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REGULATION OF FATTY ACID OXIDATION IN SKELETAL MUSCLE

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ABSTRACT

Researchers using animals are beginning to elucidate the control of fatty acid metabolism in muscle at the molecular and enzymatic level. This review examines the physiological data that has been collected from human subjects in the context of the proposed control mechanisms. A number of factors, including the availability of free fatty acids and the abundance of fatty acid transporters, may influence the rate of muscle fatty acid oxidation. However, the predominant point of control appears to be the rate at which fatty acyl–coenzyme A is transported into the mitochondria by the carnitine palmitoyl transferase system. In turn, evidence suggests that the intracellular concentration of malonyl–coenzyme A in muscle is an important regulator of carnitine palmitoyl transferase–I activity. Malonyl–coenzyme A is increased by glucose, which is likely the mechanism whereby glucose intake suppresses the transfer of fatty acids into the mitochondria for subsequent oxidation. In contrast, malonyl–coenzyme A levels decrease during exercise, which enables increased fatty acid oxidation. However, for any given carnitine palmitoyl transferase–I activity, there may be an effect of free fatty acid availability on fatty acid oxidation, particularly at low levels of free fatty acids. Nonetheless, the rate of glucose or glycogen metabolism is probably the primary regulator of the balance between glucose and fatty acid oxidation in muscle.

CONTENTS

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INTRODUCTION

Fat, stored as triglyceride (TG) in adipose tissue and mobilized in the form of plasma free fatty acids (FFAs), is the major fuel reserve and fuel source in the human body. Regulation of fat metabolism is related to the development of cardiovascular disease, insulin resistance, diabetes, nutrition, and obesity. For example, it has been recognized for many years that alterations in fat metabolism play a role in the development of cardiovascular disease (13, 14), and recent evidence suggests that increased muscle TG stores contribute to the development of insulin resistance (12). Thus, the normal control of fatty acid metabolism is not only central to the production of energy via substrate metabolism, it is also essential for good health. Skeletal muscle is a major site of fatty acid oxidation, yet the control of fatty acid oxidation in human skeletal muscle is not completely characterized. Researchers using animal models are beginning to elucidate the control of fatty acid metabolism in muscle at the molecular and enzymatic level, and it is the purpose of this review to examine the physiological data that has been collected from human subjects in the context of the proposed control mechanisms.

PATHWAY OF FATTY ACID OXIDATION

TG is the predominant lipid ingested by humans. After entering the stomach, TGs are acted on by specific lipases that release three FFAs and one glycerol molecule. FFAs readily aggregate to form micelles until they are taken up individually by the enterocytes. The FFAs inside the enterocytes are reesterified into TGs and packaged with proteins and phospholipids to form chylomicrons. Chylomicrons enter the lymphatic system and eventually the circulatory system at the thoracic duct. The chylomicrons are acted on by lipoprotein lipase, an enzyme attached to the endothelial cells lining the capillaries primarily in adipose tissue, skeletal and cardiac muscle, and the liver. FFAs are then liberated and taken up into these specific tissues. In adipose tissue, the FFAs are primarily

reesterified and stored as TGs; in other organs, such as muscle and liver, small amounts of TG are stored intracellularly. The primary sites of FFA oxidation are cardiac and skeletal muscle and liver. The liver oxidizes FFA to help fuel its various metabolic activities, with the resultant formation of ketone bodies that can be oxidized by brain and muscle when glucose is limiting. Uptake of FFA by tissues for subsequent oxidation occurs via the plasma membrane, and on entering the cell, the FFAs are esterified to coenzyme (CoA) via fatty acyl–CoA synthetase. The resulting fatty acyl–CoA is then transported through the inner membrane of the mitochondrion. This transport is mediated via carnitine and the enzyme carnitine palmitoyltransferase I (CPT-I). Once inside the mitochondrial matrix, the fatty acyl–CoA is acted on by the enzymes in the β-oxidative pathway, resulting in acetyl-CoA production. Energy produced in the β-oxidation of fatty acids and the oxidation of acetyl–CoA in the tricarboxylic acid cycle is used to generate ATP via oxidative phosphorylation.

Plasma FFAs are the major endogenous substrates within the human body. Hormone-sensitive lipase (HSL) liberates FFAs from TG stored in adipocytes; the FFAs are released into the circulation and carried to tissues in need of energy. Because in some circumstances availability of FFAs influences their uptake and oxidation within skeletal muscle, regulation of the release of FFAs (lipolysis) is pertinent to the topic of fatty acid oxidation in muscle.

REGULATION OF FATTY ACID AVAILABILITY

Fatty acids are derived primarily from two sources, endogenous TG stores located mainly in adipose tissue and exogenous TGs from foods. After a fatty meal is consumed, TGs in the form of chylomicrons and very-low-density lipoproteins (VLDL), produced from fatty acids in the liver, may be delivered either to adipose tissue for storage or to metabolically active tissues for oxidation. Exogenous forms of fatty acids can exist in various forms, but endogenous FFAs can enter the plasma only from adipose tissue. Therefore, in the postabsorptive state, the regulation of adipose tissue lipolysis is the major factor determining the circulating concentration of FFAs.

Factors Maintaining Fatty Acid Availability

FFA concentrations are the result of the balance between the rate of appearance (from lipolysis) and the rate of clearance. The principal factor regulating the rate of adipose tissue lipolysis is the activity of HSL in the adipocyte. HSL activity is regulated primarily by the circulating concentrations of insulin and epinephrine. These hormones can change during times of stress, such as starvation, trauma, or physical exercise, or after feeding, with the result being an alteration in lipolytic rates and FFA concentrations.

Regulation of Adipose Tissue Lipolysis

The rate-limiting enzyme in adipose tissue lipolysis is HSL. HSL hydrolyzes two fatty acids from the glycerol backbone, with the third being hydrolyzed by a monoglycerol lipase. The three FFA molecules then leave the adipose cell and enter the circulation, where nearly all of them bind to albumin. The remaining glycerol backbone also leaves the cell because adipose tissue does not contain the enzyme glycerol kinase (9); thus, glycerol is not reutilized for reesterification in adipocytes. Rather, glucose uptake and glycolysis to glycerol 3-phosphate is required for production of TGs in adipocytes. Because glycerol kinase is not present in adipose tissue, the rate of appearance (R_a) of glycerol is a direct indicator of adipose tissue lipolysis (81). Hence, the isotopic determination of the glycerol R_a has been used to quantify lipolytic rates in human subjects in response to several variables.

