muscle (Kiens *et al.* 1997). Within the muscle cell there will be an increased mitochondrial volume as well as mitochondrial enzyme activity (Gollnick *et al.* 1971; Morgan *et al.* 1971; Baldwin *et al.* 1972; Gollnick & Saltin, 1982; Hoppeler *et al.* 1985).

Recently, the effect of training on enzymes involved in skeletal-muscle lipid metabolism has been extensively reviewed by van der Vusse & Reneman (1996). Trained muscles express higher activities of LPL, muscle lipase, fatty acyl-CoA synthase and reductase (*EC* 1.2.1.41), carnitine acyl-transferase and 3-hydroxyacyl-CoA dehydrogenase (*EC* 1.1.1.35), which will be in favour of enhancing FA supply to the mitochondria, and subsequent oxidation (Nikkilä *et al.* 1978; Gollnick & Saltin, 1982; Kiens & Lithell, 1989; Kiens *et al.* 1989, 1993; Saltin & Åstrand, 1993). As a result, trained muscles are able to oxidize more substrate (Gollnick & Saltin, 1988) which is also expressed in an increased O₂ consumption at maximal exercise intensities (Gollnick *et al.* 1971; Morgan *et al.* 1971).

Last, trained muscles store more intracellular fat in lipid droplets which are located along the surface of the mitochondrial system, which may theoretically enhance the capacity to supply and oxidize FA derived from the intracellular lipid store (Morgan *et al.* 1969, 1971;

Hoppeler *et al.* 1973; Hoppeler *et al.* 1985; Howald *et al.* 1985; Kiens *et al.* 1993).

Increased intracellular TAG storage as well as observations from arterio-venous difference and isotope-labelling experiments indicate that highly-trained endurance athletes rely more on the utilization of intramuscular stored FA during exercise and less on the utilization of blood-borne FA (Havel *et al.* 1967; Hurley *et al.* 1986; Jansson & Kaijser, 1987; Martin *et al.* 1993). The advantage of a shift from extracellular to intracellular stores of FA is that some potential barriers in overall FA utilization, such as the endothelium and the sarcolemma, are irrelevant when intracellular TAG is utilized.

Thus, training enhances total FA oxidation, especially by increasing intramuscular fat storage and by increasing the maximal FA flux. In addition, endogenous CHO stores will be conserved during exercise in the endurance-trained individual, which prolongs the time period during which intense exercise can be performed.

Medium-chain triacylglycerol ingestion

MCT contain FA with a chain length of six, eight or ten C atoms. Generally, MCT are rapidly emptied from the stomach and taken up by the intestine (Beckers *et al.* 1992). After absorption by the enterocyte, MCT are transported in the blood to the liver, in contrast to long-chain TAG which are transported by the lymphatic system to the vena cava. MCT readily increase plasma medium-chain FA and TAG levels. In muscle, medium-chain FA are rapidly taken up by the mitochondria, not requiring the carnitine transport system. Consequently, MCT are oxidized faster and to a greater extent than long-chain TAG (Geser *et al.* 1974). This has led to the assumption that MCT may be an effective exogenous fuel for exercising muscle and that MCT ingestion may potentially enhance fat oxidation and thereby reduce CHO utilization.

Early studies have indicated that oral MCT, taken shortly before exercise, is only partly oxidized during exercise. In a study by Ivy *et al.* (1980) 30–60 g MCT were ingested with a cereal meal 1 h before exercise. However, most probably because of the relatively low oxidation of the oral MCT, no differences in CHO oxidation were found. In two other studies there was a substantial oxidation of the ingested MCT (Décombaz *et al.* 1983; Massicotte *et al.* 1992). However, in these studies the amounts of MCT ingested were relatively small. Unfortunately, the effect of MCT feedings on performance was not measured in any of these studies.

