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INTERRELATIONSHIP BETWEEN CARBOHYDRATE AND LIPID METABOLISM (GLUCOSE - FATTY ACID CYCLE)

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ABSTRACT

It is well-documented that elevated free fatty acid availability inhibits glucose oxidative metabolism both in vitro and in vivo. This reciprocal relationship between lipid and carbohydrate was described over thirty years ago by Randle in isolated rat heart and diaphragm and named glucose-free fatty acid cycle. The importance of this cycle for physiology and pathophysiology of fuel homeostasis depends on details of its operation in skeletal muscles since this tissue accounts for the largest part of glucose disposal by an insulin-sensitive mechanism in vivo. It is noteworthy that glucose-fatty acid operates in complicated milieu of extramuscular and intramuscular lipids and carbohydrate stores, mutual reciprocal relationship within intramuscular and extramuscular lipid and carbohydrate stores, FFA inhibition of glucose transport into the muscle, FFA stimulation of gluconeogenesis in the liver and FFA stimulation of β-cell responsiveness to insulin. This interaction would not have been predicted by the original Randle concept. Nowadays the mechanism of the cycle is well-documented in the myocardium, and in rat liver with a key role of glucose-originated malonyl-CoA, a product of acetyl-CoA carboxylase (ACC) reaction, and a potent inhibitor of carnitine palmitoyltransferase I (CPT I), FFA transport into mitochondria and its oxidation. However, data concerning malonyl-CoA effects on CPT I activity and FFA oxidation in human muscle are still fragmentary. Recently, it has been found that CPT I activity in humans is sensitive to training and it is markedly higher in trained than in untrained muscles. In addition, malonyl-CoA concentration in human muscle is altered in response to fasting and feeding, denervation and glucose. However, more studies are needed to establish malonyl-CoA action on CPT I activity in contracting muscles. On the other hand, an excessive FFA supply may inhibit carbohydrate metabolism affecting glucose transport - insulin signalling pathway, glucose transporter expression and/or hexokinase II activity. Many aspects of reciprocal glucose-fatty acid relationship are fundamental in the studies concerning insulin resistance, obesity as well as training effects on the muscle metabolism.

Key words: free fatty acid, glucose, muscle, fuel selection

Introduction

About 40 years ago Randle et al. (95) demonstrated that free fatty acids (FFA) effectively compete with glucose for substrate oxidation in the isolated rat heart and diaphragm.

The proposed mechanism of glucose-free fatty acid interaction has been that increased FFA provision and oxidation cause an elevation of the intramitochondrial acetyl-CoA/CoA and $NADH^+/NAD^+$ ratios with subsequent inactivation of pyruvate dehydrogenase (PDH). This in turn causes citrate accumulation, which leads to an inhibition of phosphofructokinase and subsequent elevation in glucose-6-phosphate

concentration. Increased glucose-6-phosphate would inhibit hexokinase II resulting in decreased glucose uptake. This glucose-free fatty acid competition was named the glucose-fatty acid cycle and became one of the most abundant hypotheses in physiology and pathophysiology of carbohydrate and lipid metabolism (39, 40, 89, 96).

Metabolic changes similar to those produced by addition of FFA to perfusing medium of the rat heart and diaphragm in vitro have been described in perfused heart and diaphragm of alloxan-diabetic or starved rats concomitantly with reduced insulin sensivity (48). Additionally, the coincident development of decreased insulin sensitivity in association with elevated fasting plasma FFA level has been found in obesity, acromegaly, pregnancy, and in the subjects treated with growth hormone (38, 58, 63). Therefore, the glucose-FFA cycle has been recognized to operate both in vitro and in vivo during excessive provision and oxidation of FFA.

The original Randle concept has attracted much attention since it focuses on two important issues - the relationship between lipid and carbohydrate metabolism and the mechanism of insulin resistance and glucose intolerance. In particular, high plasma FFA concentration is associated with many insulin-resistant states such as obesity, type 2 diabetes or severe trauma and sepsis (100, 145, 146).

Glucose-fatty acid cycle under physiological conditions

Fasting-refeeding transition

Under physiological conditions the operation of the glucose-fatty acid cycle has been demonstrated in the liver, heart and kidney cortex during starving-refeeding transition (for review see 120). However, the mechanism of the cycle differs from those proposed in the original Randle concept (95). Briefly, in the above-mentioned tissues in the fed state the major source of fatty acid derives directly from the diet or indirectly from triacylglycerols synthesized in the liver is estrification to triacylglycerol rather than oxidation because of profound inhibition of carnitine palmitoyltransferase I (CPT I) activity by malonyl-CoA, the first committed intermediate in fatty acid synthesis from carbohydrates. As a consequence, the degree of suppression of CPT I activity dictates the relative rate of fatty acid oxidation. During fasting malonyl-CoA concentration decreases due to shortage of carbohydrate-originated malonyl-CoA and CPT I activity increases leading to elevation in fatty acid oxidation (79, 81, 82, 83).

