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Regulatory mechanisms in the interaction between carbohydrate and lipid oxidation during exercise

L. L. Spriet and M. J. Watt
Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada

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Correspondence: Lawrence L. Spriet, Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario N1G 2W1, Canada.

Abstract

At the onset of exercise, signals from inside and outside the muscle cell increase the availability of carbohydrate (CHO) and fat to provide the fuel required for ATP production. CHO and fat oxidation are the dominant sources of aerobic ATP production and both pathways must be heavily upregulated during exercise to meet the increased energy demand. Within this paradigm, there is room for shifts between the proportion of energy that is provided from CHO and fat. It has long been known that increasing the availability of endogenous or exogenous CHO can increase the oxidation of CHO and decrease the oxidation of fat. The opposite is also true. While descriptive studies documenting these changes are numerous, the mechanisms regulating these shifts in fuel use in the face of constant energy demand have not been thoroughly elucidated. It would be expected, for example, that any fat-induced shift in CHO metabolism would target the enzymes that play key roles in regulating CHO metabolism and oxidation. Inside the muscle these could include glucose uptake (GLUT4) and phosphorylation (hexokinase), glycogenolysis (glycogen phosphorylase), glycolysis (phosphofructokinase) and conversion to acetyl CoA (pyruvate dehydrogenase). The same would be expected for a CHO-induced down regulation of fat metabolism and oxidation and might target transport of long chain fatty acids into the cell (fatty acid translocase CD36), release of fatty acids from intramuscular triacylglycerol (hormone sensitive lipase) and transport into the mitochondria (carnitine palmitoyl transferase complex). This review summarizes the work describing the interaction between CHO and fat metabolism in human skeletal muscle during exercise and presents the theories that may account for CHO/fat interaction during exercise.

Keywords carbohydrate, exercise, fat, metabolism, oxidation.

Carbohydrate (CHO) and fat are the dominant fuel sources for aerobic ATP production during exercise and the pathways that metabolize these fuels must be heavily up-regulated to meet the increased demand for energy. At the onset of exercise, signals from inside and outside the muscle cell activate the key metabolic steps leading to increased availability and metabolism of CHO and fat. While both fuels are important during exercise, the proportion of the two fuels oxidized is determined by many factors. For example, it has long been known that, in the absence of dietary interventions, increasing the exercise intensity exacerbates the reliance on CHO and increasing the duration of low and moderate exercise augments the reliance on fat. It has also been shown that increasing the availability of endogenous or exogenous CHO can increase the oxidation of CHO and that increasing the exogenous free fatty acid (FFA) availability can increase the reliance on fat.

It has been argued that a shift to an increased oxidation of fat and decreased CHO use is desirable

during exercise. CHO is the more versatile fuel as it can contribute aerobic and anaerobic energy at all aerobic power outputs and contribute anaerobic energy when the power output exceeds the aerobic range. However, the body CHO stores are not large and can be exhausted following 1–2 h of intense exercise (Hermansen *et al.* 1967). Thus, any 'sparing' of CHO by fat would be advantageous assuming that the ultimate goal of prolonged exercise is to maintain muscle glycogen stores as long as possible and for the liver to provide glucose to the working muscles while maintaining blood glucose at ~5 mM. Alternately, when muscle and liver glycogen stores are plentiful (and glucose may be added exogenously), the mobilization of fat is reduced and CHO oxidation is favoured.

While descriptive studies documenting changes in the proportion of fat and CHO utilization are numerous, the mechanisms regulating these shifts in fuel use have not been thoroughly elucidated. For instance, how does the muscle know to increase the utilization of one fuel and decrease the other in the face of a constant energy demand during exercise? It would be expected that any fat-induced down-regulation of CHO oxidation would target the key sites (transport, enzymatic reactions) regulating CHO metabolism and oxidation. Inside the muscle, these would include glucose uptake involving glucose transporters (GLUT 4), glucose phosphorylation (hexokinase, HK), glycogenolysis (glycogen phosphorylase, PHOS), glycolysis (phosphofructokinase, PFK) and conversion to acetyl-CoA (pyruvate dehydrogenase, PDH) (Spriet & Howlett, 1999). The same would be expected for CHO-induced down-regulation of fat metabolism and oxidation with targets at transport of long chain fatty acids (LCFA) into the cell (fatty acyl translocase, FAT CD36), release of FFA from intramuscular triacylglycerol (TG) (hormone sensitive lipase, HSL) and transport into the mitochondria (carnitine palmitoyl transferase I, CPTI) (Spriet 2002).