HSL is regulated via phosphorylation/dephosphorylation. For example, when epinephrine binds to its β-adrenergic receptor in the plasma membrane, it activates the membrane-bound enzyme adenylate cyclase, with the result being an increase in cAMP levels in the adipocyte. cAMP directly activates cAMP-dependent protein kinase, which phosphorylates and activates HSL. On the other hand, insulin inhibits HSL by catalyzing dephosphorylation of the enzyme. Glucagon also acts through the adenylate cyclase mechanism described above, but there is no evidence that it exhibits these effects in vivo (38).

Glucose is the predominant signal for insulin release, so an effect of glucose intake on lipolysis would be expected. The quantitative relation between glucose concentration and lipolysis was studied in healthy volunteers infused with glucose at one of three rates (84). The results showed that glycerol R_a and FFA R_a were depressed during glucose infusion at rates in excess of 1 mg kg^{-1} min^{-1}, which caused only slight increases in glucose and insulin concentration. Further increases in glucose and insulin caused more complete suppression of lipolysis. In addition, glucose caused the release of FFAs to be suppressed more than the release of glucose, indicating that reesterification of fatty acids within adipocytes was accelerated. From these data we can conclude that in humans, an increase in glucose concentration in the plasma stimulates insulin release, with concomitant increases in glucose uptake and fatty acid reesterification, and decreases in lipolysis. The inhibitory effect of glucose on lipolysis is mediated entirely by insulin (38).

Epinephrine is the primary stimulator of lipolysis, working through a β-adrenergic receptor in the plasma membrane of the adipose cell (propranolol, a β-adrenergic receptor blocker, decreases the rate of lipolysis) (31). The physiological state can affect the sensitivity to epinephrine. For example, in short-term (3 day) fasting, the lipolytic responsiveness to epinephrine infusion is enhanced (85), whereas in obesity the responsiveness to epinephrine is blunted (85).

Under most physiological circumstances, the rate of lipolysis is largely determined by the balance between the stimulatory effect of epinephrine and the inhibitory effect of insulin. For example, in short-term fasting there is a marked increase in the rate of lipolysis, which can be attributed to both a decrease in blood glucose (and thus insulin) concentration (30) and an increase in epinephrine concentration (31) and sensitivity to epinephrine (85). The striking aspect of the regulation of lipolysis is that the factors that are primary regulators (insulin and epinephrine) are not released in response to a signal related to any aspect of fatty acid metabolism. For example, acute changes in fatty acid concentrations by infusion do not affect either insulin or catecholamines or the rate of lipolysis. It could therefore be considered that insulin and catecholamines, rather than being primary regulators of lipolysis, are regulators of other physiological and metabolic processes that also affect lipolysis. From this perspective it follows that lipolysis is not regulated in relation to the requirement for fatty acids as energy substrates, but rather that fatty acid oxidation is largely determined by factors at the site of oxidation. In fact, the rate of resting lipolysis generally provides fatty acids at a rate that is far in excess of the rate required for oxidation. Fatty acids that are released from adipocytes, but not oxidized, are reesterified, thereby completing a substrate cycle.

Triglyceride-Fatty Acid Substrate Cycle

A substrate cycle exists when opposing, nonequilibrium reactions, catalyzed by different enzymes, are active simultaneously (39). These cycles require energy and produce heat but do not result in any increase in the net flux of product. The potential importance of substrate cycles in determining the flexibility and sensitivity of metabolic regulation has been emphasized (39). Glucose, phosphofructose, and phosphoenolpyruvate cycles (38, 56) and a TG–fatty acid cycle (83, 84) exist in humans and are under hormonal control (84). The net result of substrate cycling is thermogenesis and it has been argued that this is their only function. On the other hand, Newsholme & Crabtree (39) have proposed that the amplification of enzymatic control can occur.

The TG–fatty acid cycle in humans was first demonstrated in 1987 (84). This cycle consists of two types of recycling. Intracellular recycling includes lipolysis and reesterification within the adipocyte. Extracellular recycling includes intracellular recycling, but it also represents transport of FFAs to tissues for oxidation (e.g. muscle) or reesterification into TG (e.g. in liver), release of those TG to the plasma (primarily in VLDL-TG), and "reuptake" of fatty acids from circulating TG by the adipocytes. Fatty acid cycling enables rapid changes in plasma FFA oxidation in the absence of any change in lipolysis. For example, at rest, approximately 70% of FFAs released into the blood are recycled back into TGs rather than being oxidized (83). At the onset of exercise, these "extra"

FFAs can be used to supply energy to muscle. The percentage of released FFAs that is reesterified drops to 20% or less immediately at the start of exercise (83). Thus, even though lipolysis responds sluggishly to changes in the need for FFAs as energy substrates at the onset of exercise, rapid changes in FFA oxidation can occur because of the high extent of TG-FFA cycling at rest (51). Implicit in this relationship between availability and oxidation is the notion that the rate of fatty acid oxidation is regulated at the tissue level.

REGULATION OF FATTY ACID OXIDATION IN SKELETAL MUSCLE

Sites of Regulation in the Fatty Acid Pathway

When plasma FFAs enter the myocyte, the first important step in their oxidation is the formation of fatty acyl–CoA. The enzymatic regulation of CPT-I will then determine whether the fatty acyl–CoAs will be transferred into the mitochondria for subsequent oxidation. Once inside the mitochondria, the fatty acyl–CoAs are then acted on by the enzymes of the β-oxidation pathway to generate ATP. This sequence of events has three potential sites of regulation: (*a*) transport of fatty acids into the cell; (*b*) enzymatic regulation, primarily of CPT-I; and (*c*) enzymatic regulation of β-oxidation in the mitochondria.

