The Role of Glucose in the Regulation of Substrate Interaction During Exercise

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Abstract/Résumé

Glucose and fatty acids are the main energy sources for oxidative metabolism in endurance exercise. Although a reciprocal relationship exists between glucose and fatty acid contribution to energy production for a given metabolic rate, the controlling mechanism remains debatable. Randle et al.'s (1963) glucose-fatty acid cycle hypothesis provides a potential mechanism for regulating substrate interaction during exercise. The cornerstone of this hypothesis is that the rate of lipolysis, and therefore fatty acid availability, controls how glucose and fatty acids contribute to energy production. Increasing fatty acid availability attenuates carbohydrate oxidation during exercise, mainly via sparing intramuscular glycogen. However, there is little evidence for a direct inhibitory effect of fatty acids on glucose oxidation. We found that glucose directly determines the rate of fat oxidation by controlling fatty acid transport into the mitochondria. We propose that the intracellular availability of glucose, rather than fatty acids, regulates substrate interaction during exercise.

Le glucose et les acides gras sont les principales sources d'énergie du métabolisme oxydatif au cours d'un exercice d'endurance. Bien qu'à un régime énergétique donné, la contribution des substrats se fasse selon une relation réciproque, le mécanisme sous-jacent n'est pas encore bien établi. Le "cycle glucose–acide gras," un hypothèse avancée par Randle et collaborateurs (1963), constitue un mécanisme potentiel de la régulation de l'interaction des substrats métaboliques à l'effort. La pierre angulaire de cette hypothèse est que le taux

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de lipolyse et donc, la disponibilité des acides gras, contrôle la contribution respective du glucose et des acides gras dans le métabolisme énergétique. Bien que dans certaines études, on observe qu'une plus grande disponibilité d'acides gras atténue l'oxydation des hydrates de carbone en préservant principalement le glycogène musculaire, il y a peu d'études démontrant une inhibition directe de l'oxydation des sucres de la part des acides gras. Nos travaux nous amènent à proposer une hypothèse opposée à celle véhiculée par Randle et coll. En plus d'une action indirecte sur la disponibilité des acides gras, nous avons observé que le glucose détermine le taux d'oxydation des graisses en contrôlant le transport des acides gras dans la mitochondrie. Nous avançons que la disponibilité intracellulaire de glucose, et non celle des acides gras, contrôle l'interaction des substrats à l'exercice.

Substrate Contribution to Oxidative Metabolism

The present paper will focus on the regulation of substrate interaction during endurance exercise in humans. In this type of exercise, glucose and fatty acids are the two main sources for ATP production. Glucose, utilized for energy production by the muscle, may come from liver glycogen or breakdown of glycogen stored in the same muscle cell where it is oxidized. Fatty acids are supplied to the muscle from lipolysis of triacylglycerols (TG) stored in peripheral fat depots (adipose tissue) or from TG inside or between the muscle cells where they are oxidized.

Without nutritional manipulation, the relative contribution of fatty acids to oxidative metabolism decreases with increasing exercise intensity, whereas the relative contribution of glucose oxidation increases (Romijn et al., 1993). Thus, during low-intensity exercise (i.e., <30% of $\dot{V}O_2$max), plasma-derived fatty acids are the predominate energy source (see Figure 1). In moderate-exercise intensity (i.e., 30–65% of $\dot{V}O_2$max), the relative contribution of glucose from plasma and breakdown of muscle glycogen increases, whereas the relative contribution of fatty acids decreases, even though fatty acid oxidation continues to rise (see Figure 1). At this exercise intensity, fatty acids and glucose participate equally in energy production. During high-intensity exercise (i.e., >65% of $\dot{V}O_2$max), glucose becomes the main fuel for oxidation. Interestingly, although the demand for energy increases in the transition from moderate- to high-intensity exercise, fatty acid availability and oxidation decrease (see Figure 1).