The purpose of this review is to briefly summarize the work examining the reciprocal relationship between the use of CHO and fat during exercise and describe the regulatory mechanisms that may account for the communication between these two dominant pathways of energy provision. Numerous reviews have examined earlier work in this area in some detail and the reader is encouraged to consult these papers (Spriet & Dyck 1996, Spriet 1998, Rasmussen & Wolfe 1999, Spriet & Odland 1999). This paper presents the theories that may account for the interaction between fat and CHO oxidation during exercise and briefly examines the evidence supporting or refuting these theories in human skeletal muscle.

Effects of increased fat availability on the interaction between fat and CHO oxidation during exercise

Theory 1 – Classic 'glucose-fatty acid' cycle

Randle and co-workers (Randle *et al.* 1963, 1964; Garland *et al.* 1963; Garland & Randle 1963) examined the regulation of fat/CHO interaction in muscle using perfused, contracting heart muscle and incubated, resting diaphragm muscle. Increasing the availability of FFA to the muscles (1.8 mM vs. 0 mM) increased fat oxidation and reduced CHO oxidation. It also produced measurable increases in muscle acetyl-CoA, citrate and glucose 6-phosphate (G-6-P) contents. It had been previously determined that acetyl-CoA inhibited the activity of the mitochondrial enzyme PDH *in vitro*, by activating PDH kinase, the enzyme that phosphorylates PDH to its less active form. Similar *in vitro* work identified citrate as a potent inhibitor of the cytoplasmic enzyme PFK, predicting an *in vivo* effect, assuming that the FFA-induced increase in mitochondrial citrate escaped to the cytoplasm. Lastly, G-6-P had been shown to inhibit HK *in vitro*. By combining the findings from their isolated muscle experiments and the *in vitro* enzyme studies, Randle *et al.* (1963, 1964) proposed the glucose-fatty acid theory to explain the reciprocal relationship between fat and CHO oxidation. An increased FFA availability increased muscle acetyl-CoA and citrate leading to down regulation of PDH and PFK activities. The reduced flux through the glycolytic pathway caused an accumulation of G-6-P, which inhibited HK activity and ultimately decreased the uptake of glucose presumably by increasing the free glucose concentration leading to a decreased transsarcolemmal glucose gradient.

Effects of increased exogenous FFA on CHO metabolism. Attempts at examining this theory in human skeletal muscle suggest that while it is possible to alter the proportion of fat and CHO fuel oxidized during exercise, the mechanisms controlling these shifts are largely different. The original theory did not involve the regulation of muscle glycogen, as heart muscle does not rely as heavily on this fuel as skeletal muscle does. Most of the experimental work in this area has increased the availability of exogenous FFA to the muscle and examined the effects on CHO metabolism. While many models have been used to achieve this, including high fat meals and diets, short- and long-term aerobic training, caffeine ingestion, fasting, and prolonged aerobic exercise, the acute infusion of a TG solution coupled with periodic heparin administration has been most commonly used. This technique has the

advantage of acutely (~30 min) increasing the plasma (FFA) without significant alterations in other fuels, metabolites and hormones (Hargreaves *et al.* 1991, Romijn *et al.* 1995, Dyck *et al.* 1996).

During exercise at ~80% maximal O_2 uptake ($\dot{V}O_{2max}$), increased FFA availability decreased net glycogen use by ~50% in the initial 15 min of exercise and increased fat oxidation by ~15% during 30 min of exercise (Dyck *et al.* 1993, 1996, Romijn *et al.* 1995). The muscle contents of free ADP and AMP, important activators of glycogen PHOS, were significantly reduced in the high FFA condition and appeared to explain the decreased glycogen use. There were no effects on muscle citrate, acetyl-CoA and G-6-P contents or the proportion of PDH in the active form (PDHa) (Dyck *et al.* 1993, 1996). To date, no one has examined the effects of elevated FFA on glucose uptake at this intensity. Therefore, at this intense aerobic power output, the fat-induced down regulation of CHO oxidation was regulated at the level of glycogen phosphorylase.