Fatty Acid Transporters

The general assumption concerning fatty acid uptake by muscle is that the process occurs via passive diffusion, as FFAs are hydrophobic molecules and should have no problem diffusing through a hydrophobic plasma membrane. Others have argued that at physiological pH the fatty acids bound to albumin may exist in an ionized form, which may hinder their transport through the plasma membrane. Recently, researchers identified a family of plasma membrane proteins that bind FFAs in several different tissues, including skeletal muscle (7, 26, 69). These proteins are identified as plasma membrane fatty acid binding protein ($FABP_{PM}$), fatty acid translocase, and fatty acid transport protein. Although there is a positive correlation between total plasma fatty acid concentration and total fatty acid turnover, recent evidence from perfused rat skeletal muscle (66, 67) and exercising human muscle (68) reveals that unbound fatty acid uptake is a saturable process. This suggests that FFA uptake by the myocyte may involve a carrier-mediated process. For example, prior protease treatment of cells reduces FFA uptake (63), whereas conditions associated with an increased utilization of FFAs, such as fasting in rats (69) and endurance training in humans (68), increase the content of $FABP_{PM}$. In addition, there is a greater content of $FABP_{PM}$ in red oxidative skeletal muscle than in white glycolytic muscle (69). Recent evidence from giant sarcolemmal vesicles has

shown that fatty acid translocase and fatty acid transport protein are more abundant in red than in white skeletal muscles (7) and that maximal transport of fatty acids into red vesicles was 1.8-fold greater than in white vesicles. This study provides the first evidence of a fatty acid transport system in skeletal muscle because blocking FFA uptake with a variety of inhibitors was demonstrated.

It was thought that once inside the muscle cell, passive diffusion of FFAs controlled distribution, but a family of cytoplasmic fatty acid binding proteins ($FABP_C$) has been identified. This led others to speculate that the maximal diffusion capacity of FFAs will be increased approximately 17-fold by the concentration of $FABP_C$ seen in oxidative muscle fibers (72) and that $FABP_C$ may play a role in the transfer of FFAs to intracellular destinations where it is used. Others have suggested a functional relationship between large amounts of $FABP_C$ seen in oxidative muscle compared with the small content in glycolytic muscle (70).

It is thus likely that fatty acid transporters and binding proteins do exist in human skeletal muscle, but their physiological relevance has yet to be determined. Correlational evidence that oxidative fibers contain large amounts of these proteins and that alterations in protein content varies during conditions of elevated FFA oxidation may imply physiological functions. On the other hand, there is considerable circumstantial evidence that under normal resting conditions, transporters play no regulatory role. For example, as pointed out above, the rate of muscle uptake and oxidation of FFAs increases severalfold at the immediate onset of exercise, a response that would be impossible if transport had been rate limiting in the resting state. Also, acutely increasing fatty acid concentration causes a corresponding increase in fatty acid uptake in a variety of physiological settings without necessarily increasing fatty acid oxidation (e.g. 24, 50). Thus, it is more likely that fatty acid oxidation is regulated by factors inside the cell than by limitations in transport.

Enzymatic Regulation

The rate-limiting enzyme in fatty acid synthesis in liver is acetyl-CoA carboxylase (ACC), the enzyme responsible for converting acetyl-CoA to malonyl-CoA, the first intermediate in the formation of long-chain fatty acids. Inactivation of ACC occurs via phosporylation catalyzed by a variety of kinases in liver. These include AMP-activated protein kinase (AMPK), cyclic-AMP dependent protein kinase, and protein kinase C (18). Phosphorylation and hence inactivation of ACC leads to a decrease in the concentration of malonyl-CoA, a potent inhibitor of CPT-I. Thus, inhibition of long-chain fatty acid entry into the mitochondria is relieved and fatty acid oxidation increased (18, 35). Malonyl-CoA was first measured in skeletal muscle in 1983 (35). Initially, it was surprising to detect malonyl-CoA (the first committed product in fatty acid biosynthesis)

in a nonlipogenic tissue and to find that CPT-I was much more sensitive to malonyl-CoA in skeletal muscle than in liver (35). Skeletal muscle contains little, if any, fatty acid synthase, so it was unclear whether there was any function for skeletal muscle malonyl-CoA. Because malonyl-CoA could regulate the uptake of long-chain fatty acids into the mitochondria, it was suggested that malonyl-CoA played a role in regulating the rate of fatty acid oxidation in muscle (35, 74).

The role of malonyl CoA in regulating muscle fatty acid oxidation has been assessed in rats exercised on a motorized treadmill. Malonyl-CoA decreases during exercise (74), decreases more so in oxidative muscle fibers (75), and decreases as a function of exercise intensity (47). In addition, fasting also decreases skeletal muscle malonyl-CoA content (78). Two primary mechanisms can alter malonyl-CoA levels in skeletal muscle. The first is allosteric activation of ACC, and the second is covalent modification of ACC. Allosteric modulation occurs when a particular molecule noncovalently binds to the enzyme ACC, causing either an increase or a decrease in activity levels. For example, incubation of rat skeletal muscle with glucose or acetoacetate increases malonyl-CoA levels (54, 55). This is due to the increase in glucose uptake causing an increase in cytosolic acetyl-CoA and citrate (55). Citrate is the most potent activator of skeletal muscle ACC (65), and acetyl-CoA is the primary substrate for ACC. Covalent modification of skeletal muscle ACC consists of phosphorylation or dephosphorylation by kinases and phosphatases, respectively. Recently, Winder et al (79) showed that although both AMPK and cAMP-dependent protein kinase can phosphorylate ACC in vitro, only AMPK decreases ACC activity, which is consistent with their prior observation that muscle malonyl-CoA decreases in adrenodemedullated rats running on a treadmill (76). AMPK activity is increased when muscle contracts (22, 46, 47, 71, 77), and this may be due to an increase in free AMP concentrations within the myocyte, which may activate an AMPK kinase, and/or to the increase in sarcoplasmic calcium, related to contraction, which may activate calcium-dependent protein kinases. In either case, if AMPK activity increases, ACC activity will decrease, and malonyl-CoA content will decrease. AMPK has been referred to as the fuel gauge of the mammalian cell (18) because it is activated when the cell is stressed (i.e. during ATP depletion) and phosphorylates a variety of enzymes to counteract the stress.