Whereas there is clearly a reciprocal relationship between glucose and fatty acid oxidation for a given metabolic rate, the controlling mechanism of this relationship remains unclear. Over 30 years ago, Randle and coworkers presented data suggesting that the glucose-fatty acid relationship is controlled by fatty acid availability (Randle et al., 1963). The cornerstone of their hypothesis, the "glucose-fatty acid cycle," is that an increase in fatty acid oxidation increases the mitochondrial ratio of [acetyl-CoA:CoA], which suppresses pyruvate dehydrogenase (PDH) directly and phosphofructo 1-kinase and hexokinase activities indirectly via citrate and glucose-6 phosphate (G-6-P) accumulation, respectively (see Figure 2). As a result, glycolysis and glucose transport are inhibited, leading to increased blood glucose concentration (Randle et al., 1963). The hypothesis has recently been extended to include the mechanisms involved in regulating PDH (reversible phosphorylation) and phosphofructo 1-kinase (fructose 2,6-bisphosphate) (Randle et al., 1994). According to this traditional view of the glucose-fatty acid cycle, the balance between glucose and fat oxidation is determined by the intracellular availability of fatty acids.

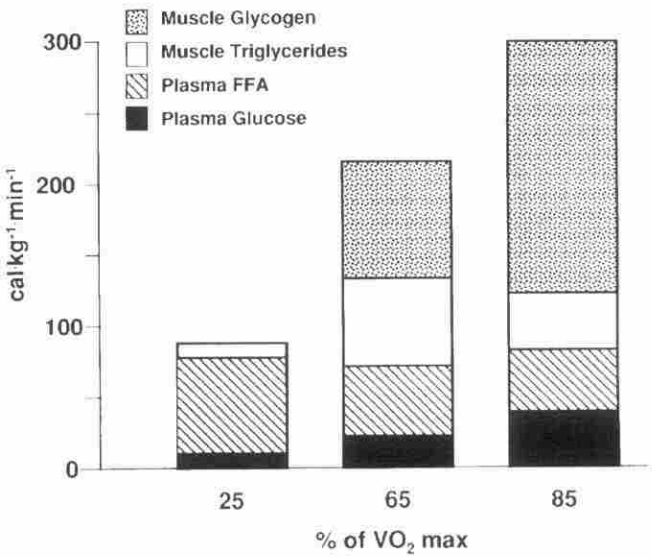

Figure 1. Maximal contribution to energy production derived from glucose and FFA taken from blood and minimal contribution of muscle triacylglycerol and glycogen stores after 30 min of exercise, expressed as a function of exercise intensity. (From "Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration," by J.A. Romijn, E.F. Coyle, L.S. Sidossis, A. Gastaldelli, J.F. Horowitz, E. Endert, and R.R. Wolfe, 1993, American Journal of Physiology, 265(28): E380–E391. Copyright 1993 by the American Physiological Society. Reprinted with permission.)

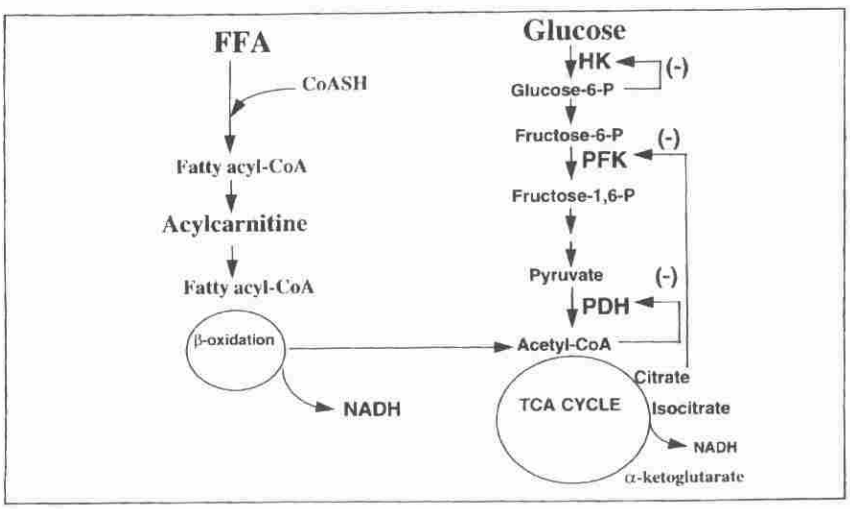

Figure 2. Schematic diagram showing the glucose-fatty acid cycle hypothesis proposed by Randle et al. (1963).