Diet composition and substrate utilization

There is a general agreement that the whole-body utilization of dietary fat and carbohydrate is balanced through a competitive system of enzyme regulation so an increased fat supply inhibits carbohydrate utilization.

It has been documented that a fat-rich diet (3 days, 50% calories from fat or 5 days, 69% calories from fat) lowers the respiratory exchange ratio (from 0.9 to 0.7), decreases muscle glycogen stores (from 437 to 192 mmol glucose/kg dry weight) in comparison with an isocaloric high-carbohydrate diet (75% calories from carbohydrates) indicating a greater whole-body reliance on fat following prolonged fat consumption (59, 133). In addition, the respiratory exchange ratio is strongly related to habitual diet composition both in sedentary and trained individuals indicating that substrate availability affects whole-body substrate utilization (42, 125). Furthermore, it has been clearly demonstrated that decreased glucose uptake by the muscle and insulin resistance directly correlates

with increased muscle fat stores, mainly saturated free fatty acids (44, 77, 92).

Similarly in rats prolonged (3-4 weeks) excess of dietary fat (41% or 66.5% of energy intake from fat) has been shown to affect carbohydrate metabolism in the muscle by a mechanism which is related to fat accumulation in the muscle, compromised glucose uptake and depressed glycogen synthetase activity in the muscle (49, 70, 122).

Effect of excess of FFA on glucose metabolism

Excess of FFA causes glucose intolerance and inhibit glucose metabolism

Early in vivo studies in man have been consistent with the operation of the glucose-fatty acid cycle. Felber and Vannotti (32) first reported that fat/heparin infusion causes glucose intolerance in normal subjects. Others have also described the influence of elevated blood FFA concentration on glucose metabolism (7, 34, 85, 114). Moreover, it has been noted that nicotinic acid, a potent inhibitor of lipolysis, increases the plasma glucose turnover and oxidation (5, 138).

The advent of the euglycemic-hyperinsulinemic clamp has allowed an explosion of studies which confirmed the inhibitory effect of lipid infusion on insulin-stimulated whole-body glucose metabolism in humans and animals (6, 12, 14, 33, 60, 74, 104, 121). However, the effect of FFA on glucose metabolism during clamp studies has been strongly related to plasma FFA concentration and sequence of insulin and FFA infusion The operation of the cycle has been demonstrated for physiological FFA concentration infused at least 2 h before insulin infusion (14, 60).

The lack of FFA infusion effect on glucose disposal during acute FFA excess

It is noteworthy that many human and animal studies have failed to detect an inhibitory effect of FFA on either oxidative or non-oxidative glucose metabolism (18, 90, 107, 114). It has been demonstrated that lipid infusion produces a greater decrease in the muscle glycogen synthesis than in glucose oxidation (65). Surprisingly, lipid infusion has been found to inhibit total carbohydrate oxidation during infusion of fructose, which metabolism is known to be largely insulin-independent (134). Furthermore, it has been described that the preferred response of human tissues to an increased plasma FFA level and oxidation is an insulin - independent reciprocal change (decrease) in intracellular FFA oxidation. The competition between lipids and carbohydrates became operative at a very high plasma FFA concentration (1 mmol/l), when the capacity for regulation within extra- and intracellular lipids had been utilized and plasma FFA oxidation accounted for all lipid oxidation (148). These results would not have been predicted by the mechanism used to explain the glucose-FFA cycle in the original Randle concept (95).

However, it is worth noting that current data on the operation of the cycle in the type 2 diabetes have not fully supported the hypothesis concerning the reciprocal regulation of glucose and FFA oxidative metabolism. Both animal and human studies have indicated that under fasting conditions glucose oxidation rather increases, whereas it decreases under insulin-stimulated circumstances (64, 66, 68, 124).

The details of the glucose-fatty acid interaction in the type 2 diabetes have been recently presented by Kelley and Mandarino (67).