When the same experiments were repeated at lower exercise power outputs (~40 and 65% $\dot{V}O_{2max}$) high fat provision appeared to down regulate CHO oxidation at more sites. Based on respiratory exchange ratio (RER) measurements, fat oxidation was increased and CHO oxidation decreased at both power outputs (Odland *et al.* 1998). Muscle glycogen use was reduced with high FFA provision, but to a smaller extent than at the higher power output and muscle glucose uptake was unaffected during whole body cycle exercise (Odland *et al.* 1998). However, another study reported no effect of high fat provision on muscle glycogen use but a reduced glucose uptake during knee extension exercise (Hargreaves *et al.* 1991). Muscle measurements revealed no effect of high fat on acetyl-CoA and G-6-P contents but a small increase in citrate and a lower PDHa (Fig. 1) (Odland *et al.* 2000). These experiments suggest fat-induced down regulation of CHO metabolism occurs at multiple sites during moderate aerobic exercise, including PHOS, PFK and PDH. However, *in vitro* work examining the inhibitory effects of citrate on PFK activity suggest that the small increase in citrate in the high fat trials would have minimal *in vivo* effect (Peters & Spriet 1995). More recent work taking the opposite approach, namely a nicotinic acid-induced decrease in FFA availability at ~60% $\dot{V}O_{2max}$, reported an increased RER, a trend towards more glycogen use and an increased PDHa (Stellingwerff *et al.* 2003). However, there were no effects on muscle citrate, acetyl-CoA or pyruvate contents.

What are the mechanisms that explain the ability of providing more exogenous fat to inhibit PHOS and PDH activities or producing the opposite effects when FFA availability is reduced?

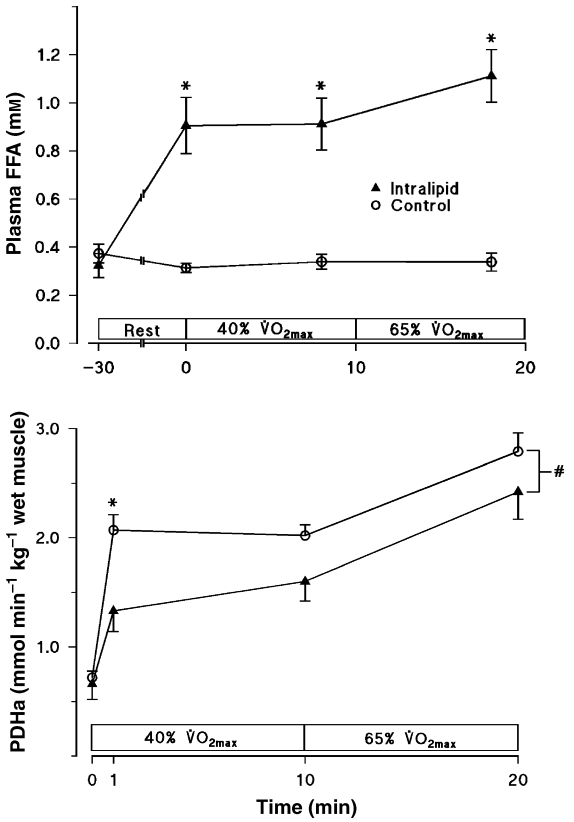

Figure 1 Plasma FFA (top) and muscle pyruvate dehydrogenase activity (bottom) of the active *a* form (PDHa) during 10 min of cycling at 40% and 10 min at 65% $\dot{V}O_{2max}$ with intralipid (and heparin) infusion or control. Values are mean ± SE. *Significant main effect between trials. Data from Odland *et al.* (2000).

Theory 2 – Mitochondrial NADH regulates fuel preference during exercise

An interesting finding of the studies that increased exogenous FFA availability was that the fall in the energy charge of the muscle that normally occurs during exercise was reduced. This was assessed by measuring muscle phosphocreatine, creatine, ATP and lactate (to predict $[H^+]$) and calculating muscle free ADP, AMP and inorganic phosphate (P_i) contents. As P_i is a substrate for PHOS, and ADP and AMP are direct allosteric regulators of the active form of PHOS, the noted reductions in these regulators could account for the decreased glycogenolysis with high fat provision (Dyck *et al.* 1996; Fig. 2). Less free ADP accumulation would also make it harder for PDH to convert to the active form during exercise at 40 and 65% $\dot{V}O_{2max}$, as a high ATP/ADP ratio activates PDH kinase (PDK) activity and decreases PDHa. However, a key question is what accounts for a more favourable energy charge during exercise with increased fat availability? We have proposed in the past that the increased fat availability in