The malonyl-CoA concentration required to inhibit CPT-1 by 50% is 2.7 ± 0.4 μM for liver and 0.034 ± 0.006 μM for skeletal muscle (35). Thus, skeletal muscle CPT-I is much more sensitive to malonyl-CoA, but the dilemma in interpreting this data is that even with this enhanced sensitivity, the concentration of malonyl-CoA at its lowest point following fasting or exercise is still high enough to cause complete inhibition of CPT-I (35). Obviously, this does

not occur. The muscle relies extensively on fatty acid oxidation during those circumstances. Hence, most of the malonyl-CoA in skeletal muscle cells may be contained within the mitochondria or may be bound to proteins in the cytosol. In any event, there is in inverse relationship between total malonyl-CoA content and fatty acid oxidation in rat muscle (36). AICAR (5-amino-4-imidazole carboxamide ribonucleoside), is taken up by the muscle cell and phosphorylated to form ZMP (an analog of 5′-AMP), increases AMPK activity, decreases ACC activity, decreases malonyl-CoA content, and increases fatty acid oxidation in a perfused rat hindlimb (36). The effect of AICAR on fatty acid oxidation increases as a function of palmitate concentration (37). Therefore, in rat skeletal muscle it appears that malonyl-CoA concentration is related to the rate of fatty acid oxidation. In fed animals at rest, malonyl-CoA content is elevated because glucose and insulin are elevated, and fatty acid oxidation is low (54). During fasting, malonyl-CoA decreases because glucose concentration falls, and fatty acid oxidation increases with increases in FFA concentrations. These relationships are shown schematically in Figures 1*a* and *b*. The following sequence of events during exercise has recently been postulated: (*a*) muscle contracts, (*b*) AMP content increases, (*c*) AMPK kinase activity increases, (*d*) AMPK activity increases, (*e*) ACC activity decreases, (*f*) malonyl-CoA decreases, and (*g*) fatty acid oxidation increases (22, 36, 47, 77). This is outlined in Figure 2.

Two studies have examined human skeletal muscle malonyl-CoA content and both found there was no change during exercise (40, 41). These studies reported human malonyl-CoA values to be one fourth to one seventh of those for rat muscle malonyl-CoA. The reason for the discrepancy between human and rat muscle has yet to be determined, but it may be a methodological problem, as different techniques were used to determine malonyl-CoA content. The human studies did not measure enzyme activities. This same group also recently measured CPT-I activity in isolated intact mitochondria from human skeletal muscle (3). They observed that total CPT-I activity was correlated with mitochondrial content of muscle and thus maximal oxygen consumption. Unfortunately, acute regulation of CPT-I is more difficult to analyze in vivo because most activators or inhibitors are lost during isolation. An alternative approach is to assess functional CPT activity in vivo (58–60). This involves comparison of the rates of oxidation of long-chain and medium-chain fatty acids. Long-chain, but not medium-chain, fatty acids require the CPT-I system for transport into the mitochondria. The relation between long-chain and medium-chain fatty acid oxidation therefore can be used as an index of the function activity of CPT-I. For example, oleate, but not octanoate, oxidation is inhibited during glucose infusion and during high-intensity exercise in humans; this indicates a decrease in functional CPT-I activity under these conditions (58).

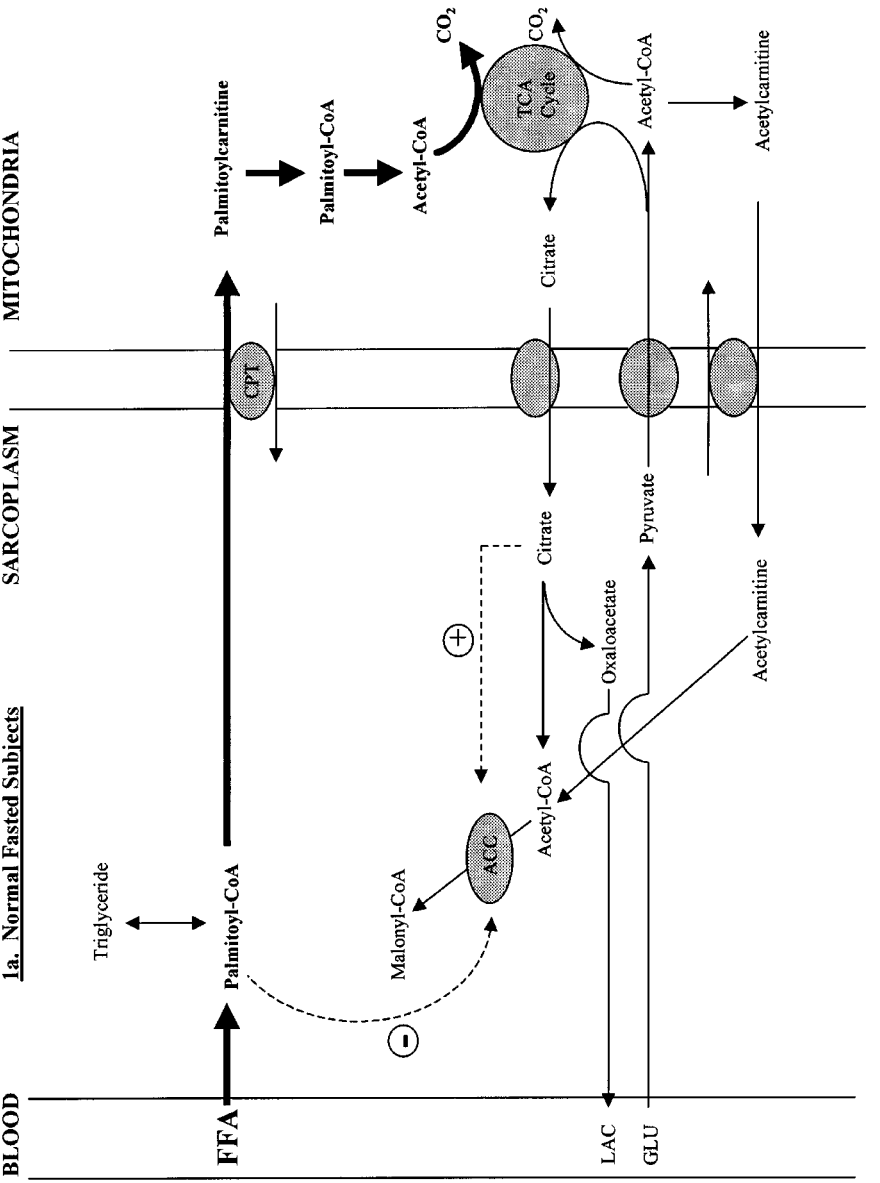

Figure 1 (*Continued*)

