AMP-activated protein kinase regulation of fatty acid oxidation in the ischaemic heart

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Abstract
The heart relies predominantly on a balance between fatty acids and glucose to generate its energy supply. There is an important interaction between the metabolic pathways of these two substrates in the heart. When circulating levels of fatty acids are high, fatty acid oxidation can dominate over glucose oxidation as a source of energy through feedback inhibition of the glucose oxidation pathway. Following an ischaemic episode, fatty acid oxidation rates increase further, resulting in an uncoupling between glycolysis and glucose oxidation. This uncoupling results in an increased proton production, which worsens ischaemic damage. Since high rates of fatty acid oxidation can contribute to ischaemic damage by inhibiting glucose oxidation, it is important to maintain proper control of fatty acid oxidation both during and following ischaemia. An important molecule that controls myocardial fatty acid oxidation is malonyl-CoA, which inhibits uptake of fatty acids into the mitochondria. The levels of malonyl-CoA in the heart are controlled both by its synthesis and degradation. Three enzymes, namely AMP-activated protein kinase (AMPK), acetyl-CoA carboxylase (ACC) and malonyl-CoA decarboxylase (MCD), appear to be extremely important in this process. AMPK causes phosphorylation and inhibition of ACC, which reduces the production of malonyl-CoA. In addition, it is suggested that AMPK also phosphorylates and activates MCD, promoting degradation of malonyl-CoA levels. As a result malonyl-CoA levels can be dramatically altered by activation of AMPK. In ischaemia, AMPK is rapidly activated and inhibits ACC, subsequently decreasing malonyl-CoA levels and increasing fatty acid oxidation rates. The consequence of this is a decrease in glucose oxidation rates. In addition to altering malonyl-CoA levels, AMPK can also increase glycolytic rates, resulting in an increased uncoupling of glycolysis from glucose oxidation and an enhanced production of protons and lactate. This decreases cardiac efficiency and contributes to the severity of ischaemic damage. Decreasing the ischaemic-induced activation of AMPK or preventing the downstream decrease in malonyl-CoA levels may be a therapeutic approach to treating ischaemic heart disease.

Introduction
The heart has a high energy demand and in general derives most of this energy (60–80%) from the oxidation of fatty acids in the mitochondria [1]. However, the use of fatty acids as a fuel operates at the expense of carbohydrate utilization of glucose and lactate. This inverse relationship between the use of fatty acids and the use of carbohydrates becomes increasingly important in the clinical setting of ischaemia. Following an ischaemic episode, the heart becomes less efficient at converting energy into contractile function (see [2] for review). This decrease in efficiency occurs because fatty acid oxidation dominates over glucose oxidation as the main source of mitochondrial metabolism and overall ATP production. During ischaemia, glucose uptake and glycolysis also accelerate. Consequently, proton production increases [3,4] due to the uncoupling of glycolysis and glucose oxidation. Contractile energy in the form of ATP is then diverted towards re-establishment of ionic homeostasis.

Several studies indicate that altering energy metabolism pharmacologically to either decrease fatty acid oxidation or increase glucose oxidation can improve cardiac recovery following an ischaemic insult [5–17].

The excessive use of fatty acids as a fuel source during and following ischaemia is primarily the result of two factors: (i) circulating fatty acid levels rise dramatically during and following ischaemia [18–20] and (ii) subcellular changes occur in the heart that result in a decreased control of fatty acid metabolism [21–23]. One important subcellular alteration is a decrease in malonyl-CoA levels (an important endogenous inhibitor of fatty acid oxidation), which results in dysregulation of the fatty acid oxidation pathway [21–24]. The levels of malonyl-CoA are determined by the rates of the two enzymes involved in its synthesis and degradation [acetyl-CoA carboxylase (ACC) and malonyl-CoA decarboxylase (MCD), respectively]. These enzymes are also controlled by an AMP-activated protein kinase (AMPK), which is activated during ischaemia, resulting in a decrease in malonyl-CoA levels and a loss in mitochondrial fatty acid uptake control.

The purpose of this review will be to discuss the role of AMPK in controlling the enzymes involved in regulating malonyl-CoA levels in both the aerobic and ischaemic heart.