More recently, several stable-isotope studies have been performed to evaluate the effect of MCT or MCT + CHO ingestion on exogenous, endogenous and total fat and CHO oxidation. These studies have shown that oral MCT are rapidly oxidized by muscle, but do not lead to glycogen sparing in active muscle cells as measured from muscle biopsy samples (Jeukendrup *et al.* 1995, 1996a,b). The fact that total fat oxidation remained the same after MCT ingestion, even in a glycogen-depleted state (Jeukendrup *et al.* 1996a), points to the fact that oral MCT most probably competes with long-chain FA and, hence, leads to a sparing of endogenous fat stores, probably intramuscular fat. This may

also explain why no endogenous CHO sparing occurred. Also, in the studies of Jeukendrup *et al.* (1995, 1996a,b), relatively small amounts of MCT were supplied to the athletes. The reason for this low amount of MCT was that ingestion of > 30 g in a short period of time induces nausea and gastrointestinal discomfort. It may be speculated that this may be caused by a relatively high cholecystokinin release after MCT intake (Douglas *et al.* 1990).

In a recent study by van Zyl *et al.* (1996), however, subjects ingested 86 g MCT during submaximal endurance exercise lasting 2 h, followed by a 40 km time trial; ingestion was as a drink containing (g/l) 43 MCT, 100 CHO + 43 MCT or 100 CHO as the control. Interestingly, they observed the poorest performance with ingestion of MCT alone, but a significantly improved performance with CHO + MCT compared with the CHO alone. No mention was made of any gastrointestinal discomfort. The authors did not measure muscle glycogen, but speculated on the basis of a reduced endogenous CHO oxidation that glycogen may have been spared and that this might explain the performance benefit observed. These findings are in contrast with the previously mentioned observations by Jeukendrup *et al.* (1996b), who observed no endogenous CHO or glycogen sparing. This has prompted Jeukendrup and co-workers (Jeukendrup *et al.* 1998) to perform a similar experiment in which the subjects ingested 85 g MCT as MCT drink, CHO + MCT drink or for control a placebo drink, during a 2 h endurance exercise at an intensity of 60% $V_{O_{2max}}$, followed by a 15 min time-trial. In this particular study, the performance test was not compounded by any physiological measurement. In contrast to the study of van Zyl *et al.* (1996), performance was not improved by the MCT + CHO treatments. A substantial number of subjects experienced gastrointestinal problems with MCT ingestion. The reason for the discrepancy in the data from these studies remains unclear. Thus, from the available data it can not be concluded that MCT ingestion is of benefit for glycogen sparing and/or improving endurance performance.

Oral fat and fat infusions

Another attempt to improve fat oxidation has been to enhance the blood long-chain FA levels by infusing lipid emulsions. This procedure has been shown to result in a significant reduction in glycogen degradation in two studies (Jansson & Kaijser, 1984; Vukovich *et al.* 1993). In line with the positive effects of fat infusion on muscle glycogen sparing, the opposite (a decline in plasma FA, induced by inhibiting lipolysis by nicotinic acid) resulted in an increased rate of muscle glycogen degradation (Bergström *et al.* 1969). An elevated level of circulating FA is thus a prerequisite for reducing the rate of endogenous CHO utilization during exercise. However, for sports practice this procedure seems to be impractical. Infusions during competition are not possible, and even if they were, they would be forbidden by the International Olympic Committee doping regulations which consider any artificial measure to enhance performance as unethical.

Oral intake of fat emulsions, also, may not be of benefit. Oral fat may inhibit the gastric emptying rate of rehydration

solutions also ingested during exercise and may lead to gastrointestinal discomfort (Brouns, 1991). Additionally, it will take a considerable time before the absorbed long-chain TAG will be available for oxidation because of passage through the lymphatic system. To our knowledge, there are currently no studies which have shown convincingly any benefit of fat ingestion shortly before or during exercise.