However, it should be stressed that the importance of the glucose-FFA cycle to the physiology and pathophysiology of fuel homeostasis is dependent on details of its operation in skeletal muscles, since this tissue accounts for the largest part of glucose

disposal by insulin-sensitive tissues in vivo (35, 71). It is noteworthy that reciprocal glucose-FFA interaction has been established in human forearm and leg muscles and in well-oxygenated rat muscles (93, 110, 136, 137, 147). The operation of the cycle has been noticed in vivo in the human heart and muscles using positron emission tomography (86).

But even now, after intensive studies, evidence for the operation of this cycle in tissues other than heart and diaphragm is controversial and the mechanism of the relationship between carbohydrate and lipid metabolism in the muscle is still under debate.

Glucose-FFA cycle operates in the milieu of complicated metabolic interaction

Effects of excessive FFA provision on hepatic glucose production, insulin secretion and sensitivity

At present it is well-known that despite the above-mentioned inhibition of glucose uptake by muscles in an excess of FFA, acutely increased plasma FFA concentration stimulates hepatic glucose production by the gluconeogenic pathway (31, 72, 108, 140). In consequence, suppression of HGP by insulin has been impaired during fat infusion even under hyperinsulinemic conditions indicating a significant role of the liver in the whole-body glucose-FFA interaction (113). Furthermore, in vivo prolonged (48 h) fat infusion and high plasma FFA concentration have been also shown to stimulate insulin secretion and to exert an insulinotropic effect both in humans and animals due to the fat-induced increase in β-cells responsiveness to glucose stimulation (11, 76, 130). Therefore, the effect of FFA on the whole-body glucose metabolism is not limited to an inhibition of glucose uptake and oxidative metabolism in muscles. The role of other tissues (e.g. liver) and FFA effect on insulin secretion and sensitivity has to be carefully considered.

FFA and insulin-stimulated glucose transport

Nowadays, it has been well documented that an excess of FFA decreases the insulin-stimulated glucose transport, however, the mechanism of this action is still under discussion. FFA have been found to affect glucose transport into the muscles suppressing glucose transporter GLUT-4 expression (69) and/or inhibiting the insulin-stimulated phosphatidylinositol 3-kinase (PI-3 kinase), an important component of the insulin signaling pathway, responsible for activation of glucose transport into the muscle cells (10, 26). On the other hand, an excess of FFA inhibits glucose transport in the muscles, affecting both glucose phosphorylation and insulin binding into the muscle (59, 105). Recently it has been demonstrated that long-chain acyl-CoA (palmitoyl-CoA, oleoyl-CoA, linoeolyl-CoA) reduce hexokinase II activity in a concentration-dependent manner in the rat soleus muscle and human hind limb muscles in vitro. In additon, acyl-CoA concentration which inhibits hexokinase II activity is similar to that determined in the muscle cytosol and the inhibitory effect is additive to the inhibition of the enzyme by glucose-6-phosphate. The above data suggest that elevation in lipid metabolites could directly interact with the insulin-mediated glucose metabolism by decreasing the rate of glucose phosphorylation and glucose-6-phosphate concentrations (123).

It is noteworthy that hexokinase II inhibition by an excess of glucose-6-phosphate is an important step in reciprocal relationship of glucose and FFA metabolism proposed in the original Randle concept (95).

FFA transport into the muscles

Knowing the hydrophobic nature of

FFA, for many years it has been argued that the acids can easily traverse the cellular membranes by simple diffusion. It has been commonly accepted that the driving force of muscular fatty acid extraction from extracellular compartment is the concentration difference. In addition, both in humans and animals FFA oxidation in muscles has been linearly related to its plasma concentration (131, 149).

On the other hand, there are numerous data indicating that exogenous FFA uptake by the muscles and other tissues is a rapid and saturable process not simply related to FFA concentration and to the rate of cellular metabolism (1, 51, 119, 127). Additionally, it has been revealed that in vivo at basal state FFA uptake into the muscle is strongly dependent on the muscles' oxidative capacity and is about 2-fold greater in red than in white quadriceps rat muscles (37).

Assuming the muscle mass (approx. 40% of body weight) and its largely fluctuating metabolic rate, skeletal muscles have to be considered as the most important tissue involved in the regulation of lipid homeostasis. Therefore, FFA transport into the muscles may be recognized as an important factor affecting fuel homeostasis and glucose-FFA interaction.