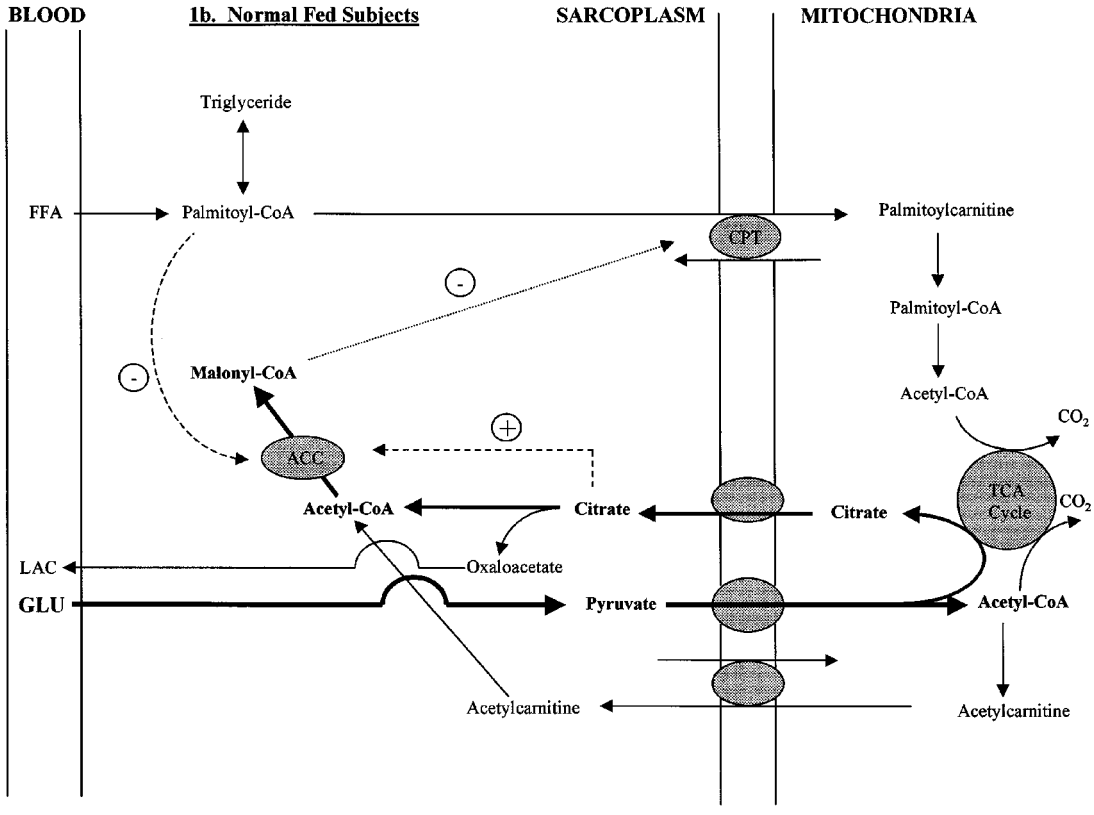

Figure 1 Proposed regulation of muscle fatty acid oxidation. (*a*) In the fasted state, palmitoyl-coenzyme (CoA) is transported into the mitochondria via the carnitine palmitoyltransferase (CPT) enzyme system for subsequent oxidation. (*b*) In the fed state, glucose metabolism causes citrate to increase in the mitochondria, which ultimately causes malonyl-CoA to increase, thereby inhibiting CPT activity and thus the transport of palmitoyl-CoA into the mitochondria. In addition, concentration of free fatty acids (FFAs) is decreased by the insulin response in the fed state, so there is less FFA available for potential transport. ACC, acetyl-CoA carboxylase; TCA, tricarboxylic acid.