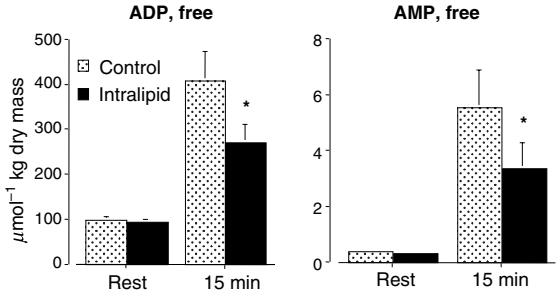

Figure 2 Muscle free ADP and AMP contents during 15 min of cycling at 85% VO_2max with intralipid (and heparin) infusion or control. Values are mean ± SE. *Significant main effect between trials. Data from Dyck *et al.* (1996).

the minutes prior to exercise and early in exercise stimulates fat metabolism to produce an increased NADH (reduced nicotinamide adenine dinucleotide) concentration in the mitochondria (Chesley *et al.* 1998, Odland *et al.* 2000). As the major inputs for aerobic ATP regeneration in the mitochondria are ADP and P_i, O_2 and NADH (reducing equivalents), it has been suggested that an increase in NADH at a given power output (energy demand) would allow for a reduced accumulation of free ADP and P_i, while maintaining a constant drive for mitochondrial respiration (Wilson 1994).

Unfortunately, this has been a difficult theory to test as mitochondrial NADH is not easy to measure in intact human skeletal muscle and much controversy surrounds the various techniques that have been attempted (Spriet & Howlett 1999). We did estimate mitochondrial (NADH) using the whole muscle homogenate technique and found that NADH was elevated at rest and at 1 min of exercise at 40% $\dot{V}O_{2max}$ when extra fat was provided (Odland *et al.* 2000). However, following 10 min at 40% $\dot{V}O_{2max}$ and again after 10 min at 65% $\dot{V}O_{2max}$, NADH was no longer higher than the control condition. It should also be stated that when the provision of FFA was reduced, there was no increase in the fall of the muscle energy status (free ADP and AMP) during 40 min of exercise, although CHO use and muscle PDHa were increased and fat use was decreased (Stellingwerff *et al.* 2003). Therefore in summary, while the mitochondrial NADH theory for explaining the reciprocal control of fuel use when fat availability is increased or decreased during exercise is attractive, there are many unexplained findings and a more thorough testing of this theory awaits improved techniques for measuring mitochondrial NADH.

Increased FFA availability during prolonged exercise. When exercise of moderate intensity is prolonged beyond 1–2 h the availability of plasma FFA increases to high levels while the availability of muscle glycogen decreases (Watt *et al.* 2002a). Not surprisingly, fat oxidation rises and CHO oxidation falls (Fig. 3). This exercise scenario provides another opportunity to study the reciprocal control of fat and CHO oxidation during exercise. We have recently shown that muscle PDHa decreases during prolonged exercise in keeping with decreased reliance on CHO (Fig. 3, Watt *et al.* 2002a). Surprisingly, no changes in the energy status of the cell

