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## TURNING DOWN LIPID OXIDATION DURING HEAVY EXERCISE - WHAT IS THE MECHANISM?

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A high potential for lipid oxidation is a sign of metabolic fitness and is important not only for exercise performance but also for health promotion. Despite considerable progress during recent years, our understanding of how lipid oxidation is controlled remains unclear. The rate of lipid oxidation reaches a peak at 50-60% of  $\dot{V}O_{2\max}$  after which the contribution of lipids decreases both in relative and absolute terms. In the high-intensity domain ( $>60\% \dot{V}O_{2\max}$ ), there is a pronounced decrease in energy state, which will stimulate the glycolytic rate in excess of the substrate requirements of mitochondrial oxidative processes. Accumulation of glycolytic products will impair lipid oxidation through an interaction with the carnitine-mediated transfer of FA into mitochondria. Another potential site of control is Acyl-CoA synthetase (ACS), which is the initial step in FA catabolism. The activity of ACS may be under control of CoASH and energy state. There is evidence that additional control points exist beyond mitochondrial influx of fatty acids. The electron transport chain (ETC) with associated feed-back control by redox state is one suggested candidate. In this review it is suggested that the control of FA oxidation during heavy exercise is distributed between ACS, CPT1, and ETC.

Key words: *carbohydrate oxidation, exercise, lipid oxidation, mitochondria, metabolic regulation*

## INTRODUCTION

The body storage of carbohydrates (CHO) is limited and excess fuel is stored as fat. This is of obvious physiological advantage due to the 9-fold higher energy density of fuel stored as fat compared with CHO. Lipid oxidation contributes significantly to whole-body energy turnover both at rest and during submaximal exercise. An increased oxidation of lipids will delay the depletion of CHO and will, therefore, improve endurance during prolonged exercise. A high potential for lipid oxidation is not only of importance for exercise performance but has obvious health benefits in reducing the risk of developing metabolic diseases *e.g.* obesity and type 2 diabetes (1, 2). An increased understanding of how lipid oxidation is controlled, and which factors are of importance in its control, is therefore of great significance.

The rate of energy turnover increases in parallel with increased exercise intensity and is during low-moderate intensity associated with an increased utilization of both fat and CHO. The availability of substrate in blood, *i.e.* the concentration of glucose and/or fatty acid (FA), is a major factor determining fuel utilization at rest and during low-intensity exercise. At higher exercise intensities there is a shift from blood borne fuels to intramuscular stores (3). There is also a shift from fat to CHO oxidation and lipid oxidation is reduced both in relative terms and in the absolute amount used. The maximal rate of fat oxidation (FATmax) (4) is reached at an exercise intensity of about 50-60 % of  $\dot{V}_{O_2 \max}$  (4, 5). Endurance training results in an increased FATmax and the exercise intensity eliciting FATmax is also moved upwards (5). The training-induced improvements in fat oxidation are likely related to muscular adaptations to training such as increased mitochondrial volume and increased capillary density.

The control mechanism for turning down FA oxidation (FAox) during heavy exercise is not fully understood. Potential sites of control include reduced intracellular availability of FA and limitations in the carnitine-mediated transport of FA into mitochondria. Furthermore, there is evidence that the interaction between CHO and fat metabolism can occur by substrate competition within mitochondria and it is possible that the electron transport chain (ETC) and mitochondrial redox state are of importance in the control of fuel utilization. The purpose of this review is to discuss the mechanisms related to the control of FAox during heavy exercise and to present some new ideas in this field of research.

## MAXIMAL RATE OF FAOX IS LOWER THAN THAT OF CHOOX

The maximal rate of CHOOx during exercise is about 2-fold higher than that of fat. The predominate source of CHO during high-intensity exercise is glycogen and depletion of muscle glycogen will therefore severely limit energy supply and maximal power output. The explanation for the difference in maximal rate of fuel utilization is not clear. One possibility is that the metabolic pathway for fat oxidation is limited by the activities of involved enzymes or by the rate of fuel