Fatty Acid Regulation of Glucose Oxidation: The Traditional View

The glucose-fatty acid cycle hypothesis was based entirely on results from in vitro experiments in rat heart and diaphragm muscle (Garland and Randle, 1964; Randle et al.,1963, 1964a). Since its introduction, this hypothesis has been extensively tested in vitro (Blackard et al., 1990; Cooper et al., 1975; Hue et al., 1988; Peters and Spriet, 1995; Philpott and Kealey, 1991), using animal (Berger et al., 1976; Jenkins et al.,1988; Lang and Dobrescu, 1992; Zorzano et al.,1985) and humans (Boden et al., 1994; Bonadonna et al., 1994; Ferrannini et al., 1983; Vaag et al., 1994; Wolfe et al., 1988; Yki Jarvinen et al., 1991) at rest, with conflicting results. Some studies (Boden et al., 1994; Bonadonna et al., 1994; Ferrannini et al., 1983) have shown that increasing fatty acid availability inhibits plasma glucose or muscle glycogen utilization, whereas others (Berger et al., 1976; Wolfe et al., 1988) found no such effect. Zorzano et al. (1985) reported an operational cycle in the rat heart but not the soleus muscle. Misinterpreted findings have further confused this area, as some investigators interpret any relationship between glucose and fatty acids as evidence of the glucose-fatty acid cycle hypothesis. In some instances, findings that were contary to the glucose-fatty acid cycle were presented or interpreted as support for this hypothesis. The existence of a relationship between glucose and fatty acids is universally accepted. What remains to be shown is which substrate, glucose or fatty acids, primarily regulates this relationship.

In addition to rest, the glucose-fatty acid cycle hypothesis has been tested during exercise and electrical stimulation. In some (Costill et al., 1977; Putman et al., 1993; Rennie et al., 1976; Romijn et al., 1995) though not all (Berger et al., 1976; Dyck and Spriet, 1994; Hargreaves et al., 1991; Ravussin et al., 1986) studies, increased fatty acid availability under these conditions resulted in decreased plasma glucose or muscle glycogen utilization, or both. In either case, fatty acid availability was raised by fat feeding or lipid and heparin infusion. Interestingly, one of the main differences between these groups of studies is the relative increase in fatty acid concentration. In the studies that showed an effect (Costill et al., 1977; Dyck et al., 1993; Putman et al., 1993; Romijn et al., 1995), fatty acid concentration was raised from 0.2–0.3 to 1.0–1.5 mmol/l, whereas fatty acids were increased from 0.5-0.7 to 1.0-1.5 mmol/l in those where no effect was found (Hargreaves et al., 1991; Ravussin et al., 1986). The modest increase in fat oxidation when fatty acid concentration was increased from 0.2–0.3 to 1.0–1.5 mmol/l may have been due to a significant increase in fatty acyl-CoA, thereby "forcing" its transport into the mitochondria.

However, even in studies where fatty acid availability affected glycogen utilization, the controlling mechanism was not that originally proposed by the glucose-fatty acid cycle hypothesis. Spriet et al. (1992) studied human participants during exercise at 80% of $\dot{V}O_2$max following placebo and caffeine ingestion to increase plasma fatty acid concentration. Muscle glycogen use decreased by 55%, but muscle citrate and acetyl-CoA contents and the acetyl-CoA-to-CoASH ratio were unaffected by caffeine ingestion. Dyck et al. (1993) examined the effect of raising plasma fatty acid concentration (via lipid and heparin infusion) on glucose oxidation during high-intensity exercise. Even though high fatty acid availability reduced muscle glycogen utilization in most (though not all) volunteers, muscle citrate, acetyl-CoA, and PDH were unaffected by this factor. Similar were findings by Putman et al. (1993), who concluded that fatty acid oxidation in the

muscle is unrelated to citrate content during exercise following diet manipulation (i.e., chronic high-fat feeding).

Holloszy and Coyle (1984) proposed that the glucose-fatty acid cycle hypothesis explains the increase in fatty acid oxidation with endurance training. However, Jansson and Kaijser (1987) and more recently Coggan et al. (1993) found that muscle G-6-P concentration was lower during exercise in the trained state, indicating that the posttraining shift in substrate utilization cannot be explained by the glucose-fatty acid cycle concept, as proposed by Randle et al. (1964b).