Intramuscular-extramuscular substrate interaction

At least some controversies concerning the operation of glucose-FFA cycle in the muscle may be due to the relationship between intra- and extramuscular substrate utilization. Li et al. (73) have shown that operation of this cycle in vitro in the rat oxidative soleus muscle is masked by intramuscular TG stores, and in the glycolytic extensor digitorum muscle (EDL) by glycogen stores. When reliance of the muscle on intramuscular substrate decreases (by an inhibition of intramuscular TG utilization with a CPT I inhibitor, or depletion of the muscle glycogen with exercise and a low-carbohydrate diet) the Randle cycle has been found to operate in both soleus and EDL muscles. It should be stressed that the effect of intramuscular TG on glucose-fatty acid interaction may be of special importance in humans, whose intramuscular TG stores are much greater than in other species, for instance rats, and in consequence may provide a substantial amount of energy both in resting and contracting muscles masking the extramuscular glucose-FFA reciprocal relationship (45).

Effect of excessive glucose provision on FFA metabolism (glucose-fatty acid cycle reversed)

It is well-documented that in the basal state, after 16-19 h fasting an intact human muscle oxidizes mostly fat with minor contribution of extracellular and intracellular carbohydrate utilization (2). However, after a meal excessive glucose provision and elevated plasma insulin level markedly affect the whole-body and muscle metabolism (20, 126). Sidossi et al. (115) have indicated that during hyperinsulinemic, hyperglycemic clamp muscle acylcarnitine formation and FFA entry into mitochondria are suppressed. Therefore, in addition to the well-known control of FFA oxidation by insulin antilipolytic activity, glucose plus insulin directly influence FFA oxidation by controlling their transport into mitochondria. This relationship and the all-important effect of excessive glucose on FFA utilization has been named the "reversed glucose-fatty acid cycle" (116).

The primary control over the ability to oxidize long-chain fatty acids in the muscles proposed by Sidossis (115) was vested in the opposing pathway of FFA biosynthesis, in such a way that when the latter was active, the former was suppressed and

vice versa. The linking molecule turned out to be malonyl-CoA, the product of the first committed step of FFA biosynthesis via acetyl-CoA carboxylase (ACC) reaction. Malonyl-CoA proved to be a potent inhibitor of carnitine palmitoyltransferase I (CPT I), essential for FFA entry into mitochondria and oxidation. In fed state (high insulin, low glucagon) the carbon flow through the lipogenic pathway is efficient in increasing PDH activity, acetyl-CoA and malonyl-CoA concentration. The latter in turn suppressed CPT I activity and fatty acid oxidation. Conversely, during fasting (low insulin, high glucagon) PDH activity is suppressed, acetyl-CoA and malonyl-CoA concentrations fall, FFA biosynthesis ceases and CPT I activity increases (4, 75, 80, 84). Under these conditions FFA entry into mitochondria and their oxidation effectively increase.

It should be pointed out that in this concept PDH activity fluctuations, due to substrate supply, corroborate the original Randle concept.

Additionally, similar mechanism of glucose-fatty acid interaction with the crucial role of malonyl-CoA was proved to operate in lipogenic tissues.

The above data indicate that both in vivo and in vitro metabolic milieu of glucose-fatty acid interaction is complicated and the experimental conditions may significantly contribute to inconsistent results of different studies.

Assuming both intramuscular and blood-borne (extramuscular) carbohydrate and fatty acid sources, FFA action on hepatic glucose production and insulin secretion, mutual interaction of glucose and FFA on its transport across muscle cell membranes, the discrepancies concerning the operation of the cycle and its mechanism are not surprising (Fig. 1).

It should be stressed that in a recent update of his tremendous contribution to fundamental aspects of carbohydrate and lipid relationship, Randle has incorporated many new ideas into the overall theory (94, 97).