Key words: acetyl-CoA carboxylase, carnitine palmitoyltransferase, malonyl-CoA, malonyl-CoA decarboxylase, mitochondria.
Abbreviations used: AMPK, AMP-activated protein kinase; ACC, acetyl-CoA carboxylase; MCD, malonyl-CoA decarboxylase; CPT-1, carnitine palmitoyltransferase-1.
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**Control of fatty acid oxidation by malonyl-CoA**

Malonyl-CoA tightly regulates fatty acid metabolism by inhibition at the level of carnitine palmitoyltransferase-1 (CPT-1), which mediates fatty acid transport into the mitochondria (Figure 1) [25]. This inhibition of CPT-1 by malonyl-CoA results in decreased mitochondrial fatty acid uptake and thus reduced fatty acid oxidation rates. In the heart, malonyl-CoA seems to play an important metabolic signalling role and consequently is degraded very rapidly with a half-life of only 1.25 min [26]. Therefore, steady-state levels of malonyl-CoA are controlled by the dynamic rates of its synthesis and degradation. Malonyl-CoA is synthesized from acetyl-CoA in the heart by ACC [23,27,28]. Degradation of malonyl-CoA occurs by the reverse reaction via decarboxylation back to acetyl-CoA by the enzyme MCD [24,29–31]. Both ACC and MCD are suggested to play an important role in influencing fatty acid oxidation rates by altering malonyl-CoA levels.

Synthesis of malonyl-CoA occurs via ACC, which exists in two isoforms distinguished by molecular mass: ACC-α (265 kDa) and ACC-β (280 kDa) [32–35]. Both isoforms are present in the heart [32,34,35], although ACC-β predominates. An association of ACC-β with the mitochondrial membrane [34,36] led to the suggestion that ACC-β is directly involved in the regulation of fatty acid oxidation [23,34,36,37]. This would allow malonyl-CoA production to occur in close physical proximity to CPT-1 and enable direct CPT-1 inhibition. Studies by our group and others have provided direct evidence that ACC-β is an important regulator of fatty acid oxidation in cardiac myocytes [1,21–23,38–40]. These studies suggest that ACC-β plays a very important role in regulating fatty acid oxidation rates in the heart.

MCD (which decarboxylates malonyl-CoA to acetyl-CoA) [41–44] is also an important modulator of intracellular malonyl-CoA levels in the cardiac myocyte. We have previously shown that increased or maintained MCD activity in conjunction with a decrease in ACC activity is likely to be responsible for the decrease in malonyl-CoA levels and increased fatty acid oxidation seen in both the post-natal heart and the reperfused ischaemic heart [24]. Subsequent to our study, similar work by Goodwin et al. [31] demonstrated that MCD may control fatty acid oxidation in the heart during contractile stimulation. Other studies show an increase in both activity and expression of MCD in streptozotocin-induced diabetic rat hearts [45] and an increase in mRNA expression during high-fat feeding, fasting and streptozotocin-induced diabetes [46]. This group of studies and several others show a correlation between MCD activity and myocardial fatty acid oxidation rates, indicating that MCD may have a role in altering energy metabolism.

Interestingly, both enzymes involved in the control of cardiac malonyl-CoA levels have been suggested to be

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**Figure 1 | Uptake of fatty acids into the mitochondria through the carnitine shuttle is inhibited by malonyl-CoA at the level of CPT-1**

The rate of fatty acid uptake influences the rates of mitochondrial fatty acid oxidation.
Phosphorylation control of ACC and MCD by AMPK alters the levels of malonyl-CoA and subsequently influences rates of fatty acid oxidation

