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The regulation of mitochondrial substrate utilization during acute exercise

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Mitochondria possess an immense capacity for energy (ATP) production and are capable of dramatically increasing fat and carbohydrate oxidation during exercise to maintain cellular ATP concentrations. This process is regulated by exercise intensity, which influences the mobilization/delivery of substrates to the mitochondria and activates several processes within mitochondria. This review focuses on the mechanisms influencing mitochondrial substrate utilization during exercise, with a particular emphasis on membrane transport as a key mechanism influencing oxidative phosphorylation. Specifically, recent evidence highlighting the regulation of fatty acid and ADP transport across mitochondrial membranes, as well as the control of carbohydrate oxidation by pyruvate dehydrogenase (PDH), will be discussed as rate-limiting steps coordinating aerobic ATP production in a highly complex manner during exercise.

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Introduction

Numerous metabolic pathways are activated at the onset of exercise to increase cellular energy production, however, a shift toward carbohydrate oxidation occurs at higher power outputs. As similar feedforward (calcium) and feedback (AMP, ADP) signals during exercise are involved in the upregulation of both fat and carbohydrate breakdown, this cannot account for the intensity-dependent regulation of substrate utilization. Instead, research has focused on mechanisms within the mitochondria, as this organelle coordinates carbohydrate and fat oxidation and is capable of vastly increasing ATP production to meet cellular demands during exercise. Many enzymes within the mitochondrial matrix involved in fat and carbohydrate oxidation are near-equilibrium, and therefore are inhibited by the accumulation of products. While both fat and carbohydrate pathways converge with the production of acetyl-CoA and NADH, the maximal capacity of pyruvate dehydrogenase (PDH) to produce these metabolites from carbohydrate sources is nearly 10-fold higher than the rate-limiting step in fatty acid oxidation (CPT-I), ultimately causing product inhibition on lipid breakdown. As a result, research has historically focused on understanding the regulation of PDH in reciprocal fuel interactions during exercise. However, additional regulation on aerobic respiration has recently been identified, including the control of mitochondrial ADP transport, and external regulation on mitochondrial lipid provision. This review will therefore focus on the regulation of mitochondrial fat and carbohydrate transport and oxidation during acute exercise, mechanisms capable of influencing substrate utilization at various power outputs.

Oxidative phosphorylation and mitochondrial ADP provision

Regulation directly within the electron transport chain (ETC) has historically not been considered, as research has largely focused on elucidating mechanisms influencing substrate provision to the ETC during exercise. However, this represents a simplistic view of the system, and the regulation of oxidative phosphorylation is likely a key event at all intensities of exercise. Recent work has therefore examined the influence of post-translational modifications on flux through the ETC during exercise, determining that muscle contraction increases glutathionylation of complex I, II, and ATP synthase [1•], events capable of altering mitochondrial respiratory function and reactive oxygen species (ROS)-producing potential *in vitro* [2]. Influenced by changes in cellular redox balance [1•,3], these modifications represent a likely mechanism involved in exercise-induced regulation of oxidative phosphorylation. In addition, deacetylation of complex I has been shown to increase activity of this respiratory complex contributing to the maintenance of ATP levels *in vivo* [4], further implicating complex I as a key regulatory point. While complex I sensitivity is increased in human muscle fibers following moderate intensity exercise [5], further suggesting direct regulation on this subunit, glutathionylation of this protein complex has yet to be determined in response to various power outputs [6•], and therefore our current understanding of intensity-dependent ETC regulation remains limited.