Caffeine

Caffeine is known to affect muscle, adipose and central nervous tissue indirectly by mediating the level of cAMP and its related Ca release from the intracellular storage sites (Leijten & van Breeman, 1984). This effect is initiated by binding of catecholamines to β -receptors of cell membranes, thereby enhancing the activity of the enzyme adenylate cyclase (EC 4.6.1.1) which catalyzes the formation of cAMP from ATP. Caffeine has been observed to enhance plasma noradrenaline (Collomp *et al.* 1991) and adrenaline levels (Berkowitz & Spector, 1971; Collomp *et al.* 1990, 1991; Graham & Spriet, 1991, 1995; Spriet *et al.* 1992). Additionally, caffeine inhibits phosphodiesterase (EC 3.1.4.1) which degrades cAMP to the non-active compound 3'5'-AMP. In this way, caffeine increases cAMP half-life and, thus, lipolysis (Beavo *et al.* 1970; Fredholm, 1980). By these actions caffeine increases the cAMP level, which maximizes the activity of the intra-adipocyte lipase and, hence, lipolysis (Zhang & Wells, 1990).

However, the notion that caffeine affects lipolysis via adrenaline has also been challenged. Chesley *et al.* (1995) infused adrenaline to a level comparable with the physiological levels after caffeine ingestion and did not observe any effect on plasma FA. Nevertheless, caffeine has been observed to enhance plasma FA in many studies in human subjects and animals (Bellet *et al.* 1965, 1968; Costill *et al.* 1978; Ivy *et al.* 1979; Acheson *et al.* 1980; Essig *et al.* 1980; Knapik *et al.* 1983; Powers *et al.* 1983; Casal & Leon, 1985; Sasaki *et al.* 1987; Arogyasami *et al.* 1989; Tarnopolsky *et al.* 1989; Dodd *et al.* 1991; Doubt & Hsieh, 1991; Spriet *et al.* 1992; Graham & Spriet, 1995). In contrast, an increased fat oxidation (by assessment of the respiratory exchange ratio) and reduced glycogen degradation were observed in only a few of these studies (Costill *et al.* 1977, 1978; Ivy *et al.* 1979; Acheson *et al.* 1980). This may indicate that the caffeine-induced elevation of FA simply occurs in addition to the relatively high exercise-induced increase in FA, which most probably already maximizes FA transport across the epithelium. These findings also indicate that the performance-enhancing effects of caffeine (Powers *et al.* 1983; Collomp *et al.* 1990, 1991; Graham & Spriet, 1991, 1995; Anselme *et al.* 1992; Pasma *et al.* 1995) are most probably related to effects on the central nervous system rather than to effects on fat oxidation and glycogen sparing. Interestingly it has recently been shown that caffeine decreases malonyl-CoA in skeletal muscle (MacLean & Winder, 1995), which may further explain why caffeine induces an increased FA oxidation when ingested under resting conditions, but not during exercise when malonyl-CoA levels in muscle cells are already appreciably reduced, among others, by down-regulation of the plasma insulin level.

There are reasons to suggest that caffeine ingestion may also indirectly counteract its effect on lipolysis and subsequent FA oxidation during exercise. Increased liver glycogen breakdown and plasma lactate levels have been observed after caffeine ingestion (Richter *et al.* 1984; Gaesser & Rich, 1985; Issekutz, 1985; Sonne & Galbo, 1985; Winder, 1985; Sasaki *et al.* 1987; Collomp *et al.* 1990, 1991; Anselme *et al.* 1992) and lactate is known to be a strong inhibitor of lipolysis (Green *et al.* 1979). Thus, it cannot be excluded that caffeine might also exert depressing effects on FA oxidation in exercising muscle cells.