transport. Some indirect findings of the metabolic response to extreme endurance exercise support this idea. During ultramarathon running, the respiratory exchange ratio (RER) is reduced and approaches 0.7 at the end of the race (6) demonstrating that fat is the sole substrate oxidized, presumably due to depleted CHO stores. The power output decreased during the run and at the end of the race was about 50-60% of  $\dot{V}_{O_2 \max}$  (6). This would suggest that the maximal rate of energy production is severely limited when fat is the sole available fuel. These findings demonstrate that the maximal rate of CHOox is higher than that of FAox and that the lower power of FAox is due to other factors than interference from CHO metabolism. This idea is supported by experiments in isolated mitochondria. In rat soleus, which is an oxidative muscle, oxidation of FA is similar to that of pyruvate whereas in EDL, which is a glycolytic muscle, FA oxidation is only 58% of that with pyruvate (7). In mitochondria isolated from human quadriceps muscle, relative FAox varies between subjects (49-93%, average 67%) and is, similar to that in rat, related to % type I fibers (8). The higher maximal rate of pyruvate oxidation in isolated mitochondria is in line with the higher maximal rate of whole-body CHOox during exercise. The shift from fat to CHOox at higher exercise intensities may therefore in part be a necessity to meet the high energy demand. However, the reduction in FAox in absolute terms during heavy exercise cannot be explained solely on the basis of a low power of the process and additional mechanisms must therefore be present.

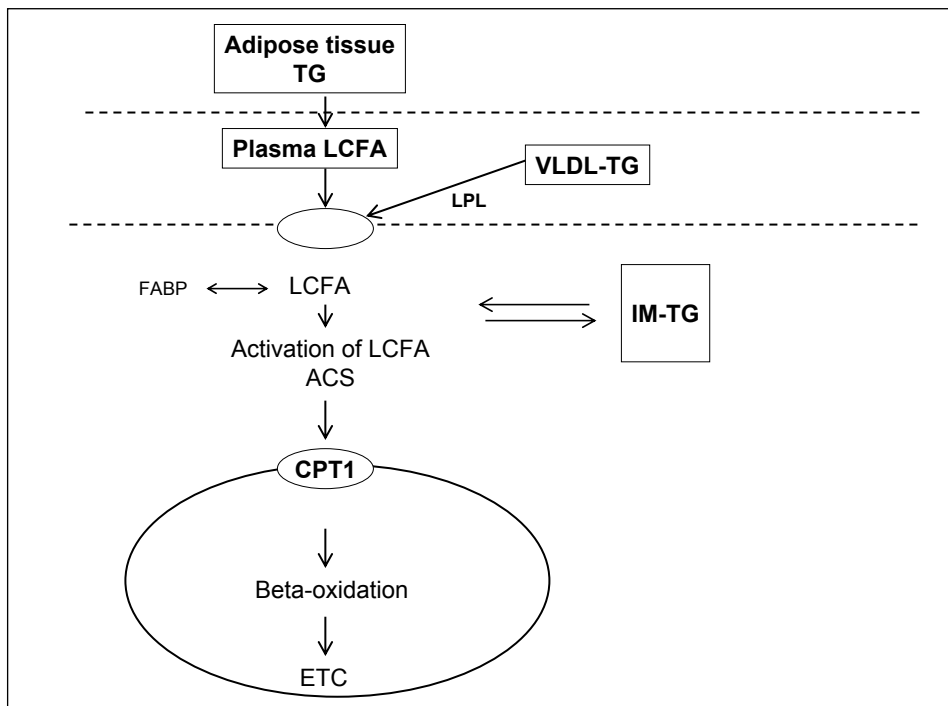
#### TURNING DOWN FA OXIDATION - WHAT IS THE MECHANISM?

Energy state or the energy charge of the adenine nucleotides (9) is a master regulator of metabolism. Energy state is reduced during exercise and it is well known that increases in ADP will stimulate key enzymes in oxidative metabolism (pyruvate dehydrogenase, tricarboxylic acid cycle and oxidative phosphorylation), which will increase oxidation of both CHO and FA. Increases in ADP (and related AMP) will also stimulate key enzymes in glycolysis (glycogen phosphorylase and phosphofructokinase). During heavy exercise, glycolysis is stimulated in excess of the fuel requirements of oxidation resulting in accumulation of glycolytic products, which may interfere with FAox.

