

Molecular basis of cardiac efficiency

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Abstract

Cardiac efficiency is defined as the ratio between cardiac work and myocardial oxygen consumption. Impairment of cardiac efficiency is believed to contribute to contractile dysfunction in cardiac disease states such as diabetic cardiomyopathy, the hyperthyroid heart, and dilated cardiomyopathy. In diabetes, reduced cardiac efficiency is a common observation and is likely to result from alterations in cardiac energy-substrate metabolism. This review will discuss potential molecular mechanisms of reduced cardiac efficiency with a focus on diabetic cardiomyopathy, highlighting recent evidence that suggests mitochondrial uncoupling to be an underlying mechanism.

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Introduction

The heart is a highly oxidative organ. It relies almost exclusively on the oxidative generation of ATP from fatty acids, glucose, lactate, and other substrates, to maintain contractile function and cellular homeostasis. Under physiological conditions, oxidative production of ATP is relatively tightly coupled to mitochondrial oxygen consumption, thus making myocardial oxygen consumption ($m\dot{V}O_2$) a useful index of ATP turnover and metabolic activity by the heart. Cardiac efficiency is defined as the ratio between cardiac work and $m\dot{V}O_2$. Reduced cardiac efficiency is believed to contribute to contractile dysfunction in cardiac diseases such as diabetic cardiomyopathy, the hyperthyroid heart, and dilated cardiomyopathy [1–3]. As $m\dot{V}O_2$ is directly related to energy metabolism, it is not surprising that research revealed a link between impairment in cardiac efficiency and alterations in cardiac energy-substrate metabolism. This review will discuss potential molecular mechanisms of reduced cardiac efficiency, with a focus on mitochondrial uncoupling as a key underlying mechanism in diabetic cardiomyopathy.

Fatty acid induced mitochondrial uncoupling

Oxidation of energy substrates occurs in mitochondria and results in the generation of a proton gradient across the inner mitochondrial membrane that is used by the F_0F_1 -ATPase* to resynthesize ATP from ADP. Thus oxygen consumption is coupled to ATP synthesis (mitochondrial coupling). If protons bypass the ATP synthase when cycling across the mitochondrial inner membrane, heat is produced instead of ATP (mitochondrial uncoupling). Under physiological conditions, a certain proportion of protons always leaks through the membrane, in part as a result of slip reactions in the proton pumping machinery and other elusive mechanisms that do not result in ATP generation [4]. In addition, basal proton conductance is halved in muscle mitochondria from mice in which adenine nucleotide translocase 1 (ANT1)* has been ablated, also suggesting that a substantial part of basal proton leak is mediated by ANT1 [5].

Uncoupling proteins (UCPs)* belong to the superfamily of mitochondrial transport proteins and can also catalyze proton conductance across the mitochondrial membrane [6]. Neither UCP1, nor its

Basic article

Heiko Bugger and E. Dale Abel

homologs UCP2 or UCP3, seem to catalyze basal proton conductance, as conductance remains the same in mitochondria from UCP isoform knockout mice [7,8]. Rather, UCPs comprise a system of inducible proton conductance. Echtay et al [6] demonstrated increased proton conductance in isolated mammalian mitochondria after the addition of exogenous superoxide for UCP1, UCP2, and UCP3 – an activation that required the presence of palmitate, thereby extending previous findings [9] that reactive oxygen species (ROS)* cause fatty acid dependent uncoupling in plant mitochondria. Superoxide-induced UCP-mediated mitochondrial uncoupling also results from endogenously produced superoxide, and such an activation of UCPs was demonstrated to occur at the matrix side of mitochondria, thereby demonstrating that mitochondria-derived ROS can induce mitochondrial uncoupling [10]. Further studies demonstrated that the lipid peroxide 4-hydroxy-2-nonenal, a frequent byproduct of increased oxygen radical generation, can also trigger uncoupling activity of both UCPs and ANT [11].