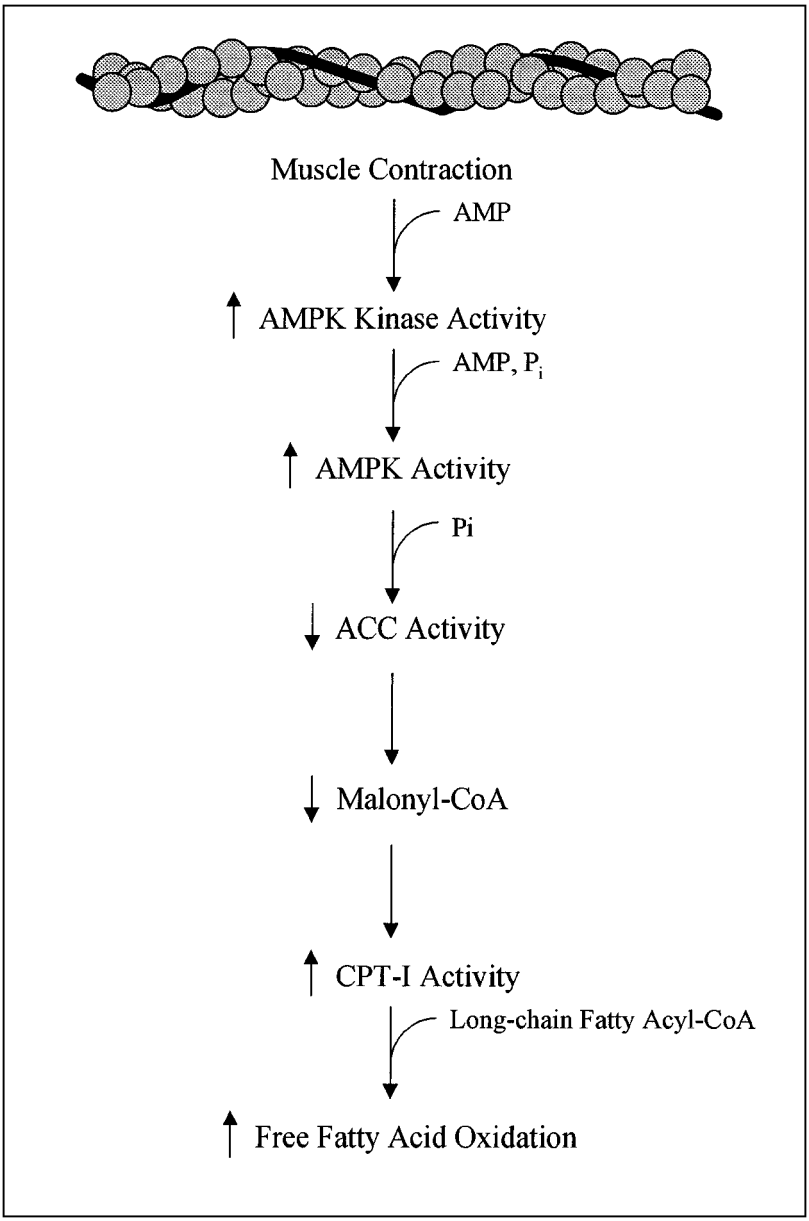

Figure 2 Proposed sequence of events causing muscle fatty acid oxidation to increase during exercise. AMPK, AMP-activated protein kinase; P_i, inorganic phosphate; ACC, acetyl-CoA carboxylase; CPT-1, carnitine palmitoyltransferase-I.

Intramuscular Triglyceride Pool

Skeletal muscle contains significant amounts of intracellular TG (TG_m), and in vivo isotopic studies indicate that intracellular TG utilization during prolonged submaximal exercise may account for 20%–25% of energy expenditure (28, 50). Several studies found decreases in muscle TG content following exercise (15, 21, 43), but others did not find such a decrease (25, 26, 73). A recent study reported that TG_m did not decrease with glycogen-depleting exercise, although TG_m did decrease in the postexercise recovery period (27). These authors suggested that TG_m utilization during the recovery phase provides energy for glycogen resynthesis. Recently, Peters et al (42) found HSL present in greater quantities within oxidative muscle fibers than in glycolytic fibers; they also found that TG_m hydrolysis was increased when rat muscle was incubated with epinephrine. Thus, although it is evident that the TG_m pool may be a significant source of energy, the large variability in muscle biopsy determinations of TG_m (73) makes it difficult to draw conclusions regarding TG_m utilization during exercise or other conditions. It does appear that a TG–fatty acid cycle does exist within skeletal muscle (17), and future isotopic studies designed to directly measure TG_m utilization in vivo are necessary to quantify the significance of the TG_m pool as an energy source.

Effects of Exercise on Muscle Fatty Acid Oxidation

Changes that occur in fat oxidation in the transition from rest to exercise can be attributed almost entirely to muscle metabolism. When evaluating the metabolic response to exercise, it is necessary to consider the duration and intensity of exercise, as well as the training status of the individual (25). Both untrained (83) and well-trained subjects exercising at low-intensity effort (25% VO_{2max}) rely almost entirely on an increase in fat oxidation to supply the necessary energy requirements. Fatty acids used for oxidation are derived primarily from plasma and have been released as a consequence of peripheral lipolysis. During moderate-intensity exercise (65% VO_{2max}), fatty acids from plasma decrease in their relative contribution to total energy expenditure, and the fatty acids derived from intramuscular lipolysis are about equally as important as plasma FFAs (49). Together, plasma and intramuscular fatty acids provide approximately 40% of the energy during exercise at 65% VO_{2max}. During higher-intensity exercise (greater than 80% VO_{2max}), the rate of plasma FFA oxidation is actually decreased below the value at 65% VO_{2max}, and the intramuscular TGs become the major source of fatty acids for oxidation (49). A fall in the rate of appearance of plasma FFAs during high-intensity exercise occurs concomitantly with the decrease in fatty acid oxidation. A limitation in the availability of FFAs may limit the FFA oxidation to some extent during high-intensity exercise, but this apparently is only of minor importance. When plasma FFA concentrations were

increased by intravenous infusions of long-chain triacylglycerols and heparin during exercise at 85% VO_{2max}, fat oxidation was significantly increased by 27% whereas carbohydrate oxidation decreased by 11% (50). However, rates of fat oxidation under these conditions were still suppressed relative to lipid utilization during exercise at moderate intensity, which suggests that factors in addition to plasma FFA concentration attenuate FFA oxidation during high-intensity exercise. The major limitation in FFA oxidation is likely due to a limitation of the transport of fatty acids into the mitochondria via carnitine acyl transferase I (58). Thus, during low-intensity exercise, fat predominates as an energy substrate, whereas during high-intensity exercise, carbohydrate metabolism dominates. Nonetheless, even at 80% VO_{2max}, the total rate of fat oxidation is still markedly elevated over the resting value.

The level of physical conditioning (25) can significantly influence the contribution of lipids to the energy cost of exercise. Endurance training results in physiological adaptations that enhance fuel mobilization and utilization. Studies assessing the effect of training on substrate oxidation during exercise in response to training interventions show an increase in the contribution of total lipids to energy costs after training (21). In untrained individuals, during prolonged submaximal (e.g. 40% VO_{2max}) exercise, changes in the rate of carbohydrate and fat oxidation occur progressively over time. In contrast, trained individuals are able to maintain a relatively stable metabolic state for several hours during low-intensity work (28). Walking for 4 h was fueled primarily by fat oxidation (7.51 ± 0.26 μmol kg^{-1} min^{-1}) in trained subjects exercising at 28% VO_{2max} (28). In untrained subjects working at the same absolute intensity, much of the energy cost was also derived from lipid sources (5.67 ± 0.51 μmol kg^{-1} min^{-1}), although a lesser amount of intramuscular TGs contributed to the fuel mix in these subjects compared with the trained subjects (28).

As mentioned above, in rats running at a submaximal pace on a motorized treadmill, malonyl-CoA decreases in skeletal muscle (74) and decreases earlier in oxidative fibers versus glycolytic fibers (75). The decrease in malonyl-CoA is attenuated when glucose is infused during exercise (11), or with endurance training (23). Malonyl-CoA decreases as a function of exercise intensity (47) and remains depressed during the postexercise recovery period (46). This decrease in malonyl-CoA is associated with an increase in AMPK activity and a decrease in ACC activity (22, 47, 77). It appears that this intracellular signaling cascade is contraction dependent, because nerve stimulation results in the same intracellular changes (10, 22, 71), denervation increases malonyl-CoA content (54), and adrenodemedullated rats running on a treadmill still have a decrease in muscle malonyl-CoA (76).