The movement of electrons through the ETC generates a proton motive force, utilized to produce ATP from the hydrolysis of ADP by ATP synthase. In this respect, the movement of ADP into the mitochondrial matrix is a key event during exercise, and it is not surprising that extensive regulation on ADP transport has recently been identified. Despite the increase in mitochondrial respiratory flux during exercise as a result of the rise in cytosolic ADP, it has consistently been shown that mitochondrial ADP sensitivity is attenuated in humans [5,7] and rodents [8•,9•,10••] following acute exercise. While the functional consequences of this event remain unknown, the reduction in ADP sensitivity is likely influenced by exercise-induced modifications on the mitochondrial inner membrane protein adenine nucleotide translocase (ANT). Specifically, an acute bout of exercise decreases lysine acetylation of ANT [11], a post-translational event which correlates with a decline in ADP sensitivity [12]. In addition, *in vitro* work has determined redox-mediated glutathionylation of ANT is capable of altering activity [13], and as exercise-induced reductions in ADP sensitivity are absent in mice with attenuated mitochondrial ROS, redox balance appears directly linked to the regulation of mitochondrial ADP sensitivity [8•]. While it remains unknown if ROS-mediated regulation of ANT occurs as a result of glutathionylation or acetylation, redox signaling has been shown to influence histone acetylation [14], raising the possibility that these mechanisms are coordinated. While ATP synthase is also influenced by redox modifications and could in theory contribute to the impairment in ADP sensitivity with acute exercise [1•,15], redox regulation of ATP synthase has been shown to alter maximal capacity [15], which does not occur following acute exercise [8•,9•,10••]. In addition, while mitochondrial creatine kinase (MiCK) was classically thought to enhance ADP transport by concentrating ADP within the intermembrane space, mice lacking MiCK are not compromised during exercise [9•] and therefore ANT-mediated ADP transport appears to be of greater importance.

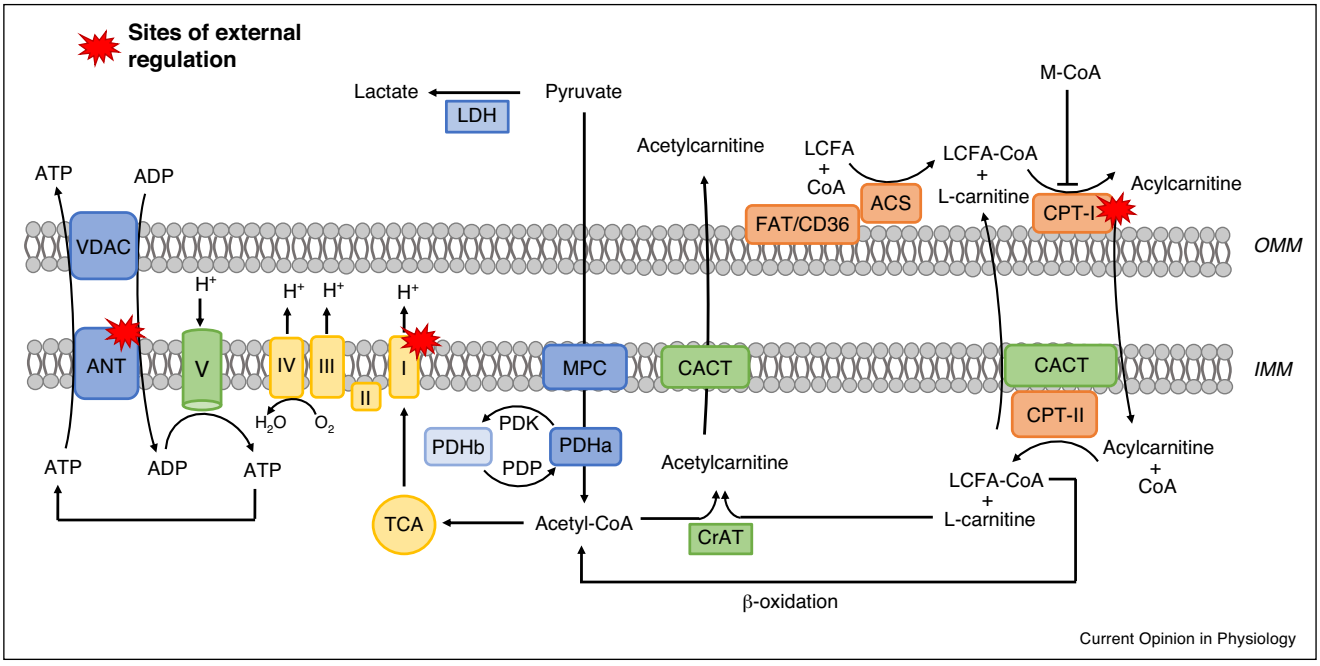
While the regulation of ADP sensitivity is not intensity dependent [10••], it is likely that a temporal relationship based on exercise duration exists, particularly as mitochondrial ROS production has been shown to increase in a delayed manner after 30 min of muscle contraction [16]. As ADP concentrations begin to plateau after the onset of exercise [17], it is possible that an impairment in mitochondrial ADP sensitivity during prolonged exercise is important to increase cytosolic ADP concentrations, allosterically maintaining glycolytic flux as glycogen granules become smaller; however, this remains to be determined. In support of a potential relationship between cytosolic ADP concentrations and impairments in mitochondrial ADP sensitivity, it has recently been established that women have lower ADP sensitivity than men at rest [18], in association with a greater rise in free ADP during exercise [19]. While this response could be related to the differences in substrate utilization during exercise between males and females [20], a direct comparison between metabolite concentrations, substrate utilization, and impairments in ADP sensitivity during exercise has yet to be performed. Regardless, ADP sensitivity is not regulated in an intensity-dependent manner [10••], and therefore does not appear to account for the shift in substrate utilization during exercise, particularly as oxidative pathways are shared between fat and carbohydrates sources.