Glucose Regulation of Fatty Acid Oxidation: The Glucose-Fatty Acid Cycle Reversed

From the above discussion, we can conclude that the glucose-fatty acid relationship is not controlled by fatty acid availability. Thus, we logically propose the reverse of the traditional glucose-fatty acid cycle (i.e., that the availability of glucose, rather than fatty acids, controls the rate of fatty acid oxidation). Glucose availability might determine fatty acid oxidation by regulating its availability or directly controlling its oxidation.

Glucose infusion increases the rate of oxidizing this substance and slows the same process for fatty acids. The inhibitory effect of glucose on fatty acid oxidation has been attributed to insulin-induced inhibition of lipolysis and consequently decreased fatty acid availability (Coppack et al., 1994). However, we recently showed that glucose infusion inhibits fatty acid oxidation even with constant fatty acid availability, suggesting a direct effect of glucose on fatty acid oxidation.

REST STUDIES

We studied five healthy volunteers in the basal state and during a hyperinsulinemic-hyperglycemic clamp (plasma insulin = 1,789 pmol/L, plasma glucose = 7.7 mmol/L; Sidossis and Wolfe, 1996). To isolate the effect of hyperinsulinemia-hyperglycemia on fatty acid oxidation, plasma FFA concentration was held constant in both trials via lipid and heparin infusion. Glucose and fat oxidation were quantified using indirect calorimetry and stable isotopes (1-^{13}C-oleate). Glucose oxidation increased from 6.2 ± 0.8 in basal to 22.3 ± 1.4 $\mu mol.kg^{-1} \cdot min^{-1}$ during the clamp ($p < .01$). Total (indirect calorimetry) and plasma fatty acid oxidation (isotopic determination) decreased from 2.6 ± 0.2 to 0.4 ± 0.3 ($p < .01$) and 2.2 ± 0.2 to 1.4 ± 0.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ ($p < .05$), respectively. We concluded that when glucose availability increases, this substance is preferentially oxidized over fatty acids, even when fatty acid availability remains constant. Our findings suggest that contrary to the prediction of the glucose-fatty acid cycle hypothesis, the intracellular availability of glucose (rather than FFA) determines the nature of substrate oxidation in resting individuals. However, we could not determine the exact mechanism by which glucose regulates fatty acid oxidation in humans.

McGarry et al. (1977), based on rat liver studies, proposed a potential mechanism by which increased glucose availability and oxidation might directly inhibit fatty acid oxidation. Activated long-chain fatty acids (LCFA) must bind to carnitine, a reaction catalyzed by the enzyme carnitine acyltransferase I (CAT I), to gain access into the mitochondrial matrix (see Figure 3). The product of this reaction, fatty acyl-carnitine, is transported across the inner mitochondrial membrane via

the carnitine-acylcarnitine translocase mechanism (Pande, 1975). Based on findings from a series of studies in vitro, McGarry et al. (1977, 1983, 1989) suggested that in the carbohydrate-fed state, when the insulin:glucagon ratio increases, the decrease in cytosolic cyclic 3', 5'-adenosine monophosphate concentration, coupled with an increase in pyruvate availability, results in increased formation of malonyl-CoA, a potent CAT I inhibitor (McGarry et al., 1977). Thus, when cytosolic malonyl-CoA concentration increases, LCFA uptake by the mitochondria is inhibited (McGarry et al., 1977), and fatty acid oxidation consequently decreases. As starvation progresses (low insulin:glucagon ratio), malonyl-CoA concentration decreases, which relieves CAT I inhibition, thereby allowing the flow of long-chain fatty acyl-CoA into the mitochondria for oxidation (see Figure 3).

We tested the hypothesis that glucose limits fat oxidation by inhibiting fatty acid entry into the mitochondria in resting volunteers (Sidossis et al., 1996). We gave constant infusions of [1-^{13}C] oleate, a long-chain fatty acid and [1-^{14}C] octanoate, a medium-chain fatty acid, for 3 hr in seven individuals (basal). Immediately following the basal period, a hyperinsulinemic (insulin infusion = 120 mU $\cdot$ m^{-2} $\cdot$ min^{-1}), hyperglycemic (plasma glucose = 7.8 mmol/l) clamp was started and continued for 5 hr. During the last 3 hr of the clamp, we reinfused [1-^{13}C] oleate and [1-^{14}C] octanoate. We measured intracellular acylcarnitine concentrations in muscle biopsies obtained before and after the clamp. Plasma oleate enrichment and FFA concentration were kept constant via variable infusions of lipids and heparin. The rationale of the study design is outlined in Figure 4. Oleate, but not octanoate, requires carnitine binding to gain access to the mitochondrial matrix. Hence, if glucose limits long-chain fatty acid entrance into the mitochondria, then during the clamp, long-chain acylcarnitine formation should be decreased, causing a decrease in oleate, but not octanoate, oxidation (see Figure 4). In fact, oleate