AMPK activation

ACETYL CoA

MCD

MALONYL CoA

Increased fatty acid oxidation

AMPK and regulation of malonyl-CoA levels

AMPK is a serine/threonine kinase which consists of three subunits, α, β and γ (see [52] for a comprehensive review of AMPK). The α catalytic subunit includes the kinase domain and is responsible for the transfer of a high-energy phosphate from ATP to specific protein targets [52]. The β and γ subunits act as regulatory components of the AMPK heterotrimer and each subunit has several distinct isoforms, including α1, α2, β1, β2, γ1, γ2 and γ3 [52]. AMPK is activated by metabolic breakdown products such as the AMP/ATP ratio and the creatine/phosphocreatine ratio [53]. However, cardiac AMPK activity can be changed under conditions where significant alterations in the AMP/ATP ratio do not occur [54,55]. In particular, insulin inhibits AMPK activity in isolated perfused hearts under conditions where the AMP/ATP and creatine/phosphocreatine ratios do not change [7,55,56], which may indicate an involvement of an upstream kinase AMPK kinase. AMPK kinase phosphorylates AMPK on Thr-172 and causes a significant increase in AMPK activity (greater than 50-fold; reviewed in [52]). Increased AMPK activity has been shown to increase phosphorylation of several targets including 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, hormone-sensitive lipase, glycogen synthase [52] and endothelial nitric oxide synthase [57].

In the heart, AMPK is as an important metabolic sensor, which is activated under conditions of metabolic stress such as hypoxia, or under conditions where ATP is depleted. AMPK is an important modulator of fatty acid metabolism in the myocardium by accelerating fatty acid oxidation when ATP is low, and decreasing fatty acid oxidation when ATP supply is high. The modulation of fatty acid metabolism occurs by potential phosphorylation of two key enzymes involved in the control of malonyl-CoA levels: ACC and MCD.

Cardiac ACC phosphorylation by AMPK appears to involve the regulation of fatty acid oxidation at the level of CPT-1. A close correlation exists between increased AMPK activity, decreased ACC activity and increased fatty acid oxidation in isolated working rat hearts [21,22,39,56]. It has been well established that AMPK is able to phosphorylate both isoforms of ACC [47–49] and we have shown that cardiac ACC co-purifies with the α2 isoform of the catalytic subunit of AMPK [58]. These studies suggest a tight association of AMPK with ACC in the heart and phosphorylation/dephosphorylation control of ACC. Recent work by Park et al. [59] shows a strong correlation between AMPK activity, ACC activity and phosphorylated ACC protein in electrically stimulated gastrocnemius muscle, providing further proof that AMPK phosphorylates and inactivates ACC. This inactivation results in a decrease in the synthesis of malonyl-CoA and thus relieves the inhibition of CPT-1-mediated uptake of mitochondrial fatty acids.

Since AMPK seems to be indirectly involved in the production of malonyl-CoA, several studies have also investigated whether MCD is a substrate for AMPK. The ability of AMPK to control both the production of malonyl-CoA (via ACC) and the degradation of malonyl-CoA (via MCD) is an attractive hypothesis. Our early studies have shown that purified AMPK does not modify MCD activity in vitro [30]. However, work by Saha et al. [50] suggests that 5-amino-4-imidazole carboxamide riboside (AICAR) activation of AMPK can increase MCD activity 2-fold in rat extensor digitorum longus muscle. The authors also suggest that muscle contraction-induced activation of AMPK leads to phosphorylation and activation of MCD. In contrast, it has also been shown that neither contraction-induced nor AICAR-induced AMPK activation of three rat fast twitch muscle types have any effect on MCD activity [60]. However, more recently Park et al. [51] have shown an increase in both AMPK and MCD activity in exercising muscle and liver, and more importantly that MCD activity could be reduced when incubated with protein phosphatase 2A. These results suggest that AMPK does phosphorylate MCD in exercising rat muscle and liver. The reasons for these discrepancies are unknown and thus the question of whether AMPK can phosphorylate cardiac MCD remains unclear. Recent studies from our laboratory have shown that AMPK activation in cardiac cells results in translocation of overexpressed MCD from...
the cytoplasmic space to the mitochondria (N. Sambandam, J.R.B. Dyck and G.D. Lopaschuk, unpublished work). Whether this involves the phosphorylation of AMPK has yet to be established. Phosphorylation control of MCD and ACC by AMPK becomes especially important in the setting of ischaemia. AMPK activity dramatically increases during ischaemia, causing alterations in malonyl-CoA levels through the phosphorylation of these two key regulatory enzymes.