Mitochondrial carbohydrate transport and oxidation

At higher intensities of exercise, allosteric activation of the glycolytic enzymes phosphorylase (PHOS) and phosphofructokinase (PFK) result in a large increase in pyruvate, which, in combination with activation of PDH, dramatically increases flux through PDH. Current understanding of mechanisms influencing PDH flux largely stem from classical literature identifying kinases and phosphatases as key covalent regulators of PDH, whereby dephosphorylation events activate PDH in an intensity-dependent manner, ultimately supplying acetyl-CoA during carbohydrate oxidation [17,21•]. Emerging evidence suggests PDH may also be regulated by post-translational glutathionylation on cysteine residues [22] and acetylation on lysine residues [21•], events which have been shown to influence *in vitro* PDH activity [23], PDH-derived ROS production [22], and mitochondrial pyruvate transport [2]. However, changes in PDH acetylation have not been identified during various power outputs in humans [21•], and as flux through PDH is closely matched with the intensity-dependent activity of dephosphorylated PDHa [17], the influence of these post-translational modifications on carbohydrate oxidation is debatable.

As the capacity of PDH to produce acetyl-CoA is much higher than flux through the tricarboxylic acid cycle (TCA), the products of PDH (acetyl-CoA and NADH) accumulate in an intensity-dependent manner. To prevent back inhibition on enzymatic flux, mitochondrial carnitine acetyltransferase (CrAT) is capable of buffering excess acetyl-CoA at the expense of free carnitine (Figure 1). This process is clearly important for sustaining carbohydrate oxidation during exercise, as mice lacking CrAT present with increased PDH phosphorylation, a compensatory increase in glycogen and fat breakdown, and impaired exercise performance [24]. In humans, considerable interest has focused on carnitine supplementation as a mechanism to augment acetyl-CoA buffering capacity and sustain carbohydrate oxidation, thereby delaying fatigue, during high intensity exercise. While increasing skeletal muscle carnitine content upregulated PDH activity at higher power outputs [25], this did not occur in exercise-trained individuals [26], suggesting the effects of training on matching PDH and TCA

Figure 1



Sites of regulation implicated in mitochondrial substrate utilization. Mitochondrial lipid transport is mediated by FAT/CD36, ACS, and CPT-I; a process requiring carnitine, and inhibited by M-CoA. Lipid and carbohydrate pathways converge at PDH, where CrAT is capable of buffering excess acetyl-CoA at the expense of free carnitine. Reducing equivalents derived from lipid and carbohydrate sources undergo a series of reactions in the electron transport chain (complex I–V) to produce ATP. External regulation of this process exists at the level of CPT-I, ANT, and within the electron transport chain (complex I), denoted by red stars. ADP, adenosine diphosphate; ANT, adenine nucleotide translocase; ACS, acyl-CoA synthetase; ATP, adenosine triphosphate; CACT, carnitine acylcarnitine translocase; CPT-I, carnitine palmitoyltransferase-I; CPT-II, carnitine palmitoyltransferase-II; CrAT, carnitine acetyltransferase; ETC, electron transport chain; FAT/CD36, fatty acid translocase/cluster of differentiation 36; IMM, inner mitochondrial membrane; LCFA, long-chain fatty acid; LDH, lactate dehydrogenase; MPC, mitochondrial pyruvate carrier; OMM, outer mitochondrial membrane; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PDP, pyruvate dehydrogenase phosphatase; TCA, tricarboxylic acid cycle; VDAC, voltage-dependent anion channel.