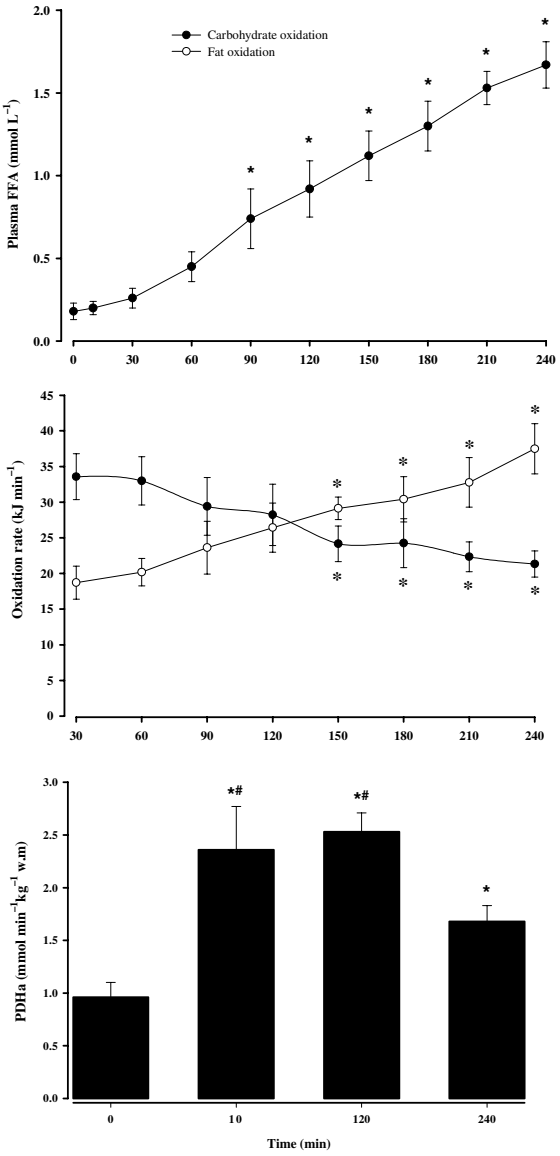

Figure 3 Plasma FFA (top), fuel oxidation rates (centre) and muscle pyruvate dehydrogenase activity of the active *a* form (PDHa, bottom) during 4 h of cycling at 57% $\dot{V}O_{2max}$. Values are mean ± SE. *Significantly different from 0 time in top and bottom panels and significantly different from 30 min in centre panel. #Significantly different from 240 min. Data from Watt *et al.* (2002a).

or decreases in pyruvate accompanied the decrease in PDHa or the decreased glycogenolysis. It is possible that a reduction in the availability of substrate is responsible for the decreased PHOS and PDHa activities, as glycogen is nearing depletion and the production of pyruvate is decreased although it does not translate into a decreased content in the muscle. However, an alternate possibility to explain the decreased PDHa involving the upregulation of PDK activity has been suggested.

Theory 3 – High fat availability upregulates PDK activity and decreases PDHa

Situations that chronically decrease CHO availability and increase the reliance of skeletal muscle on fat produce increases in the mRNA and protein of the PDK 4 isoform and the activity of PDK (Peters *et al.* 2001). This ultimately leads to a decreased fraction of PDH in the active form and documented decreases in whole body CHO oxidation at rest (Peters *et al.* 2001). While these changes occur over hours and days, it is not currently known whether more rapid fat-induced up regulation of PDK activity could occur during prolonged exercise. Could these changes explain the down regulation of PDH that occurs during 4 h of moderate exercise? Pilegaard *et al.* (2000, 2002) have reported upregulation of PDK 4 mRNA during the latter stages of prolonged exercise and early in the recovery period following prolonged exercise. This theory needs testing with direct measures of PDK and PDHa activities and PDK isoform mRNA and protein in human skeletal muscle during prolonged exercise.

Increased intramuscular fat availability. The most common method used to alter intramuscular fat (IMTG) availability is via dietary manipulation. IMTG has been demonstrated to increase by 50–80% following both long- (Kiens *et al.* 1987) and short-term (Jansson & Kaisjer 1982, Starling *et al.* 1997) consumption of high fat diets (50–70% of total energy supplied by fat), whereas when dietary fat intake was reduced from 22 to 2% of caloric intake, IMTG was decreased after 1 week (Coyle *et al.* 2001). A number of lines of evidence imply a regulatory role for IMTG on CHO metabolism during exercise. Those studies that have determined fuel oxidation rates during moderate intensity exercise following long-term high fat diets report reduced reliance on CHO as a fuel (Schrauwen *et al.* 2000, Helge *et al.* 2001). Muscle glycogen utilization was lower in subjects consuming a high fat compared with a high CHO diet, whereas exogenous glucose uptake was similar (Helge *et al.* 2001). In agreement, when IMTG concentration was reduced by negligible (2%) dietary fat consumption for 7 days, whole body CHO oxidation and muscle glycogen utilization were higher, and whole body glucose uptake remained unchanged (Coyle *et al.* 2001). Taken together, these limited data suggest IMTG is without effect on muscle glucose uptake during exercise but may influence muscle glycogen utilization. Importantly, the interpretation of these diet studies is confounded by concomitant changes in muscle glycogen availability, which contributes to altered patterns of fat utilization, and possible diet-training interactions.

Aside from the aforementioned dietary manipulations there exists no direct evidence of an acute effect of increased IMTG on CHO metabolism. The finding of greater pre-exercise IMTG and decreased RER during moderate exercise in well-trained men who consumed a 12-h high fat diet suggests acute changes in IMTG may decrease CHO oxidation (Starling *et al.* 1997). Finally, pre-exercise IMTG was positively related to IMTG utilization during heavy resistance exercise, suggesting a role of IMTG on its own catabolism (Essen-Gustavsson & Tesch 1990). Clearly, to elucidate the possible interaction between endogenous fat and CHO fuel metabolism, future studies must employ interventions that induce acute changes in IMTG independent of alterations in the availability of other substrates (e.g. muscle glycogen and plasma FFA).