The metabolic pathway of FA oxidation includes several potential sites of control (*Fig. 1*). The most plausible factors explaining the turn-down of FAox include: reduced intracellular availability of FA, reduced flux of FA into mitochondria and possibly substrate interference within mitochondria.

#### FA AVAILABILITY

Oxidation of FA at rest and during low-intensity exercise is to a large extent influenced by the concentration of FA in plasma and thus the availability of FA in



*Fig. 1.* Schematic view of the metabolic pathway of lipid oxidation. ACS, acyl-CoA synthetase; CPT1, carnitine palmitoyl transferase; ETC, electron transport chain; LCFA, long chain fatty acid; IM-TG, intramuscular triglyceride; LPL, lipoprotein lipase; VLDL-TG, triglycerides associated with very low-density lipoprotein particles.

the cytosol. A reduced FA concentration in the cytosol due to limited sarcolemmal transport of FA could therefore be involved in the shift from fat to CHO oxidation at high exercise intensities. Measurement of intracellular FA concentration is associated with methodological problems due to the binding of FA to proteins and lipids. Nevertheless, measurements of the muscle contents of FA in muscle biopsies taken at rest and after exercise (10) demonstrate that muscle content of FA was reduced after 40 min of exercise at 65% of  $\dot{V}_{O_2 \max}$  compared with that at rest. However, when the exercise intensity was increased to 90%  $\dot{V}_{O_2 \max}$  FA increased significantly (vs. 65%  $\dot{V}_{O_2 \max}$ ) and was not different from that at rest. The observed reduction in FAox at 90%  $\dot{V}_{O_2 \max}$  (judged from the increase in RER) could thus not be explained by reduced muscle content of FA. Furthermore, in another study where plasma FA was elevated by infusion of intralipid and heparin the reduction in FAox at 85%  $\dot{V}_{O_2 \max}$  could only partially be prevented (11). These findings demonstrate that FA availability, although an important determinant of fuel utilization at rest and low-moderate intensity exercise, is not the major factor behind the fuel shift at higher intensities. Instead focus of the discussion has been

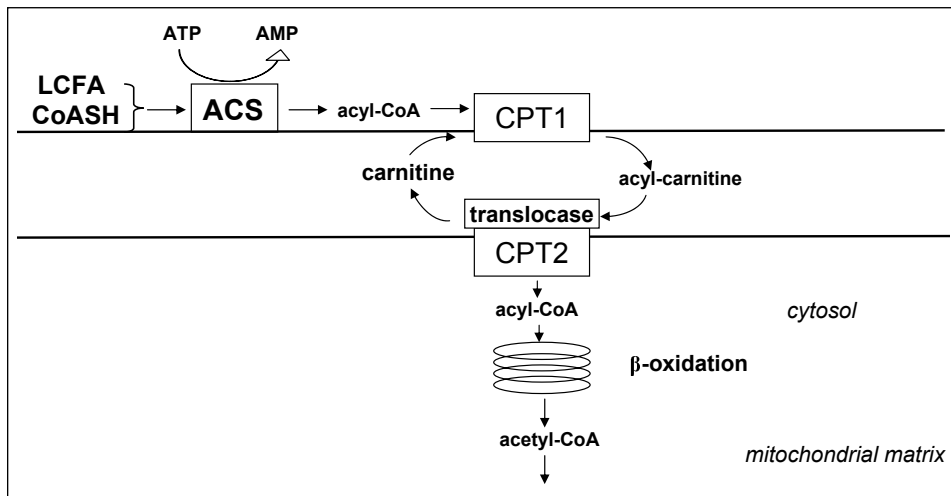


Fig. 2. Schematic view of the transport of long chain fatty acids through the mitochondrial membrane. ACS, acyl-CoA synthetase; CoASH, free Coenzyme A; CPT1 and CPT2, carnitine palmitoyl transferase; LCFA, long chain fatty acid.