flux override the ability of increased carnitine content to buffer acetyl-CoA. While the accumulation of acetyl-CoA can cause product inhibition of PDH, it also represents a convergence point between fat and carbohydrate interactions, as increased production from PDH can attenuate lipid oxidation. In addition, buffering of excess acetyl-CoA at higher power outputs is capable of indirectly impairing lipid transport by decreasing carnitine content, a necessary substrate for mitochondrial fatty acid provision, indicating coordination between carbohydrate and fatty acid oxidative pathways.

Mitochondrial lipid transport

The provision of lipids to the mitochondrial matrix is a highly regulated process integral to fuel selection (Figure 1), mediated by the rate-limiting enzyme carnitine palmityoltransferase (CPT-I) [27,28]. As overexpression [29] and underexpression [30] of CPT-I dramatically alter rates of fat oxidation, and as exercise influences the oxidation of CPT-I-dependent, but not independent fatty acids [10••,31], CPT-I is clearly an essential enzyme for mitochondrial lipid transport. Furthermore, CPT-I is the only lipid enzyme subject to external regulation, as malonyl-CoA (M-CoA) is a naturally occurring inhibitor of CPT-I. In response to exercise, M-CoA content is decreased in rodent skeletal muscle [32], and therefore classical literature has largely focused on reductions in M-CoA content as an important regulatory point increasing fat oxidation during exercise. However, while M-CoA clearly inhibits CPT-I in human skeletal muscle, M-CoA content is not reduced during exercise at various power outputs in humans [33]. Moreover, classic experiments in isolated mitochondria have determined that concentrations of M-CoA required to inhibit CPT-I flux appear orders of magnitude lower than resting M-CoA content in human skeletal muscle [34]. These data suggest additional regulation exists within the CPT-I M-CoA axis, as even pronounced reductions in M-CoA content would not be expected to decrease the inhibitory effect on CPT-I, and therefore would have little influence on rates of skeletal muscle fatty acid oxidation. However, M-CoA sensitivity has historically been examined in isolated mitochondria and recent literature has determined that permeabilized muscle fiber preparations are more indicative of the *in vivo* environment, in which the concentrations of M-CoA required to inhibit CPT-I flux

are ~15-fold higher than previously determined. Regardless, it is clear that alternative mechanisms-of-action independent of M-CoA content must exist for the increase flux through CPT-I during exercise. While classical allosteric regulators of enzymes involved in carbohydrate breakdown do not influence CPT-I enzymatic properties (e.g. ADP, AMP), recent work has highlighted that the sensitivity of CPT-I to M-CoA is decreased during exercise [35,36], and in addition, exercise-induced increases in palmitoyl-CoA (P-CoA) [37] are further capable of overriding the inhibitory effect of M-CoA [35,38]. These cellular events regulating CPT-I during exercise therefore provide two independent mechanisms capable of increasing CPT-I flux in the absence of reductions in M-CoA content [8•,10••,35,36]. Moreover, in addition to attenuating the inhibitory effects of M-CoA, the increase in P-CoA content during exercise would independently increase CPT-I flux, given that P-CoA is a substrate for CPT-I [35,38]. As lipid delivery to skeletal muscle is proposed to decrease in response to high intensity exercise [39,40], it remains possible that a decline in P-CoA concentration is capable of attenuating CPT-I flux through this dual mechanism, therefore contributing to the reduction in fat oxidation above ~65% VO_2max.