Effects of increased CHO availability and metabolism on fat oxidation during exercise

Although several studies have demonstrated that increasing the availability of CHO before and during exercise increases CHO utilization (Coyle *et al.* 1997, Horowitz *et al.* 1997), few studies have examined the biochemical mechanisms underlying this shift in fuel selection that occurs in these exercise situations.

Increased exogenous glucose availability

It has long been recognized that pre-exercise CHO ingestion reduces fat oxidation during a subsequent bout of low to moderate exercise, but not at exercise intensities above ~75% $\dot{V}O_{2max}$. There is accumulating evidence to support an inhibitory role of increased exogenous glucose availability on fat utilization during exercise. It appears that the reduction in fat metabolism following glucose ingestion is due to the coordinated effects of decreased fatty acid availability secondary to decreased adipose lipolysis and fatty acid oxidation at the muscle.

Two studies from Coyle's laboratory measured adipose tissue lipolysis during low-moderate intensity exercise when subjects were fasted or 60 min after

CHO ingestion (Coyle *et al.* 1997, Horowitz *et al.* 1997). During exercise in the fasted state adipose tissue lipolysis exceeded skeletal muscle fat oxidation, whereas CHO ingestion resulted in elevated plasma insulin, reduced adipose tissue lipolysis and decreased fat oxidation that equalled the reduction in lipolysis, thus indicating a limitation of FFA availability for fat oxidation. However, when plasma FFA availability was restored by intravenous lipid and heparin infusion, fatty acid oxidation was increased by 30% but not fully restored, suggesting that the inhibitory effect of CHO ingestion also resides at the level of oxidation (Horowitz *et al.* 1997). Indeed, pre-exercise CHO ingestion increased glycolytic flux and CHO oxidation and reduced both plasma-derived fatty acid and IMTG oxidation (Coyle *et al.* 1997). The finding of reduced long chain palmitate uptake (which is dependent on CPT1), but not the medium chain fatty acid octanate (which is largely independent of mitochondrial transport) supports an inhibitory effect of CHO oxidation on fat oxidation. The affected regulatory steps may be either FFA transport into muscle (FAT/CD36) and/or into the mitochondria (CPT1) (Coyle *et al.* 1997). Such a mechanism is also evident during other situations characterized by increased glucose availability and glycolytic flux such as high intensity exercise (Sidossis *et al.* 1997) and during a hyperinsulinaemic–hyperglycaemic clamp (Sidossis *et al.* 1996, Sidossis and Wolfe, 1996).

Increasing the exercise power output is another situation where changes in CHO availability (glycolytic flux) alter fat oxidation. Fat utilization increases from rest during low to moderate intensity exercise (40–65% $\dot{V}O_{2max}$), however, fatty acid oxidation decreases at power outputs above ~75% $\dot{V}O_{2max}$ (Romijn *et al.* 1993, Sidossis *et al.* 1997). Blood glucose levels, muscle glycogenolysis, glycolytic flux, PDH activation and CHO oxidation are increased during exercise at higher compared with moderate exercise power outputs (Wahren *et al.* 1971, Gollnick *et al.* 1974, Howlett *et al.* 1998). It has been suggested that increased CHO oxidation elevates contents of malonyl-CoA (M-CoA), which may inhibit fatty acid oxidation during heavy to intense exercise. This has not been substantiated by experimental evidence in human skeletal muscle (refer to Theory 4). Instead, the decreased pH observed at intense exercise power outputs may provide the link between increased glycolytic flux and reduced fat oxidation. Studies in mitochondria isolated from resting human skeletal muscle showed that small decreases in pH induce large reductions in CPTI activity (Starritt *et al.* 2000). In view of the lowered pH at moderate and intense exercise power outputs (Howlett *et al.* 1998), this could lead to decreased CPTI activity and decreased fatty acid oxidation.

Theory 4 – Carbohydrate oxidation decreases fat oxidation via M-CoA inhibition of CPTI activity

As previously mentioned, CPTI appears to play an important role in the interaction between fat and CHO metabolism in skeletal muscle. CPTI is located on the outer mitochondrial membrane and converts acyl-CoA to acylcarnitine, which is then transported through the inner mitochondrial membrane via a carnitine–acylcarnitine translocase in exchange for free carnitine. The acylcarnitine is then reconverted to acyl-CoA and made available for β-oxidation by the action of CPT II on the inside of the mitochondria (McGarry & Brown 1997).