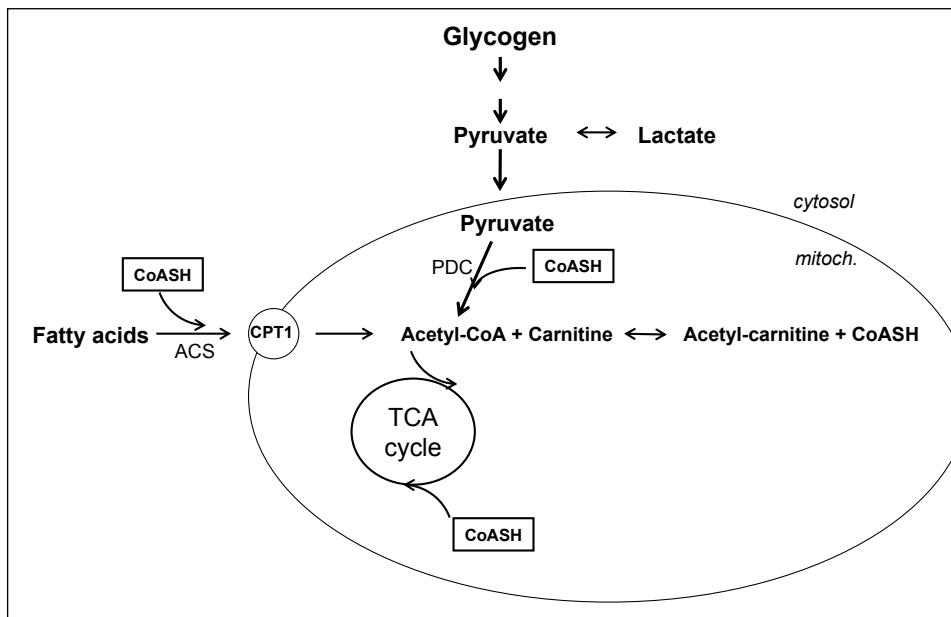
shifted and there is currently general consensus that mitochondrial factors are of major importance in the control (12-14).

#### TRANSPORT OF FA INTO MITOCHONDRIA

The major form of FA used in fuel oxidation is long-chain fatty acids (LCFA). LCFA are transported into mitochondria with a complex system involving a) an initial activation step by which FA is linked to CoASH forming acyl-CoA, and b) a carnitine mediated transport system (Fig. 2). Key enzymes in this transport process are acyl-CoA synthetase (ACS) and carnitine palmitoyltransferase (CPT1).

#### LIMITATIONS OF ACS ACTIVITY

Activation of FA by ACS is an energy-dependent reaction linked to hydrolysis of ATP to AMP. A control switch located at this initial step of FA catabolism would be advantageous since this would reduce trapping of CoASH as acyl-CoA. Studies in rat cardiac tissue have indeed demonstrated that the ACS reaction is inhibited by AMP and thus that ACS may be a target for control by energy state (15). However, the concentration of AMP required to inhibit ACS is quite high ( $K_i = 0.2$  mM, (15)) and several magnitudes higher than the concentration of the metabolic active form of AMP in skeletal muscle (about 1  $\mu$ M). The sensitivity of



*Fig. 3.* Converging metabolic pathways to Acetyl-CoA. ACS, acyl-CoA synthetase; CoASH, free Coenzyme A; CPT1, carnitine palmitoyl transferase; LCFA, long chain fatty acid; PDC, pyruvate dehydrogenase complex; TCA cycle, tricarboxylic acid cycle.

ACS for AMP in skeletal muscle is not known and further studies are required to investigate if ACS can function as an energy sensor and reduce FA oxidation during conditions of energy deficiency.

Another possibility is that the activity of ACS is controlled by the concentration of the free Coenzyme A (CoASH), which is a substrate to ACS. In cardiac tissue it was estimated that the concentration of free cytosolic CoASH is close to the  $K_m$  value (15) and thus that variations in the cytosolic CoASH concentration could be a powerful regulator of lipid oxidation. During high-intensity exercise glycolytic flux is increased several fold resulting in increased pyruvate and binding of CoASH into acetyl-CoA. Experiments in humans show that CoASH decreases by about 30-45% after exercise at 75-90% of  $\dot{V}_{O_2 \max}$  (16 - 18), whereas other studies in human (19) and rat muscle (20) show that CoASH remains unchanged despite 2-3 times elevation of acetyl-CoA. Measurements of total CoA (the sum of CoASH, acyl-CoA, acetyl-CoA) in homogenates from rat cardiac tissue demonstrated that most of the CoA is located in mitochondria (15). Measurements of whole tissue concentration of CoASH may therefore give limited information about the cytosolic concentration of CoASH, which is the relevant pool for FA activation by ACS. CoASH is a substrate of the mitochondrial enzymes PDC (pyruvate dehydrogenase complex) and oxoglutarate dehydrogenase (a key enzyme in the TCA cycle) (*Fig. 3*). A