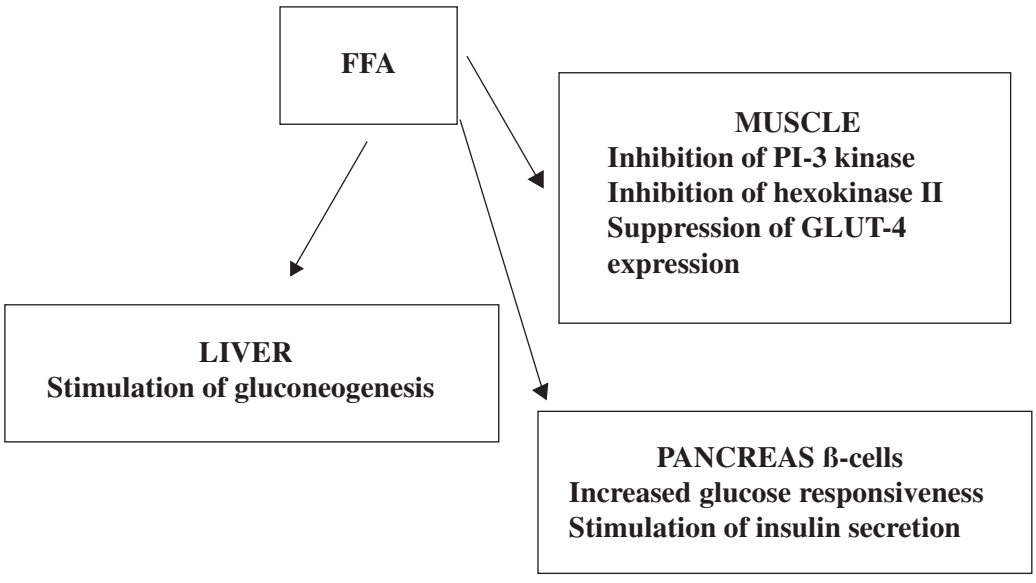

Fig. 1. *The influence of FFA on glucose metabolism which may affect reciprocal FFA-glucose interactions*

Glucose-FFA cycle in contracting muscles

The reciprocal relationship between carbohydrate and lipid metabolism during muscular contraction has been extensively studied but it is still a matter of controversy (53). It is worth noting that Brooks and Mercier (17) have described the balance of carbohydrate and fat utilization during exercise and named it "crossover" concept. Briefly, with increasing exercise intensity carbohydrate oxidation progressively increases concomitantly with decreasing fat oxidation, with a cross point depending on the diet and/or training status. However, although increased carbohydrate utilization with increasing exercise intensity is commonly accepted, there is no agreement concerning the relationship between exercise intensity and fat utilization. Recent data have indicated that in trained subjects fat oxidation increases significantly up to the intensity of 65 % $\dot{V}O_2$max and thereafter decreases (106). Similarly, in moderately-trained women fat oxidation steadily increases up to the intensity of approx. 75% $\dot{V}O_2$max, and thereafter decreases (3). In the view of these facts a successive decline in fat utilization in the "crossover" concept has to be re-evaluated.

Glucose - FFA cycle operates in the working muscle

Corn oil given per os 3 h before heparin injection causes less liver and muscle glycogen store depletion, higher muscle citrate content, higher blood glucose and insulin concentrations and lower blood glucagon level in exercising rats in comparison with controls. In human muscle glycogen-sparing effect of elevated FFA plasma concentration due to depressed glycogen phosphorylase activity has been noted during exhausting cycling (85% $\dot{V}O_2$max) (29). Moreover, time to exhaustion during physical exercise was significantly longer in the oil-fed rats than in their counterparts fed a standard diet (52, 102). Reduction of fat availability with nicotinic acid -a potent inhibitor of adipose tissue lipolysis brought about an elevation in the glucagon level, acceleration of the glucose flux and increases in glucose and lactate oxidation during 90 min moderate exercise (16). Similarly, fat feeding (90 g heavy whipping cream) and lipid infusion resulted in sparing of the muscle glycogen in humans during 60 min of cycling at 60% $\dot{V}O_2$max (135). Furthermore, low muscle glycogen stores increase whole-body fat oxidation both at rest and during prolonged exercise (145 min at 70% $\dot{V}O_2$max) (139). Additionally, Zorrano et al. (150) has provided an evidence of free fatty acid-induced inhibition of glycolysis in the rat skeletal muscles during recovery from exercise.

Glucose-FFA cycle does not operate in working muscles

The lack of the effects of fat/heparin infusion on fuel selection has been demonstrated in sedentary subjects during 2.5 h cycling at 44% $\dot{V}O_2$max (99). Jeukendrup et al. (61) have not revealed any influence of the medium-chain triacylglycerol (MCT) ingestion on carbohydrate oxidation during 180 min cycling at 50% $\dot{V}O_2$ max in trained triathletes or cyclists. Similarly, in well-trained cyclists Horowitz et al. (54) failed to show any effect of MCT ingestion on glycogen oxidation during 30 min cycling at 84% $\dot{V}O_2$max. The lack of FFA effect on glycogen metabolism and glucose - fatty acid interaction has been reported in healthy male volunteers during intensive knee extension exercise (80 % MVC) (50). In this study it has been postulated that muscle carbohydrate metabolism is affected only when plasma concentration of lipids and lipid metabolism increase, being due to direct inhibition of glucose transport by FFA rather than to the classical glucose-fatty acid cycle.