It is widely believed that CPTI is the rate-limiting enzyme in this complex. *In vitro* work has established that the activity of skeletal muscle CPTI can be reversibly inhibited by M-CoA, while CPT II is unaffected, as was initially demonstrated in many other tissues (McGarry *et al.* 1978, 1983, Berthon *et al.* 1998). M-CoA is produced in the cytoplasm by acetyl-CoA carboxylase (ACC) and is the first committed intermediate produced during fatty acid synthesis. It is a well-established regulator of fatty acid oxidation in lipogenic tissues such as adipose tissue and liver (McGarry & Brown 1997). When CHO supply is abundant and lipid is synthesized in these tissues, high levels of M-CoA inhibit CPTI activity and the transport of lipid into the mitochondria.

Measurable amounts of M-CoA have been detected in rat (Winder *et al.* 1989, Saha *et al.* 1995) and human skeletal muscle (Odland *et al.* 1996, 1998, Dean *et al.* 2000), and a muscle isoform of ACC (ACC_β or ACC_2), which appears to be regulated differently than hepatic ACC has also been discovered in skeletal muscle (Ha *et al.* 1996). As skeletal muscle is not a lipogenic tissue, the role that M-CoA plays in regulating the entry of LCFA into the mitochondria during exercise is unclear. The unique aspect of this tissue is that exercise situations require simultaneous increases in both fat and CHO oxidation to meet the large increase in energy demand. This is quite different than the reciprocal changes in CHO and fat oxidation that occur in other tissues or resting skeletal muscle when the demand for energy is essentially constant.

Resting M-CoA levels are believed to be high enough to limit FFA transport into the mitochondria. It is clear that M-CoA levels decrease during muscle contractions in rodent skeletal muscle, when energy production from fat oxidation is increasing (Winder *et al.* 1989). This suggests that the decrease in M-CoA may be important in relieving the inhibition of CPTI that normally exists in resting skeletal muscle and increasing FFA transport into the mitochondria during aerobic exercise. The exercise-induced decrease in M-CoA is likely due to AMP kinase (AMPK) mediated phosphorylation of

ACC_β, which decreases ACC activity. AMPK is activated during exercise by increased free AMP and decreased phosphocreatine (Hutber *et al.* 1997, Vavvas *et al.* 1997). This suggested explanation for the decrease in M-CoA is certainly consistent with activation of AMPK (Fujii *et al.* 2000) and decreased ACC activity (Dean *et al.* 2000) at exercise intensities above 70% $\dot{V}O_{2max}$.

Although increased M-CoA contents have been directly related to decreased FFA oxidation rates in perfused working heart muscle (Saddik *et al.* 1993), only correlational data is currently available regarding the role of M-CoA in rodent skeletal muscle. Measurements of M-CoA in human skeletal muscle during exercise demonstrated that M-CoA was largely unaffected by exercise at varying power outputs (35–100% $\dot{V}O_{2max}$) and rates of fat oxidation (Odland *et al.* 1996, 1998, Dean *et al.* 2000; Fig. 4). These data may not be surprising given that acetyl-CoA and citrate accumulate during exercise (Dyck *et al.* 1993, Howlett *et al.* 1998). The increased acetyl-CoA contents may be the result of increased citrate export into the cytoplasm and conversion to acetyl-CoA via citrate lyase, or export of accumulating acetylcarnitine and then conversion to acetyl-CoA via carnitine acyltransferase. It is possible that the increased acetyl CoA may stimulate the activity of ACC_β, as it is the primary substrate for this enzyme. Moreover, increased cytosolic citrate (an allosteric activator of ACC_β) may offset the potential phosphorylation-induced reduction in ACC activity and M-CoA during exercise. Although these preliminary data do not support a regulatory role for M-CoA in fat oxidation during exercise, the interpretation of these data from muscle biopsy samples are confounded by the existence of cytoplasmic and mitochondrial concentrations of M-CoA and key regulators of M-CoA production, including acetyl CoA and citrate. Future studies are required to elucidate the regulation and role of CTPI and M-CoA in the control of fat metabolism during exercise.