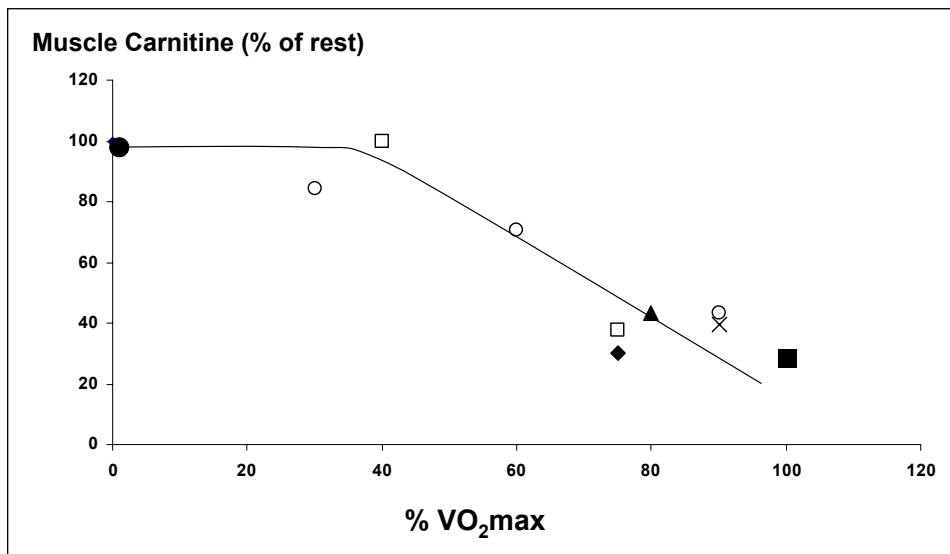


Fig. 4. Muscle carnitine during exercise at different intensities. Data points are mean values from different studies. Squares, data from (22); Open circles, data from (16); Filled romb data from (17); Filled triangle, data from (19), X, data from (23); Filled circle, values at rest (all studies).

conserved pool of mitochondrial CoASH is therefore necessary to maintain adequate flux of pyruvate oxidation and TCA cycle. A potential scenario is that the cytosolic concentration of CoASH is reduced during exercise at high intensity, thus limiting ACS activity and FA oxidation, but that the mitochondrial concentration of CoASH is maintained. Further studies are required to investigate whether ACS activity is limited by the concentration of CoASH in skeletal muscle and if this mechanism may restrict lipid oxidation at high exercise intensities.

#### LIMITATIONS OF CPT1 ACTIVITY

LCFA uses the carnitine-mediated transport system, whereas medium chain FA (MCFA) bypass this system and is independent of CPT1 activity. Studies in humans demonstrate that LCFA oxidation is reduced when exercise intensity is increased, whereas that of MCFA, is maintained (21) giving support for the view that CPT1 is an important control point of LCFA oxidation.

High-intensity exercise will stimulate glycolytic flux and result in accumulation of glycolytic products *i.e.* lactate, pyruvate, hydrogen ions and acetyl-CoA (16). Due to the equilibrium nature of the reaction catalyzed by carnitine-acetyl transferase, acetyl-groups will combine with carnitine by mass-action and form acetyl-carnitine (Fig. 3). The functional role of this reaction may relate to the necessity to