**Ischaemia-induced alterations in cardiac metabolism**

There is a dynamic interplay between the oxidation of fatty acids and glucose metabolism in the heart. Acetyl-CoA derived from fatty acids can inhibit glucose oxidation at the level of the pyruvate dehydrogenase complex. Therefore, increased fatty acid oxidation decreases glucose oxidation. This occurs even though glycolytic rates in the heart can remain high, promoting the proton production observed during and following ischaemia. This is physiologically relevant since free circulating fatty acid levels increase during and following ischaemia due to increased hormone-sensitive lipase activity and breakdown of triacylglycerols [18–20]. Excessive fatty acid levels are detrimental to both the ischaemic heart [61,62] and the reperfused ischaemic heart [63,64]. While fatty acids have the potential to have direct lipotoxic effects on the heart [65–67], excessively high rates of fatty acid oxidation at the expense of glucose oxidation appear to be a key contributor to the adverse effects of fatty acids during and following ischaemia (see [68] for review).

During severe ischaemia, when oxygen is unavailable, the heart relies on glycolysis to produce ATP. Other energy-producing metabolic pathways are incapable of proceeding in the absence of oxygen. However, glycolysis results in the production of protons when uncoupled from the proton-consuming glucose-oxidation pathway. The reduction in coronary flow during ischaemia causes deleterious by-products of glycolysis, such as lactate and protons, to accumulate intracellularly. This excessive production of protons in the heart [3,4] can lead to an accumulation of sodium and calcium ions [69]. During reperfusion the clearance of these protons causes a decrease in cardiac efficiency as more energy is expended to re-establish a normal intracellular pH (see [68] for a review). If glucose oxidation levels are increased during reperfusion, the production of protons is lessened and improved coupling between glycolysis and glucose oxidation occurs. This in turn promotes improved cardiac recovery by increasing the efficiency of the heart to convert energy into contractile function.

**Alterations in AMPK activity during ischaemia/reperfusion**

During ischaemia/reperfusion AMPK has a dual role of increasing both fatty acid oxidation and glycolysis to increase overall energy production in response to high energy demand (Figure 3). Ischaemia causes a rapid increase in AMPK phosphorylation and activity [21,22,70]. AMPK activation may occur in order to increase myocyte glucose utilization by increasing both glucose uptake and glycolysis ([71–73]; Figure 3). For example, AMPK activation promotes the translocation of GLUT-4 to the sarcolemma of the myocyte to increase glucose uptake [74]. The glycolytic pathway is accelerated by the phosphorylation and activation of phosphofructokinase-2 by AMPK [73], which produces fructose 2,6-bisphosphate (a potent activator of glycolysis).

However, as well as accelerating the use of glucose, AMPK also has a potent effect on fatty acid oxidation rates. Activation of AMPK during ischaemia results in the use of fatty acids as the main source of residual oxidative metabolism. The consequence of this is an accelerated production of deleterious glycolytic by-products, particularly lactate and protons. These by-products are formed due to fatty acid inhibition of glucose oxidation, while glycolytic rates remain high.

AMPK activity persists during reperfusion of the myocardium [21,22] and during this time the heart continues to be exposed to high circulating levels of fatty acids. Fatty acid oxidation rates recover and provide the majority of the tricarboxylic acid cycle acetyl-CoA, again at the expense of glucose oxidation [13,61,75]. Glycolytic rates remain elevated [76,77], causing greater uncoupling between glycolysis and glucose oxidation and resulting in proton production [9,78] and reduced pH recovery following reperfusion [79].
AMPK plays an important role in regulating malonyl-CoA levels through the phosphorylation of ACC and the proposed phosphorylation of MCD. The activity of these enzymes is important in determining the steady-state malonyl-CoA levels in the myocardium. As levels of malonyl-CoA are responsible for inhibition of CPT-1 and control of fatty acid uptake into the mitochondria, malonyl-CoA is an important metabolic mediator. AMPK is physiologically important as a sensor of metabolic demand by modulating malonyl-CoA levels. AMPK accelerates both fatty acid oxidation and glycolysis in an attempt to replenish ATP production during ischaemia/reperfusion. Unfortunately, this dual role of AMPK leads to a greater uncoupling of glycolysis and glucose oxidation in the heart, leading to excessive proton and lactate production. AMPK may therefore be an important pharmacological target for improving cardiac efficiency following ischaemia.

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