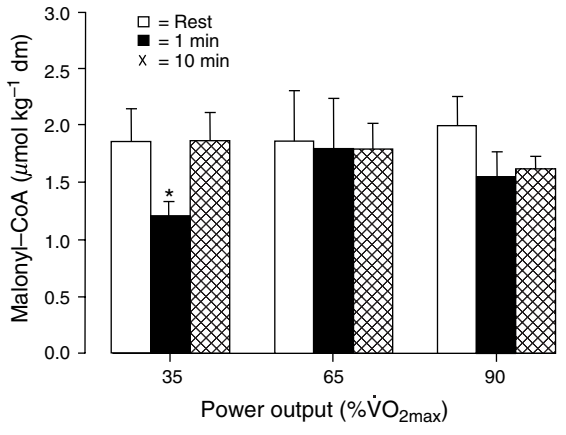

Figure 4 Skeletal muscle malonyl CoA content during 10 min of cycling at various exercise power outputs. Values are mean ± SEM. *Significantly different from 0 and 10 min. Data from Odland *et al.* (1998).

Theory 5 – Increased CHO availability decreases FFA availability and fat oxidation secondary to increased insulin

Glucose ingestion prior to exercise may inhibit fat oxidation secondary to increased plasma insulin levels. Glucose ingestion before exercise results in increased plasma glucose and insulin, PDH activity and reduced plasma FFA (Marmy-Comus *et al.* 1996, Watt *et al.* 2002b), presumably via decreased adipose tissue lipolysis (Horowitz *et al.* 1997). The altered metabolic and hormonal milieu is largely maintained during subsequent exercise, resulting in greater whole-body CHO oxidation as estimated by RER measures (Marmy-Comus *et al.* 1996, Watt *et al.* 2002b). In a similar study, Coyle *et al.* (1997) demonstrated reduced entry of LCFA into the muscle and the mitochondria following CHO ingestion in endurance-trained men. The potential exists that decreased mitochondria LCFA uptake results in cytosolic accumulation of LCFA-CoA and subsequent allosteric inhibition of HSL (Jepson & Yeaman 1992) and reduced IMTG hydrolysis. Indeed, glycerol released from the muscle (an index of IMTG hydrolysis) was reduced during hyperinsulinaemia (Enoksson *et al.* 1998, Jacob *et al.* 1999) and insulin suppressed IMTG hydrolysis and fatty acid oxidation in the isolated contracting rodent soleus (Dyck *et al.* 2001). Taken together, these data suggest that the elevated insulin associated with increased CHO availability may decrease IMTG hydrolysis secondary to increased LCFA-CoA accumulation and fatty acid oxidation. Alternately, the CHO-induced increase in insulin may offset any LCFA-CoA accumulation in the muscle by simply reducing the FFA availability. At the present time, these suggestions have not been directly tested in human skeletal muscle.

Increased muscle glycogen availability. In contrast to the numerous studies that have demonstrated a regulatory role of skeletal muscle glycogen content on both muscle glycogenolysis and glucose uptake, there is negligible information pertaining to the influence of muscle glycogen on FFA uptake and IMTG metabolism. Blomstrand & Saltin (1999) reported similar rates of FFA uptake between exercising legs, 12 h after glycogen depleting exercise in one leg. In the same study, glycerol release from the low-glycogen leg was ~60% greater than the control leg, suggesting a possible increase in IMTG hydrolysis with low muscle glycogen. These data are consistent with decreased muscle glycogen and

increased whole body fat metabolism following a 12-h high fat diet (Starling *et al.* 1997). Given that muscle glycogen can bind to glycogen phosphorylase and increase its activity (Johnson 1992), the possibility exists that glycogen may influence the regulatory enzymes of fat metabolism, which sets up the possibility for future study in this field.

Conclusion

Carbohydrate and fat are the primary metabolic substrates oxidized during aerobic exercise. The notions that increasing the availability of CHO can increase the oxidation of CHO and that increasing the exogenous FFA availability can increase fat oxidation are well supported. We have presented data to suggest that increasing fat availability decreases CHO oxidation. However, during exercise the classic 'glucose-fatty acid cycle' is unlikely to explain this interaction. Instead, increasing fat availability may increase NADH and buffer the fall in the cellular energy charge, resulting in reduced glycogenolysis and PDH activation. Alternatively, increased FFA may upregulate PDK activity and thereby attenuate PDHa, a paradigm that may occur as exercise is prolonged. There is evidence indicating that altering exogenous CHO availability can decrease fat oxidation, possibly via an increase in plasma insulin and decreased FFA availability and also by decreasing the rate of fat transport into the muscle and/or the mitochondria. Future studies examining membrane fat transport, muscle TG lipolysis (HSL activity) and mitochondrial fat transport (CPTI activity) in human skeletal muscle and the effects of altered endogenous fuel availability (IMTG, muscle glycogen) on the interaction between CHO and fat metabolism during exercise are clearly warranted.

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