In addition to M-CoA, several other theories implicate lipid transport as a key mechanism in reciprocal fuel interactions during exercise, including pH-mediated reductions in CPT-I activity [41], a decline in lipid delivery [39,40], and most notably, an intensity-dependent reduction in intramuscular carnitine content [42]. As a necessary substrate for CPT-I, a reduction in carnitine content is proposed to limit CPT-I flux [42,43], and work in mice has indeed identified a marked decline in fat oxidation following pharmacological carnitine depletion [44]. However, while skeletal muscle carnitine content is decreased at low intensities of exercise, rates of fat oxidation are optimal [42], challenging the prominent theory of carnitine-mediated inhibition of CPT-I flux. While it is possible that carnitine concentrations remain saturating at lower power outputs, this notion has largely been unproven. However, recently work from our laboratory in mouse permeabilized muscle fibers has established that carnitine content is well above saturating levels required for maximal CPT-I activity at rest [10••]. Furthermore, while carnitine content is mildly decreased at low intensities of exercise, this does not appear to limit CPT-I flux when considering CPT-I enzymatic properties. In contrast, the dramatic reduction in carnitine content following high intensity exercise, in combination with an ~30% attenuation in CPT-I sensitivity to carnitine, would be predicted to inhibit CPT-I flux *in vivo*. While these findings suggest an increase in carnitine content would have little effect on fat oxidation at low intensities of exercise, a previous report determined that 24-weeks of carnitine supplementation in humans lead to metabolic adaptations consistent with an increase in fat oxidation during low intensity exercise [25]. CPT-I sensitivity to carnitine has yet to be examined in human skeletal muscle in response to exercise and dietary manipulation, however, CPT-I flux and carnitine sensitivity nevertheless constitute an attractive regulatory point implicated in the shift in substrate selection during exercise.

While complex regulation of CPT-I sensitivity to M-CoA and carnitine clearly exists, the cellular events governing these changes remain unknown. Unlike ANT, CPT-I substrate sensitivity is not influenced by redox regulation during exercise [8•]. Alternatively, CPT-I contains several serine residues subject to phosphorylation in an AMPK-independent manner [35,45], and therefore it is likely that kinases associated with acute exercise (CaMKII, ERK, p38 MAPK) phosphorylate CPT-I and influence substrate sensitivity. In addition, cytoskeletal interactions are likely involved, as pharmacological disruption of intermediate filaments decreased CPT-I sensitivity to M-CoA in permeabilized muscle fibers, an effect which occurs with exercise [35]. While CPT-I is important in mediating lipid transport, additional pathways upstream of CPT-I also exert a role in this process during exercise. As a protein located on the outer mitochondrial membrane, fatty acid translocase/cluster of differentiation 36 (FAT/CD36) facilitates the transport of fatty acids to acyl-CoA synthetase (ACS) upstream of CPT-I [46,47]. While FAT/CD36 is not essential to support lipid transport, it enhances this process during exercise, as impairments in palmitate oxidation at rest in mice lacking FAT/CD36 are exacerbated following muscle stimulation [48], and overexpression of FAT/CD36 is capable of increasing palmitate oxidation in isolated mitochondria [49]. As FAT/CD36 is known to translocate to the mitochondrial membrane in a duration-dependent manner during exercise [36,50], this therefore may be important to increase lipid transport during prolonged, low intensity exercise. It also remains likely that FAT/CD36 is capable of concentrating P-CoA in close proximity to CPT-I, further promoting mitochondrial lipid transport and attenuating M-CoA inhibition. However, to date, little is known regarding the acute signaling events mediating FAT/CD36 translocation, or the influence of exercise intensity in this process. Similar to CPT-I, it does not appear this event is dependent on AMPK or cellular metabolites (AMP, ADP) during exercise [50], further highlighting the importance of other cellular events in the complex regulation of mitochondrial lipid transport.

Conclusion

While the intensity-dependent regulation of substrate utilization during exercise has long been examined, recent evidence clearly indicates the importance of regulation within mitochondria in this process. Controlled by substrate-product interactions, enzyme sensitivity, and post-translational modifications, the proteins involved

in substrate transport and oxidation are important for coordinating fat and carbohydrate utilization. CPT-I, PDH, and ANT are known to be key proteins subject to extensive regulation, particularly as CPT-I sensitivity to M-CoA and carnitine is dramatically influence by exercise. However, many unanswered questions remain, and future research is clearly needed for a more complete understanding of the mechanisms within mitochondria influencing the reciprocal relationship between fat and carbohydrate utilization during exercise.

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Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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