Two studies examined malonyl-CoA in human skeletal muscle and found no change in malonyl-CoA content with exercise (40, 41). These studies did not

measure enzyme activities, and future studies will need to address methodological concerns, nutritional states of subjects, and potential species differences. Even with the difficulty in detecting changes in human muscle malonyl-CoA, the human studies of fat metabolism during exercise (cited above) correlate well with the enzymatic data from rats.

The rate of FFA oxidation in exercising subjects is elevated during submaximal exercise (49) but eventually begins to diminish with high-intensity exercise. It has been postulated that malonyl-CoA may increase during high-intensity exercise because of the increase in glycolysis and the subsequent increase in acetyl-CoA, although in that same study no change in malonyl-CoA was detected (41). In contrast to that notion, Rasmussen & Winder have shown that malonyl-CoA decreases as a function of exercise intensity, with malonyl-CoA being at its lowest level at the highest intensity of exercise (47). This apparently paradoxical response, relative to observed changes in fatty acid oxidation, can be explained by the fact that covalent modification of ACC by AMPK overrides the allosteric activation of ACC produced by citrate and acetyl-CoA. In vitro, ACC is inactivated when phosphorylated and an increase of acetyl-CoA in the physiologic range does not activate the enzyme (65, 77). Hence, during exercise, signaling mechanisms employing covalent modification of enzymes determine the activity of ACC, and thus malonyl-CoA content. Because malonyl-CoA levels do not increase during high-intensity exercise (see above), other regulatory factors must exert an effect on CPT-I activity. One possibility may be that the accumulation of acetylcarnitine during intense exercise (41) prevents long-chain acyl-CoA from reacting with carnitine to form acylcarnitine.

Interactions Between FFAs and Glucose

The metabolism of fatty acids by muscle is inexorably linked to the metabolism of carbohydrates, because by necessity if the oxidation of fatty acids decreases, the oxidation of glucose or glycogen increases to meet the energy requirements of the cell. However, the nature of the relationship is not certain. The most popular hypothesis, called the glucose–fatty acid, was put forward in 1963 by Randle et al (44), and this explanation of the nature of the relationship between glucose and fatty acids is still favored by many. The central aspect of the hypothesis is that the availability of FFA dictates the rate of fat oxidation in the muscle and that fatty acid oxidation directly inhibits glycogen and glucose metabolism (44).

Over the ensuing years, a number of specific mechanisms have been hypothesized to explain the glucose–fatty acid cycle (45). Thus, it has been proposed that the inhibitory effects of fatty acids on glucose oxidation are due to (*a*) the inhibition of pyruvate dehydrogenase, mediated by an increased ratio of acetyl-CoA to CoA, (*b*) the inhibition of phosphofructokinase, mediated by an increase in

citrate, and (*c*) the inhibition of hexokinase, mediated by glucose-6-phosphate (45).

The glucose–fatty acid cycle provides a potential explanation for substrate interactions in a variety of circumstances. For example, high plasma fatty acid concentrations occur in many insulin-resistant states, such as obesity, type 2 diabetes, or severe trauma and sepsis. The glucose–fatty acid cycle potentially provides a link between high fatty acid concentrations and insulin resistance, because the high fatty acid concentrations should inhibit glucose oxidation and thus uptake by virtue of increased fatty acid oxidation.

The original glucose–fatty acid cycle was based on results from in vitro experiments on rat heart and diaphragm muscle metabolism (44). Several in vitro studies have been done since that time, with conflicting results. Whereas some studies showed an inhibitory effect of fatty acids on glucose oxidation in rat skeletal muscle (48), others found no such effect, (2, 52). Maizels et al (33) proposed that a fatty acid effect on glucose oxidation may occur only in red muscle under some circumstances, such as when the rate of glycolysis is increased. Thus, it appears that under certain circumstances, the glucose–fatty acid cycle functions in specific tissues when assessed in vitro. However, it is not clear how these in vitro results relate to the situation in vivo.

In humans there is no direct support for the mechanisms originally proposed by Randle et al (44) to account for control of glucose metabolism by fatty acids. In studies in which fatty acid concentrations were altered, corresponding changes were not observed in concentrations of either muscle citrate or glucose-6-phosphate (3, 4, 16, 19). Nonetheless, the glucose–fatty acid cycle has received widespread acceptance as an explanation for substrate interactions in humans (1, 6, 64) because, in certain circumstances, elevating the fatty acid concentration increases fatty acid oxidation and decreases glucose oxidation. Most evidence in this regard comes from experiments in which the euglycemic-hyperinsulinemic clamp procedure was used on humans. With this procedure, the effect of fatty acids on glucose oxidation in humans has been assessed by acutely elevating the plasma fatty acid concentration by infusing, in the setting of euglycemia-hyperinsulinemia, a lipid emulsion plus heparin. In this experimental setting, it has generally, but not always, been found that the amount of glucose infusion necessary to maintain euglycemia at any particular insulin concentration is less when the fatty acid concentration is high (64). Furthermore, in this circumstance, glucose oxidation also generally decreases (64), leading to the conclusion that fatty acids inhibit glucose oxidation (i.e. a validation of a central component of the glucose–fatty acid cycle). However, there is no evidence that, once glucose is in the cell, there is any impairment of glucose oxidation by fatty acids. In contrast, the evidence consistently reveals

that FFAs have no affect on the percentage of glucose uptake that is oxidized (64, 80). Rather, when glucose uptake is maintained at a constant high rate, a 10-fold increase in FFA concentration had no affect on glucose oxidation (80).

In contrast to the traditional perspective of the glucose–fatty acid cycle, we suggest that the primary determinant of substrate oxidation in muscle is the availability of glucose, not FFAs (82). Thus, the effect of the acute elevation of glucose availability and oxidation on fatty acid oxidation was tested in the setting of constant fatty acid concentration (60). In this study it was found that increased availability of glucose inhibited fat oxidation, despite the constant availability of fatty acids. This result is contrary to that predicted by the traditional glucose–fatty acid cycle and leads to the conclusion that the intracellular availability of glucose (rather than fatty acids) determines the nature of substrate oxidation in humans.