In the mammalian heart, both UCP2 and UCP3 are expressed [12,13]. Their expression correlates with serum fatty acid concentrations and rates of myocardial fatty acid oxidation [14]. UCP3 and UCP2 (in part), are positively regulated by the transcriptional regulator of the fatty acid oxidative pathway, peroxisome proliferator-activated receptor α (PPAR α)*, as indicated by reduced expression of UCP2 and UCP3 in PPAR α null mice and increased expression of UCP3 in rats and mice treated with the PPAR α agonist, WY-14,643 [12,15]. The physiological significance of changes in the abundance of cardiac UCPs remains to be elucidated, but the existence of UCP-mediated uncoupling in the heart has been demonstrated recently, as discussed below.

In the postnatal mammalian heart, ATP is generated mainly from the oxidation of fatty acids (60–70%) and to a lesser extent from glucose, lactate, and other substrates (30–40%). Early studies in healthy rats demonstrated that increasing the cardiac uptake of fatty acids by infusions of Intralipid resulted in increased $m\dot{V}O_2$, but no increase in cardiac mechanical activity – that is, reduced cardiac efficiency [16]. Similar observations have been made in obesity and type 2 diabetes, in which conditions the heart also oxidizes relatively more fatty acids and less carbohydrates, leading to reduced cardiac efficiency. Peterson et al [17] showed that impaired glucose tolerance correlated with increased fatty acid utilization, and that an increase in body mass index was associated with increased $m\dot{V}O_2$ and reduced cardiac efficiency in obese and insulin-resistant women. Similarly, increased myocardial fatty acid oxidation and oxygen consumption are associated with unchanged or reduced contractile function, resulting in reduced

cardiac efficiency, in obese and diabetic ob/ob and db/db mice [1,18,19]. As both fatty acid oxidation and oxygen consumption occur in mitochondria, the findings of these studies suggest that the basis for impaired cardiac efficiency may be located within mitochondria. Work from our group showed that both ob/ob and db/db hearts pre-perfused with buffer containing glucose as the only substrate have impaired rates of ADP-stimulated mitochondrial oxygen consumption and ATP synthesis, probably caused by reduced expression of oxidative phosphorylation (OXPHOS) proteins*, but show normal ATP: oxygen ratios, indicating normal mitochondrial coupling [13,20]. In contrast, the addition of high concentrations of palmitate to the perfusion medium resulted in an increase in palmitoyl-carnitine-supported mitochondrial respiration back to wildtype levels, accompanied by reduced ATP: oxygen ratios, suggesting that the “normalization” of oxygen consumption was the result of uncoupled respiration. These data suggest that mitochondrial uncoupling was induced by fatty acids in the perfusion buffer. Further studies using proton leak kinetics demonstrated that proton conductance was increased in mitochondria of db/db but not wildtype hearts, and that addition of the UCP inhibitor, guanosine diphosphate, reduced proton conductance in db/db heart mitochondria, suggesting the presence of UCP-mediated uncoupling in db/db hearts [20]. Studies using the ANT inhibitor, atractyloside, showed that a small component of proton leak could also be attributed to ANT. Interestingly, the expression of UCP3 and that of ANT were not increased in db/db mice, suggesting an expression-independent mechanism of direct activation, consistent with the inducible nature of proton conductance by UCPs. Indeed, db/db hearts showed increased mitochondrial production of hydrogen peroxide and increased levels of 4-hydroxy-2-nonenal. As hydrogen peroxide production in isolated heart mitochondria is higher with palmitoyl carnitine as the substrate than with pyruvate, and because superoxide and 4-hydroxy-2-nonenal can directly activate UCPs, we propose the following mechanism for reduced cardiac efficiency in obesity and type 2 diabetes [6,11,21] (*Figure 1*).