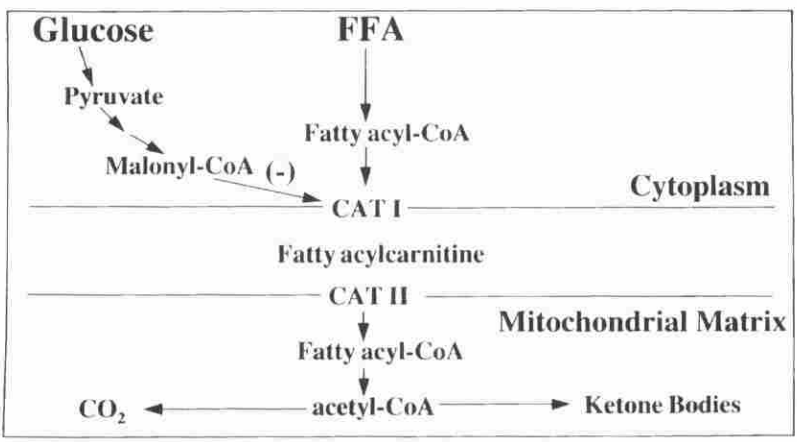

Figure 3. Schematic diagram showing the mechanism of glucose regulation of fatty acid oxidation proposed by McGarry and coworkers (1977). (Reproduced from "Glucose plus insulin regulate fat oxidation by controlling the rate of fatty acid entry into the mitochondria," by L.S. Sidossis, C.A. Stuart, G.I. Shulman, G.D. Lopaschuk, and R.R. Wolfe, 1996, Journal of Clinical Investigation, 98(10): 2244-2250, by copyright permission of The Rockefeller University Press.)

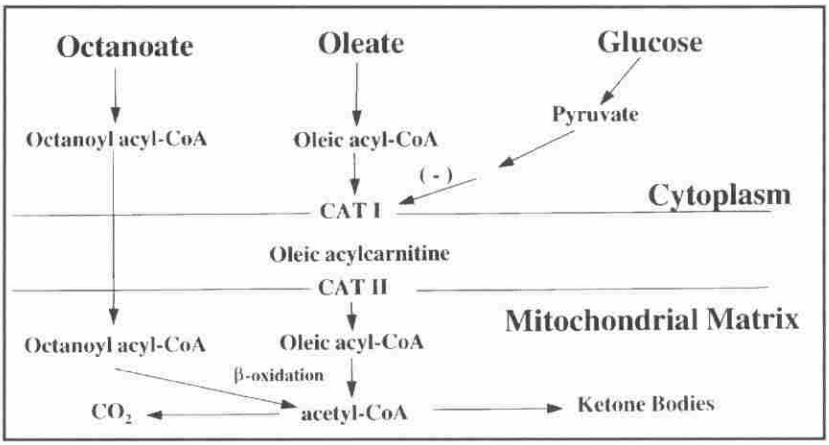

Figure 4. Rationale of study design to examine the effect of glycolytic flux on long-chain fatty acid transport into the mitochondrial matrix.