defend/buffer the mitochondrial level of CoASH and thus maintaining an adequate function of PDC and TCA cycle (16). Although total carnitine remains constant during exercise, there is a large decrease in free carnitine from about 5-6 mM at rest to 1-3 mM after heavy exercise (16, 17, 22, 23) corresponding to about 30% of the initial level (*Fig. 4*). Carnitine is required for the transport of LCFA into mitochondria and a decreased concentration may therefore limit lipid oxidation, an idea supported by the inverse correlation between lipid oxidation and muscle levels of acetyl-carnitine (12). The sensitivity of CPT1 to carnitine (the  $K_m$  value) has been determined by measuring the rate of palmitoyl-carnitine formation at different levels of carnitine. With this method the  $K_m$  for carnitine was determined to be about 0.5 mM for mitochondria isolated from both rat and human skeletal muscle (24). When the kinetics of CPT1 were measured with a more physiological approach using an integrated system analyzing the rate of FA oxidation at different carnitine levels, the observed  $K_m$  value of CPT1 for carnitine was somewhat lower (about 0.2-0.4 mM) (25). It can be calculated that, with a  $K_m$  of 0.2-0.5 mM and a decrease in free carnitine from 5.5 mM to 2 mM, CPT1 activity would decrease by only 5-13%. However, in analogy with CoASH it is possible that the concentration of carnitine in the cytosolic compartment is much lower than that in the whole tissue. Therefore, it cannot be excluded that the concentration of carnitine may influence lipid utilization. This idea is supported by recent experiments in humans where muscle carnitine was elevated by 15% by administration of carnitine during hyperinsulinaemic conditions (26). During conditions of elevated muscle carnitine there was evidence of an increased glycogen storage and acyl-CoA content suggesting an increased fat oxidation at rest (26). The results indicate that muscle carnitine levels may restrict FAox oxidation even at the high carnitine levels present in resting muscle.

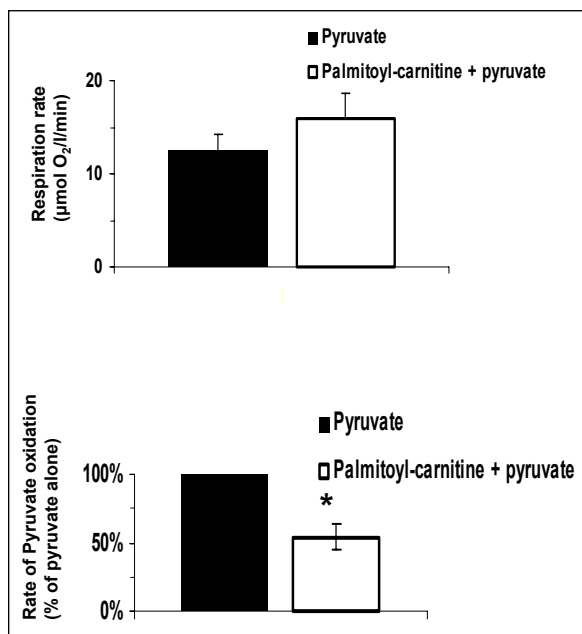
The activity of CPT1 has a strong pH dependency. In the presence of malonyl-CoA (an inhibitor of CPT1) the activity of CPT1 was reduced by 40% when pH decreased from 7.0 to 6.8 (27). This degree of acidosis corresponds to that observed in human muscle during exercise at 75%  $\dot{V}_{O_2 \max}$  (28), which is an exercise intensity where fuel utilization is switched from FA to CHO.

To summarize, there is a theoretical base and experimental evidence that transport of LCFA into mitochondria is a limiting step in FA oxidation. CPT1 is a well recognised control point and the effect of reduced carnitine levels and acidosis on this enzyme may, at least in part, explain the decrease in FAox during heavy exercise. Further studies are required to investigate the regulatory properties of ACS in skeletal muscle and the possibility that the activity of ACS is reduced by energetic stress or by limiting concentrations of substrate (CoASH).

#### SUBSTRATE COMPETITION WITHIN MITOCHONDRIA

Interaction between lipid and CHO oxidation has been studied at the whole body level, at the tissue level, in cultured myotubes (29) and in cultured heart





*Fig. 5.* Interaction between pyruvate and fatty acids in isolated mitochondria. Upper part: Respiration rate of isolated mitochondria with pyruvate (1 mM) or with pyruvate (1mM) + palmitoyl-carnitine (PC; 10 µM). Lower part: Rate of pyruvate oxidation in the absence and presence of 10 µM PC. Mitochondria were isolated from rat soleus muscle and respiration measured in an oxygraph during maximal ADP stimulation. The rate of pyruvate oxidation in the absence and presence of PC was measured as the rate of produced <sup>14</sup>CO<sub>2</sub> using pyruvate (1-<sup>14</sup>C). CO<sub>2</sub> was trapped in NaOH. \*, *P* < 0.05 Student *t*-test. The figure is derived from unpublished results.

cells (30). Only few studies have investigated substrate interaction in isolated mitochondria. Mitochondria isolated from soleus muscle in rats showed a 50% inhibition of FAox (oleate) in the presence of 2 mM pyruvate (31). However, the mitochondria were not stimulated with ADP and exhibited a non-physiological low rate of respiration. It is therefore unclear if the presence of pyruvate may reduce FAox during high rates of mitochondrial respiration.