L-Carnitine

In man, carnitine is obtained from the diet, particularly from red meat. In addition, carnitine is synthesized in the body from intracellular trimethyllysine which requires methionine for the methylation process. This biosynthetic process occurs mainly in liver and to a lesser extent in kidney and brain (Hoppel & Davis, 1986), after which L-carnitine is released into the circulation, then taken up by muscle. L-Carnitine is lost from the body daily in small amounts via urine and stool. The primary function of L-carnitine is the transfer of long-chain FA across the mitochondrial membrane (Fritz, 1968), to enter the oxidation pathway.

Addition of L-carnitine to the incubation medium has been shown to markedly enhance the long-chain FA oxidation of isolated mitochondria (Fritz, 1968). This has led to the speculative assumption that oral L-carnitine intake should lead to enhanced fat oxidation in athletes or in people wanting to lose weight. However, there is no solid scientific evidence that this is the case, despite the enormous number of positive performance claims made in advertisements for this nutritional aid, as under normal conditions tissue carnitine levels are relatively high and do not form a constraint on FA oxidation.

Oral L-carnitine has been observed to increase the plasma L-carnitine level, while uptake in muscle remained unchanged (Soop *et al.* 1988). This observation fits well with the finding that L-carnitine is taken up against a concentration gradient (plasma 40–60 μmol , muscle 3–4 mmol ; Engel & Rebouche, 1984). This gradient is so large that even a substantial oral intake would not result in a measurable change in this situation. As a result of increased plasma levels and unchanged muscle uptake, urinary carnitine excretion increases manyfold (Wagenmakers, 1991).

In addition, there are no indications that heavy exercise results in a substantial loss of carnitine from muscle cells. No differences in resting carnitine levels have been observed between training and non-training individuals (Janssen *et al.* 1989). These findings, as well as those of other well-controlled recent studies (Trappe *et al.* 1994; Vukovich *et al.* 1994a,b; Maassen *et al.* 1995), failed to show an effect of L-carnitine supplementation on FA oxidation in muscle during exercise (for comprehensive review, see Wagenmakers, 1991).

High-fat diet

High-fat diets are claimed to enhance the capacity to oxidize FA and have attained considerable interest as a potential tool to improve performance in endurance athletes. In rats, a high-fat diet has been observed to increase LPL activity significantly, compared with animals fed on a high-CHO diet (Pratt, 1989). However, this observation has to be interpreted with caution and may be explained by a strong up-regulation of LPL activity with the combination of high fat–low CHO used in one group and a down-regulation in the other group, receiving high CHO–low fat. Thus, most probably, such a striking difference may not appear when a high-fat diet is compared with a normal mixed diet.

An increased LPL activity as well as an increased deposition of intracellular fat in muscle may explain a greater availability of FA to the mitochondria after a high-fat diet and also may explain the lower respiratory exchange ratio (Bergström *et al.* 1967; Jansson & Kaijser, 1982; Hurley *et al.* 1986; Storlien *et al.* 1991). In rats, a high-fat diet also induced an improved performance (Miller *et al.* 1984). However, there may be significant species differences in FA handling. As such, human studies are of critical importance in order to draw any conclusions.

Johannessen *et al.* (1981) studied seven male subjects who ingested a high-fat diet in either solid or liquid form (76 % energy from fat) during 4 d, or a high-CHO diet (76 % energy from CHO). This dietary regimen was followed by a run endurance test until exhaustion. The running test consisted of alternating blocks of 30 min running followed by 10 min rest. Performance was significantly reduced by approximately 40 % after this short-term high-fat diet. Jansson & Kaijser (1982) investigated the effect of a high-fat diet for 5 d (69 % energy as fat) followed by 5 d on a high-CHO diet (75 % energy as CHO) on muscle substrate utilization in twenty subjects. FA utilization was estimated by measuring arterio–venous differences and by measurement of substrate concentrations in muscle biopsy samples. Although they observed a lower respiratory exchange ratio after the high-fat diet and an increased FA extraction by muscle, there was no consistent effect on muscle glycogen utilization. The study included both males and females, was not randomized in treatment order and the diet duration was very short. No performance measures were taken. Phinney *et al.* (1983) studied five cyclists who had to perform an endurance capacity test until exhaustion after a high-fat diet for 4 weeks. The authors claimed that the high-fat diet caused a significant improvement in performance. However, the individual performance data show that only two of five cyclists improved their performance, one of these two by 57 %! Two cyclists showed a decreased performance and one cyclist remained at the same level. That the overall result was positive was largely the result of the single subject who showed the rather unrealistic 57 % performance increase after 1 month on a high-fat diet. Furthermore, no cross-over design was used in this study. Lambert *et al.* (1994) studied five well-trained cyclists for a period of 14 d who ingested either a high-fat diet (67 % energy as fat) or a high-CHO diet (74 % energy as CHO). The high-fat diet led to a