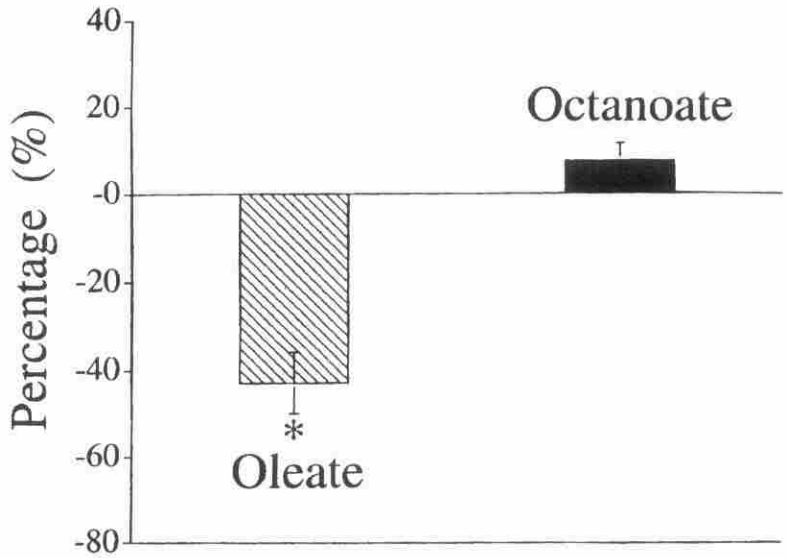

Figure 5. Percent difference in [1-^{13}C] oleate (long-chain fatty acid) and [1-^{14}C] octanoate (medium-chain fatty acid) oxidation in the transition from the basal state to hyperinsulinemia-hyperglycemia. $M \pm SE$ for 7 volunteers. $^{*}p < .05$ from baseline. (Reproduced from "Glucose plus insulin regulate fat oxidation by controlling the rate of fatty acid entry into the mitochondria," by L.S. Sidossis, C.A. Stuart, G.I. Shulman, G.D. Lopaschuk, and R.R. Wolfe, 1996, Journal of Clinical Investigation, 98(10): 2244-2250, by copyright permission of The Rockefeller University Press.)

oxidation decreased, whereas octanoate oxidation remained unchanged in the transition from basal to clamp (see Figure 5). Long-chain acylcarnitine concentration decreased from 855 ± 271 in the basal state to 376 ± 83 nmol/g dry weight during the clamp ($p < .05$). Based on these data, we concluded that glucose determines fatty acid oxidation in resting individuals by controlling the rate of long-chain fatty acid entry into the mitochondria.

EXERCISE STUDIES

Romijn et al. (1993) determined that fatty acid oxidation decreases when exercise intensity increases from 65 to 85% of $\dot{V}O_2$max, even though energy demands increase (see Figure 1). Romijn et al. partially attributed the decrease in fatty acid oxidation to limited fatty acid availability, as plasma fatty acid concentration significantly decreased during exercise at 85% of $\dot{V}O_2$max. To directly examine the role of FFA availability on fatty acid oxidation during high-intensity exercise, Romijn et al. (1995) studied well-trained athletes during exercise at 85% of $\dot{V}O_2$max with "normal" and "high" plasma fatty acid availability. Plasma fatty acid concentration was increased by lipid and heparin infusions. The increased fatty acid availability moderately increased fat oxidation but did not restore fatty acid oxidation to levels during exercise at 65% of $\dot{V}O_2$max. The authors concluded that although suboptimal fatty acid availability to the muscle impairs fatty acid oxidation during high-intensity exercise, fatty acid availability is only partially responsible for the decrease in fatty acid oxidation under these circumstances.

Based on our findings that glucose rather than fatty acids may regulate substrate utilization in resting volunteers (Sidossis and Wolfe, 1996; Sidossis et al., 1996), we examined the hypothesis that fatty acid oxidation decreases during high-intensity exercise because of inhibited fatty acid entry into the mitochondria (Sidossis et al., 1997). We studied six healthy individuals during exercise at 40% of $\dot{V}O_2$peak for 60 min and at 80% for 30 min on two different occasions. [1-^{13}C] oleate and [1-^{14}C] octanoate were infused for the duration of the studies. Oleate but not octanoate requires carnitine binding to gain access to the mitochondrial matrix; hence, if glucose or insulin limits long-chain fatty acid entrance into the mitochondria, then when glycolytic flux is accelerated during high-intensity exercise, oleate but not octanoate oxidation should be decreased. Plasma oleate and total FFA availability were maintained in the two experiments via lipid and heparin infusions during exercise at 80% of $\dot{V}O_2$peak. Oleate oxidation decreased from 2.8 ± 0.6 (40% of $\dot{V}O_2$peak) to 1.8 ± 0.2 μmol · kg^{-1} · min^{-1} (80% of $\dot{V}O_2$peak, $p < .05$), whereas octanoate oxidation increased from 1.0e^{-05} ± 1.0e^{-06} (40% of $\dot{V}O_2$peak) to 1.3e^{-05} ± 5.1e^{-06} μmol · kg^{-1} · min^{-1} (80% of $\dot{V}O_2$peak, $p < .05$). Furthermore, the percent of oxidized oleate uptake—a reflection of intracellular metabolism—significantly decreased, whereas the percent of octanoate oxidized was similar during exercise at 40 and 80% of $\dot{V}O_2$peak (see Figure 6). These data suggest that in addition to suboptimal FFA availability, fatty acid oxidation is likely limited during high-intensity exercise because long-chain fatty acid is direcly inhibited from entering into the mitochondria (Sidossis et al., 1997).