To assess the mechanism by which glucose inhibits fatty acid oxidation, the hypothesis that glucose, insulin, or both inhibit entrance of fatty acids into the mitochondria was investigated (59). We infusioned [1-^{13}C]oleate, a long-chain fatty acid, and [1-^{14}C]octanoate, a medium-chain fatty acid, at constant rates in the basal state and during a hyperglycemic and hyperinsulinemic state. Enrichment of plasma oleate with [^{13}C]oleate and fatty acid concentration were maintained by infusing Intralipid® and heparin. Oleate, but not octanoate, requires carnitine acyltransferase to gain access to the mitochondrial matrix. During the transition from basal to clamp conditions, oleate oxidation decreased more than 50%, but octanoate oxidation remained unchanged. It was concluded that glucose or insulin directly limits fatty acid oxidation by restricting uptake of long-chain fatty acids into the metabolic pathways in the mitochondria. This conclusion was supported by a decrease in intramuscular acylcarnitine concentration from 843 ± 390 nmol/g (dry wt) in the basal state to 296 ± 77 nmol/g (dry wt) during the clamp procedure (59).

Although the traditional glucose–fatty acid cycle is flawed, it would not have endured so long if it had absolutely no merit. Indeed, in many circumstances, some fatty acid effects on glucose can be shown, even if glucose availability predominates in importance. The modest increase in fatty acid oxidation during exercise when fatty acid concentrations were elevated is a good example. Thus, whereas glucose availability predominates in determining the mix between oxidation of glucose and fatty acids, there is a reciprocal relationship in which fatty acid availability also plays a role, albeit a minor one. This is to be expected, considering that glucose inhibits fatty acid oxidation by limiting their entry into mitochondria by inhibiting carnitine acyltransferase. For any given enzyme activity, within some range of substrate concentration, a greater concentration of

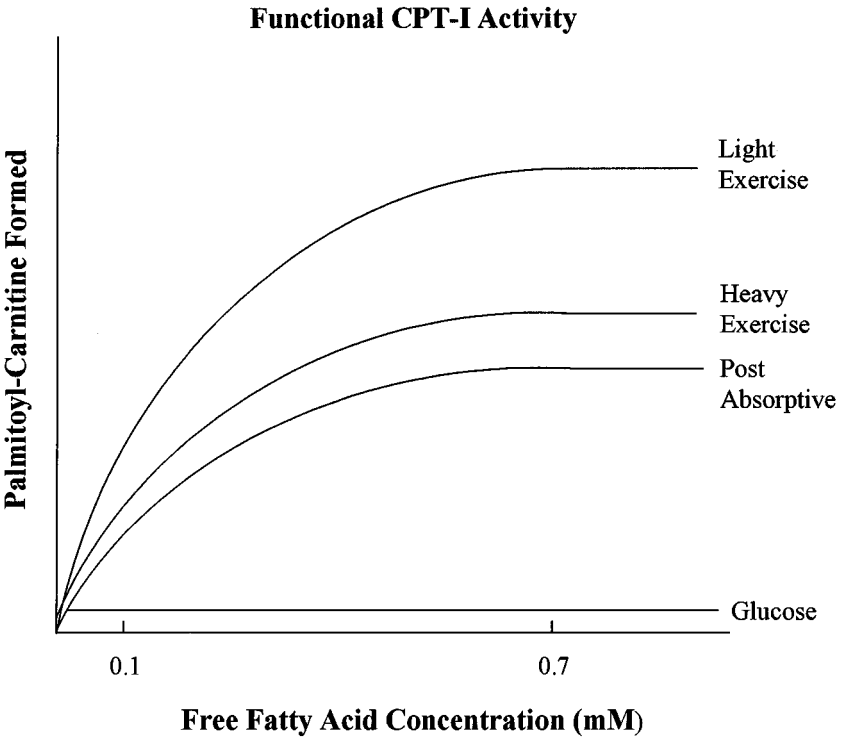

Figure 3 Schematic representation of relation between free fatty acid (FFAs) concentration and the rate of which palmitoyl-carnitine is formed. Unless carnitine palmitoyltransferase (CPT) activity is entirely suppressed (with high-dose glucose), changes in FFA concentration will affect the rate of formation of palmitoyl-carnitine at any given activity of CPT, but the ultimate rate of fatty acid oxidation will predominantly be determined by the fractional activity of CPT-I.

fatty acids will cause more fatty acyl–CoA to be transferred to the mitochondria unless the enzyme activity is completely suppressed. This can be seen in the schematic diagram of functional CPT-I activity shown in Figure 3. Thus, the physiological circumstance determines the V_{max} of the CPT-I enzyme. At any given V_{max}, FFA availability will have an effect on the rate of formation of palmitoyl-carnitine formed and, thus, FFA oxidation, up to a concentration of about 0.7 mM. Increases in plasma FFA concentration above 0.7 mM have no effect on fatty acid oxidation under any circumstance. From this schematic diagram it is clear how glucose can predominate in the control of substrate metabolism, while changes in FFA availability may also affect the rate of FFA oxidation if the glucose availability is not great enough to completely inhibit CPT activity.

CONCLUSION

A number of factors, including the availability of FFAs and the abundance of fatty acid transporters, may influence the rate of muscle fatty acid oxidation. However, the predominant point of control appears to be the rate at which fatty acyl–CoA is transported into the mitochondria by the CPT system. In turn, evidence suggests that the intracellular concentration of malonyl-CoA in muscle is an important regulator of CPT-I activity. Malonyl-CoA is increased by glucose, which is likely the mechanism whereby glucose intake suppresses the transfer of fatty acids into the mitochondria for subsequent oxidation. In contrast, malonyl-CoA levels decrease during exercise, which enables increased fatty acid oxidation. However, for any given CPT-I activity, there may be an effect of FFA availability on fatty acid oxidation, particularly at low levels of FFAs. Nonetheless, in general terms, the rate of glucose or glycogen metabolism is likely to be the primary regulator of the balance between glucose and fatty acid oxidation in muscle.

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