reduction in the muscle glycogen content of approximately 50 % (121 (SE 4) and 68 (SE 4) mmol/kg wet weight for high-CHO and high-fat treatments respectively). In a high-intensity cycling test to exhaustion (85 % $V_{O_{2max}}$) there were no statistically significant differences between the treatments, although the mean values were quite different in terms of athletic performance times (8.3 (SE 2.3) and 12.5 (SE 3.8) min for high-fat and high-CHO diets respectively). During a low-intensity performance trial, which followed the high-intensity trial after a rest period of 20 min, time to exhaustion was significantly prolonged. However, despite the fact that exhaustion occurred, the heart rate observed was only 142 (SE 7) beats/min in the high-fat diet and 143 (SE 8) beats/min with the high-CHO diet, compared with a heart rate of > 180 beats/min in the high-intensity trial. The fact that the preload (the high-intensity test) was not standardized, and that heart-rate response does not reflect the stress of exercise-induced exhaustion, points to the possibility that variables other than the difference in the diet alone, e.g. motivational, may have influenced the performance results. The very large difference in time to exhaustion in the low-intensity (50 % peak power output) trial (79.7 (SE 7.6) min v. 42.5 (SE 6.8) min for high-fat and high-CHO diets respectively) further underlines this suggestion. It can be questioned whether such a large performance difference can be caused only by 14 d on a high-fat diet.

Muioio *et al.* (1994) tested runners on a treadmill after a 3-week diet intervention and observed a significant increase in time to exhaustion from 76 to 91 min while running at an intensity of 75–85 % $V_{O_{2max}}$. Moreover, the 'high fat diet' consisted of 50 % energy as CHO and 38 % energy as fat, which is comparable with a normal mixed diet consumed by many athletes. Thus, since there was no genuine high-fat diet and no change in fat oxidation was observed, it is unclear whether this performance capacity improvement is the result of fat in the diet.

The most recent studies are those of Helge *et al.* (1996) who studied the effect of combined training and diet on performance progression in twenty untrained subjects, divided into two groups of ten. These subjects performed endurance training for a period of 7 weeks, three to four times per week, while ingesting diets containing either 65 % energy as CHO or 62 % energy as fat. This period was followed by another training period of 1 week, while ingesting the CHO-rich diet alone. The results showed that $V_{O_{2max}}$ increased by 11 % in both diet groups. Performance progression, however, was significantly better with the high-CHO diet (from 35.2 (SE 4.5) min to 102.4 (SE 5.0) min with the high-CHO diet and from 35.7 (SE 3.8) min to 65.2 (SE 7.2) min with the high-fat diet. After the final week on the CHO diet, the performance improvement in the previously CHO-treated groups was maintained, while the group previously receiving the fat-rich diet further improved their endurance performance (from 65.2 (SE 7.2) min to 76.7 (SE 8.7) min. However, this was still below the achieved performance of the CHO-diet group, i.e. 103.6 (SE 7.2) min. Heart rate and noradrenaline levels were highest while on the high-fat diet. These results indicate that a high-fat diet is detrimental with respect to training and performance progression at the beginning of an

endurance training programme. This was the case despite the observation that the high-fat diet resulted in a 25 % increase in 3-hydroxyacyl-CoA dehydrogenase, one of the key enzymes in FA oxidation, in the high-fat diet group compared with no change in the CHO-diet group. It should be emphasized, however, that these findings do not allow for a generalization towards highly-trained individuals.