Jansson and Kaijer (1987) and Coggan et al. (1993) dismissed the notion that changes in substrate utilization after endurance training can be explained by the glucose-fatty acid cycle hypothesis. Since endurance training reduces the rate

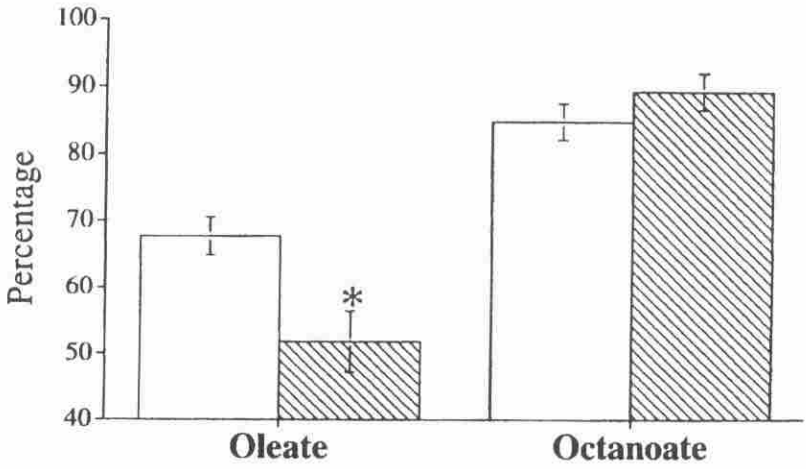

Figure 6. Percent oleate and octanoate tracer uptake oxidized during exercise at 40 (open bars) and 80% of $\dot{V}O_2$peak (hatched bars). $M \pm SE$ for 6 volunteers. $p < .05$ vs. exercise at 40% of $\dot{V}O_2$ peak. (From "Regulation of plasma fatty acid oxidation during low- and high-intensity exercise," by L.S. Sidossis and R.R. Wolfe, 1996, American Journal of Physiology, 265(28): E733-E738. Copyright 1996 by the American Physiological Society. Reprinted with permission.)

of carbohydrate flux during exercise, we examined the hypothesis that increased fatty acid oxidation during exercise after endurance training results from accelerated entry of fatty acids into the mitochondria. To test this hypothesis, sedentary and endurance-trained men (5 of each) exercised on a cycle ergometer at a $\dot{V}O_2$ of ~2.0 L/min, representing 80 and 40% of $\dot{V}O_2$peak, respectively. [1-^{13}C] oleate and [1-^{14}C] octanoate were infused during both studies. Carbohydrate oxidation was significantly higher in the sedentary group (196 ± 9 vs. 102 ± 17 μmol · kg^{-1} · min^{-1}, $p < .05$). Oleate oxidation was higher in the trained (3.8 ± 0.6 vs. 1.9 ± 0.3 μmol · kg^{-1} · min^{-1}, $p < .05$), whereas octanoate oxidation did not differ between both groups. The percentage of oleate taken up by tissues and oxidized was higher among trained individuals (76 ± 7% vs. 58 ± 3%, $p < .05$). However, the percentage octanoate taken up and oxidized was not different (82 ± 3% vs. 85 ± 4%). Since octanoate, unlike oleate, can freely diffuse across the mitochondrial membrane, these results suggest that the difference in fatty acid oxidation between trained and untrained individuals may be due to enhanced fatty acid entry into the mitochondria.

In summary, our work on the regulation of substrate interaction at rest and during exercise has led to a perspective contrary to that suggested in the original glucose-fatty acid cycle hypothesis. We have found that glucose, in addition to indirectly limiting fatty acid availability, directly determines the rate of fat oxidation by controlling fatty acid transport into the mitochondria. We therefore propose that it is the intracellular availability of glucose, rather than fatty acids, that regulates substrate interaction during exercise.

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