We have recently examined substrate interaction in well-coupled mitochondria during conditions of maximal ADP stimulation. When mitochondria, isolated from rat soleus muscle, were respiring with both palmitoyl-carnitine (PC) and pyruvate, oxygen consumption was 26% higher than that with pyruvate alone *Fig. 5A*. Parallel measurements with tracers (pyruvate 1-<sup>14</sup>C) showed that pyruvate oxidation was reduced by about 50% in the presence of PC (*Fig. 5B*). The <sup>14</sup>CO<sub>2</sub> produced from pyruvate (1-<sup>14</sup>C) reflects decarboxylation catalysed by the pyruvate dehydrogenase complex. The mechanism for the observed inhibition of pyruvate oxidation by PC could be that β-oxidation of PC elevates mitochondrial concentrations of acetyl-CoA and NADH, which are known to inhibit PDC, the first step of pyruvate oxidation. PDC is activated by increased pyruvate concentration and the observed removal of the inhibitory effect of PC when the concentrations of pyruvate was increased from 1 to 5 mM is thus consistent with this hypothesis. However, this cannot explain how pyruvate can inhibit oxidation of PC (31).

A new and more intriguing explanation however, is that the electron transport chain (ETC) is an additional site of competition between fat and CHO oxidation.

The basis for this hypothesis is that catabolism of CHO to acetyl-CoA produces redox equivalents (NADH) feeding electrons into complex I, whereas catabolism of FA to acetyl-CoA produces redox equivalents feeding electrons into both complex I and II. Differences in the relative activities of complex I and II and in the feed-back control by redox state on PDC and  $\beta$ -oxidation may provide a theoretical base for a control at the level of the ETC. The hypothesis of substrate competition at ETC combined with feed-back control by the redox state can explain the inhibitory effect of PC on oxidation of pyruvate. Furthermore it can also explain the reduced rate of FA oxidation in the presence of pyruvate as observed in isolated mitochondria ((31) and our own preliminary observations), in cultured heart cells (30) and in cultured myotubes (29). The hypothesis of ETC being a site of control needs to be confirmed by further experiments.

#### PERSPECTIVES

Measurement of ATP/O<sub>2</sub> ratio in isolated mitochondria and of whole body oxygen demand demonstrate that the yield of ATP is about 10 % higher during CHO oxidation than during FA oxidation for the same amount of oxygen utilized. The higher ATP yield for CHOox is an obvious advantage when oxygen availability becomes critical. Measurements of muscle redox state demonstrate that, metabolism is influenced by oxygen availability already during submaximal exercise (*i.e.* 75% of  $\dot{V}_{O_{2max}}$ , (32)). The mechanism for the shift from lipid to CHO oxidation during heavy exercise may therefore be related to a decreased availability of oxygen. A similar shift from lipid to CHO oxidation occurs during exercise at high altitude (33, 34) and supports the idea that oxygen availability influences fuel utilization. The mechanism by which decreased O<sub>2</sub> availability could affect fuel utilization during exercise is unclear but it seems likely that reduced energy state could be involved as a mediator. The cross-over from FAox to CHOox at high intensities is important to maintain a high rate of energy production and to increase the efficiency of oxidative phosphorylation in terms of ATP/O<sub>2</sub> yield.

The down-regulation of FAox during heavy exercise is a phenomenon known for many years. Control of FAox can occur at several levels and carnitine-mediated transport of FA into the mitochondrial matrix is an accepted site of control. In this review it is suggested that FAox is controlled also at the initial step of FA catabolism *i.e.* ACS and by substrate competition at the electron transport chain. Further studies are required to investigate these hypotheses.

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