In addition, it has been found that in endurance-trained men substrate oxidation during moderate exercise (50% $\dot{V}O_2$max) can be determined by carbohydrate availability and glycolytic flux directly controlling fatty acid entry into mitochondria and their oxidation, since glucose or sucrose ingestion before or during exercise significantly decreases lipid mobilization from adipose tissue and FFA oxidation from both extra and intramuscular stores (24, 41). The effect of glycolytic flux on FFA entry into mitochondria has been postulated to affect lipid utilization also during high-intensity exercise (80% $\dot{V}O_2$max) (117). It should be pointed out that the effect of glycolytic flux on FFA entry into mitochondria has been postulated by Sidossis et al. (116).

On the other hand, in vitro studies of Turcotte et al. 1995 (127) have revealed that circulating palmitate oxidation is not altered by glycogen depletion in contracting skeletal muscle. Similarly, it was found that muscle glycogen depletion increases blood-borne glucose utilization, but does not affect plasma FFA oxidation in the rat contracting muscles (103, 128).

These findings may suggest that during muscular work the regulation within the pathways of carbohydrate metabolism takes precedence over that between the pathways of lipid and carbohydrate metabolism.

Muscle glycogen stores and fuel selection during contraction

In the effort to understand the mechanism controlling fuel selection in the muscle a special role of muscle glycogen stores could not be omitted. It is well known that during prolonged contractions, depletion of muscle glycogen may lead to exhaustion even though fat is readily available probably due to inability to maintain the Krebs cycle intermediates (112). Thus, it appears that when intramuscular carbohydrate availability is limited fat combustion cannot take place sufficiently fast to deliver the necessary energy for contractions and the glucose- fatty acid cycle does not operate (103).

Training effects on glucose-fatty acid interaction

One of the hallmark effect of endurance training is a decrease in the total carbohydrate utilization and an increase in total fat oxidation during exercise of the same absolute intensity, with subsequent sparing of muscular glycogen stores (43). Van Loon et al. (132) have demonstrated that in trained cyclists and triathletes exercising 120 min at 50% $\dot{V}O_2$max fat oxidation contributes more to energy expenditure than carbohydrates in comparison with their untrained counterparts. In addition, a fatty meal consumed 4.5-5 h before exercise causing an increase in the plasma FFA concentration decreases (by 40%) the rate of muscle glycogen depletion in runners during 30 min of a treadmill exercise (70% $\dot{V}O_2$max) (23). Similarly, the medium-chain triacylglycerol (MCT) ingestion decreased directly or indirectly (via lactate) oxidation of muscle glycogen during moderate physical activity in well-trained cyclists (151). These findings may indicate existence of the glucose-FFA cycle during exercise.

It is worth noting that recent studies have demonstrated an increased reliance of working muscles on intramuscular TG stores in trained humans (21, 55, 62, 78, 117). Therefore, it could not be excluded that there is a reciprocal relationship between the intramuscular TG and glycogen stores. in trained subjects.

On the other hand, in rats chronic electrical stimulation increased FFA transporter number in the sarcolemmal membrane and in consequence exogenous FFA transport into the muscle cells and caused greater reliance of contracting muscles on

extracellular FFA oxidation (15). In addition, in trained rat muscles during contraction increased exogenous FFA oxidation and sparing of intramuscular TG stores have been reported (28). Therefore, it could not be excluded that the regulation within fat stores takes precedence over the regulation between lipids and carbohydrates. It should be pointed out that a similar relationship has been demonstrated in resting human muscles (148).

The mechanism of glucose-FFA interaction in the muscle

FFA influence on PDH activity, citrate and glucose-6-phosphate concentration in the muscles

The original mechanism of the cycle, the effect of excessive FFA provision on glucose-6-phosphate and citrate concentrations and PDH activity in resting human muscle has been questioned (12,13). Also in the rat there was no effect of FFA on the muscle citrate, glucose-6-phosphate and phosphofructokinase activity. (9). In mice an inhibition of FFA oxidation by Etomoxir has been shown to increase PDH activity and glucose metabolism in the heart and liver, but not in the skeletal muscls (18). In addition, in exercising humans citrate concentration in *vastus lateralis* muscle was not affected by 2 h cycling at 60% $\dot{V}O_2$max, indicating a lack of the mechanism proposed in the classical glucose-fatty acids concept (22).

More recent studies have demonstrated that a high-fat, low-carbohydrate diet resulting in the excessive FFA supply elevates muscle PDH kinase activity, responsible for PDH phosphorylation and its subsequent inactivation indicating that excessive fat provision depresses oxidative glucose metabolism and this is in keeping with the glucose-fatty acid cycle (36, 91). Unfortunately, in the above studies the response of the muscle enzymes involved in fat metabolism (CPT I, β-HAD) did not increase following the high-fat diet suggesting the lack of the effect of fat availability on FFA oxidation.