Recently, Van Zyl and co-workers (CG Van Zyl, K Murphy, JA Hawley, J Goedecke, TD Noakes, SC Dennis, unpublished results) studied five endurance-trained cyclists who ingested in random order either a high-fat diet (65 % energy as fat) or a habitual diet (29 % energy as fat) for 10 d followed by a high-CHO diet (65 % energy as CHO) for 3 d. These subjects then performed a 150 min ride at 70 % $V_{O_{2,max}}$, followed by a 20 km time trial, during which a 100 g CHO + 43 g MCT/l solution was ingested. As a result of this combined dietary treatment, the authors observed a significantly improved performance. Time-trial performance was improved by 80 s ($P < 0.05$) after the 7 d high-fat-3 d high-CHO regimen.

To the best of our knowledge no other human studies on the effect of high-fat diets are available at this moment. Seen against the bulk of the evidence that CHO ingestion improves endurance performance tasks, it remains speculative to state that a high-fat diet, which down-regulates CHO metabolism as well as decreases glycogen stores in muscle and liver, may lead to better results. The fact that high-fat diets are unpalatable restricts most attempts to study its effects in human subjects to a duration of several weeks at maximum. On the one hand, this may be too short-term to achieve measurable adaptation effects. On the other hand, long-term trials may result in adverse health effects on the cardiovascular system, especially in less-well-trained subjects, due to overexposure of lipids to the body. Interestingly, in the available human fat-diet performance studies, no systematic measurements of changes in lipoproteins were undertaken. Recently, Leddy *et al.* (1997) reported data on twelve male and thirteen female runners, divided in subgroups, who raised daily fat intake from 16 to either 30 or 40 % energy as fat for 4 weeks. This increase in fat was not associated with changes in LDL-cholesterol, apolipoprotein B or apolipoprotein A1: apolipoprotein B, but raised HDL-cholesterol. This study indicates that changing from a high-CHO diet to a diet that has a fat content comparable with that of many sedentary individuals, is not related with negative side effects for well-trained athletes. Since most high-fat diets tested and sometimes recommended to athletes have a substantial higher fat content, i.e. 50–65 % energy as fat, additional studies are required to evaluate the possible effects on cardiovascular risk factors.

The above-mentioned findings by Van Zyl and co-workers point to the fact that a combination of a short-term high fat diet followed by a high-CHO diet may improve endurance performance. However, more studies with a greater number of subjects need to be done before any well-founded recommendations on this type of nutritional regimen can be made. Interesting in this context are the observations made by Helge & Kiens (1997) that ingesting a high-fat diet for 7 weeks, irrespective of training, increases 3-hydroxyacyl-CoA-dehydrogenase activity in

muscle, and that this effect is not observed in subjects following the same training programme but ingesting a CHO-rich diet. This suggests that diet per se can influence endurance-exercise-induced adaptations in muscle.

Conclusion

Although a number of intervention possibilities to enhance FA oxidation during exercise, with the goal to improve endurance capacity, have been studied, it appears that so far only regular endurance training can be classified as being successful in this respect. Although some very recent data obtained after following a combined dietary intervention (CHO-rich diet → short-term high-fat diet → high-CHO diet → competition) show improvements in performance during low-intensity exercise, the bulk of evidence points to the fact that high-intensity exercise performance is best achieved after being on a diet which is relatively high in CHO and low in fat.

Statements that L-carnitine, caffeine, MCT feedings, oral TAG feedings and high-fat diets may improve endurance performance of endurance athletes during high-intensity events cannot at present be supported by consistent and solid scientific evidence.

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