Malonyl-CoA in muscles under resting conditions

In vitro studies on the rat skeletal muscle have revealed that CPT I activity is very sensitive to the malonyl-CoA inhibition and being fully reduced inhibited at the malonyl-CoA concentrations exisiting in intact cells (109). On the other hand, it has been found that the rat malonyl-CoA content is sensitive to fasting and refeeding (143). In the isolated rat soleus muscle malonyl-CoA content has been found to increase in response to insulin and denervation (111). In the presence of glucose, insulin acutely elevates malonyl-CoA by increasing the cytosolic concentration of citrate, an allosteric activator of acetyl-CoA carboxylase (ACC), the enzyme, which catalyzes malonyl-CoA synthesis, and a potent inhibitor of phosphofructokinase (110).

Recent studies have indicated that the elevation in malonyl-CoA content in the rat muscles during refeeding after fasting is not due to the increase in the activity of either ACC, or cytosolic citrate. The most likely cause of increased malonyl-CoA was recognized as a decrease in the cytosolic concentration of LCFA-CoA (long-chain fatty acid-CoA) - an allosteric inhibitor of acetyl-CoA carboxylase (19).

Despite all discrepancies it is likely that at least in the rat muscles regulation of ACC activity, a key enzyme in malonyl-CoA synthesis plays a crucial role in the fuel selection.

The operation of the glucose-FFA cycle via malonyl-CoA action in the human muscle is less clear. The basal malonyl-CoA concentration in human *vastus lateralis* muscle is much lower than in the red

portion of the rat gastrocnemius muscle (87). In addition, Starrit et al. (118) using the intact *vastus lateralis* muscle mitochondria have found that CPT I sensitivity to malonyl-CoA is less than those reported for the rat skeletal muscles. However, in vivo studies have revealed that both citrate and malonyl-CoA concentration in the human *vastus lateralis* muscle significantly increases in response to an infusion of glucose and insulin at a high rate (insulin at 1.0 mU/kg/min, glucose at 8.1 mg/kg/min) together with significant (by 41%) elevation in the whole-body fatty acid oxidation. Futhermore, an inverse, significant correlation has been demonstrated between the malonyl-CoA concentration in skeletal muscles and the whole-body fatty acid oxidation (8).

Because in the above studies PDH activity in the muscles was not evaluated, therefore, it is still unknown whether excessive glucose availability counterregulates both CPT I and PDH activity in the muscles as it was observed in the liver.

Malonyl-CoA in contracting muscles

The data concerning the malonyl-CoA response to contractile activity in rat muscles seems to support its crucial role in the regulation of lipid and carbohydrate metabolism. Sciatic nerve stimulation in vivo acutely decreases malonyl-CoA content in the rat muscle (27). It has been revealed that malonylo-CoA concentration decreases in working fast-twitch oxidative muscle fibers (98). Interestingly, the decrease in malonyl-CoA level in contracting muscle has been noted before the depletion of muscle and liver glycogen occurred and before a decline in blood glucose concentration. Glucose infusion prevented the drop in the liver malonyl-CoA and attenuated the progressive decline in the muscle malonyl-CoA in exercising rats (30). Exercise has been found to affect muscle PDH activity. Increased duration of exercise led to reduced PDH activity, probably due to elevated FFA oxidation (25). Therefore, it could be presumed that in exercising rat muscles the regulation of carbohydrate and lipid metabolism is similar to that found in the liver with interactive regulation of CPT I and PDH activity with crucial role of malonyl-CoA in this process.

However, there is lack of studies which simultaneously determined malonyl-CoA concentration, and CPT I and PDH activity in contracting rat muscles and at present the operation of this mechanism could be only speculated upon. Nevertheless, it has been speculated that insulin, glucose and contractile activity regulate the concentration of malonyl-CoA in the muscle and malonyl-CoA was recognized as a component of fuel-sensing and signaling mechanism (Table 1). Data concerning malonyl-CoA response to a single exercise in human muscles are scarce and they have not supported its key role in the regulation of carbohydrate and lipid metabolism. Odland et al. (87, 88) have demonstrated that malonyl-CoA concentration in human vastus lateralis remained unchanged during exercise varying form 35 to 90% $\dot{V}O_2max$.

Effect of exercise training effect on the malonyl-CoA content in muscles

Data concerning the training effect on CPT I activity and malonyl-CoA concentration in rat muscles are scarce and contradictory. An early study of Guzman and Castro (47) has demonstrated that 8-week endurance training (treadmill running, 45 min/day, 5 days a week) induces a significant elevation in CPT I activity in the hindlimb muscles. Furthermore, after training the enzyme sensitivity to pH has been markedly attenuated, despite similar susceptibility to malonyl-CoA inhibition. The above data may indicate that after endurance

Table 1. *Malonyl-CoA as a key metabolite in the regulation of oxidative FFA and glucose metabolism*

Increased carbohydrate availability (high insulin, low glucagon)
1. Increased PDH activity, acetyl-CoA and citrate formation 2. Stimulation of acetyl-CoA carboxylase (ACC) activity by citrate 3. Increased malonyl-CoA production 4. Inhibition of CPT I activity by malonyl-CoA, FFA transport into mitochondria and oxidation favours glucose metabolism
Depressed carbohydrate availability (low insulin, high glucagon)
1. Decreased PDH activity, acetyl-CoA and citrate formation 2. Lower activity of acetyl-CoA carboxylase due to shortage of citrate 3. Low malonyl-CoA production 4. Elevation in CPT I activity, increased FFA transport into mitochondria and oxidation 5. The inhibition of acetyl-CoA activity by acyl-CoA further depresses malonyl-CoA formation and favours FFA metabolism

training FFA utilization in the muscle may be sustained even at lowered pH – e.i. during high-intensity exercise, reflecting greater reliance of trained muscle on FFA oxidation.

On the contrary, Hutber et al. (56) have revealed that a decrease in the malonyl-CoA concentration in the red quadriceps muscle in response to prolonged exercise in trained rats is less than that in their untrained counterparts. Moreover, training has prevented the exercise-induced increase in the constant for the citrate activation of acetyl-CoA carboxylase (ACC), a key enzyme in malonyl-CoA synthesis resulting in higher ACC activity after than before training. The above findings may indicate that the training-induced greater FFA utilization is not due to lowered malonyl-CoA synthesis and subsequent elevation in CPT I activity. On the other hand, in trained rats pronounced muscle glycogen sparing has been observed suggesting greater reliance of the working muscle on FFA oxidation.

Despite the above-mentioned low malonyl-CoA content in human muscles and the lack of its response to exercise, CPT I was shown to be senstive to training. Recently, it has been shown that in aerobically trained individuals CPT I activity in the *vastus lateralis* muscle is by 89% higher than in untrained subjects (118). In addition, in trained subjects CPT I is more sensitive to the inhibition by the malonyl-CoA. The concentration of malonyl-CoA inhibiting 50% of CPT I activity in trained subjects equals 0.17 mM, whilst in untrained ones 0.42 mM. Furthermore, CPT I activity is very sensitive to pH changes in the muscle and even a small decline in pH (from 7.0 to 6.8) results in significant (about 2-fold) reduction in CPT I activity both in trained and untrained subjects. Moreover, the enzyme is inhibited by low concentration of acetylcarnitine, but this effect is abolished when the acetylcarnitine concentration is increased to the level expected to occur in skeletal muscle during increased FFA utilization (e.g. exercise). The above data may indicate that the aerobic training effect promoting FFA oxidation in the muscles may be due to the training - induced changes in CPT I regulation by malonyl-CoA and intramuscular pH.

In summary, there is no doubt that fuel selection between lipids and carbohydrate plays a fundamental role in the regulation

of the whole-body metabolism in response to starving-refeeding transition and during increased energy demands (e.g. exercise). However, according to the current data the mechanism of this relationship is extremely complicated. Both extramuscular and intramuscular fat and carbohydrate contribution in covering the energy demands, reciprocal relationship between extramuscular and intramuscular fat or carbohydrate metabolism, the diet and training effect on energy stores and fuel selection, liver contribution to glucose production, FFA effect on insulin secretion and glucose transport as well as hormonal milieu in response to increased carbohydrate provision have to be included to the overall concept of glucose - fatty acid interaction.

In lipogenic tissue such as liver the mechanism of glucose-fatty acid cycle seems to operate via regulation of PDH and ACC activity by carbohydrate provision and subsequent fluctuations in malonyl-CoA concentration, a potent inhibitor of CPT I activity and FFA entry into mitochondria.

However, the data concerning the mechanism of the cycle operation in skeletal muscles are still fragmentary, although they suggest a mechanism similar to that in the liver with crucial roles of PDH, ACC, and malonyl-CoA concentration as well as and CPT I regulation in response to substrate availability.

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