Increased fatty acid oxidation in the diabetic heart may lead to increased production of mitochondrial ROS, resulting in activation of uncoupling proteins, which leads to increased oxygen consumption but reduced ATP synthesis. The deficit in ATP production may not allow an increase in cardiac work or may even lead to reduced cardiac work despite increased oxygen consumption, resulting in reduced cardiac efficiency. In combination with impaired mitochondrial oxidative capacity resulting from the downregulation of OXPHOS proteins, mitochondrial uncoupling may contribute to the development of contractile dysfunction as a result of a relative energy

Basic article

Molecular basis of cardiac efficiency

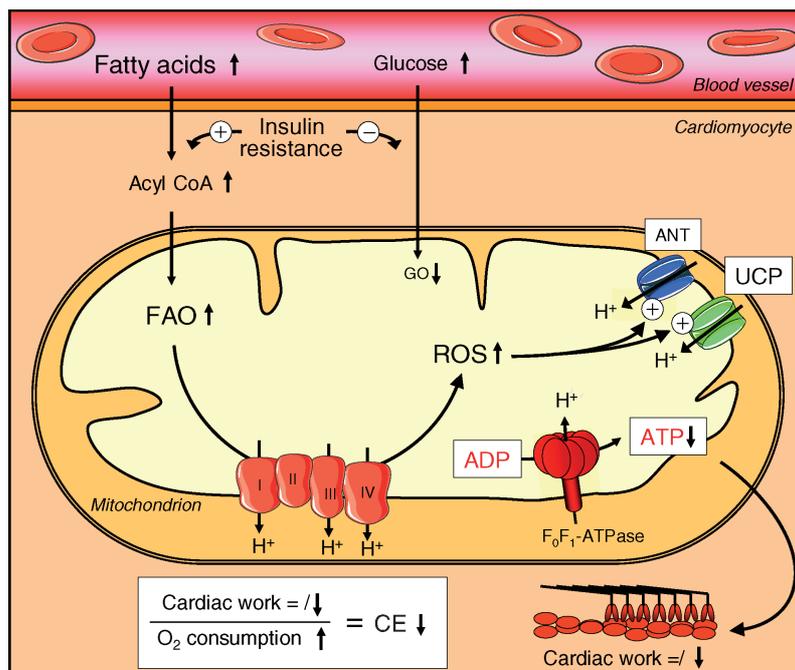


Figure 1. Proposed model for fatty acid induced mitochondrial uncoupling in type 2 diabetes. Increased serum fatty acid levels or cardiac insulin resistance, or both, leads to increased cardiac uptake and oxidation of fatty acids. Increased fatty acid oxidation (FAO) may result in increased mitochondrial generation of reactive oxygen species (ROS), which may induce mitochondrial uncoupling by activating uncoupling proteins (UCP) and adenine nucleotide translocase (ANT), ultimately resulting in reduced synthesis of ATP. Oxygen consumption may be increased as a result of mitochondrial uncoupling and increased oxygen cost for ATP production resulting from the preferential oxidation of fatty acids. Increased oxygen consumption in the absence of increased cardiac work caused by a relative energy deficit reduces cardiac efficiency (CE; white box). Acyl CoA, acyl coenzyme A; GO, glucose oxidation; ↑, increased; ↓, decreased; =/↓, the same or decreased.

deficit. The increase in fatty acid utilization in diabetic hearts may originate from increased uptake of fatty acid as a consequence of increased serum fatty acid concentrations or from reduced glucose utilization as a result of cardiac insulin resistance, which can reciprocally increase fatty acid utilization. Increased $m\dot{V}O_2$ would result from both mitochondrial uncoupling and the higher oxygen cost for the production of ATP that are caused by the preferential metabolism of fatty acids (see below).

The question remains why only db/db hearts, and not wildtype hearts, show increased ROS production and mitochondrial uncoupling when pre-perfused with high concentrations of fatty acids. This may result from differences in the enzymatic equipment (increased abundance or activity of the enzymes of fatty acid oxidation) or imbalances in the expression of OXPHOS subunits in diabetic animals, which may predispose diabetic heart mitochondria to increased generation of ROS [13,20]. Greater production of ROS, simply as a consequence of increased fatty acid oxidation, may alone not be sufficient to overwhelm the antioxidant defense and to activate UCPs. According to this hypothesis, in wildtype animals, increased $m\dot{V}O_2$ after lipid infusion would not result from fatty acid induced mitochondrial uncoupling (because these animals have no predisposition), but rather from the higher oxygen

cost for ATP synthesis when fatty acids are preferentially oxidized.

Reduced cardiac efficiency is also observed in experimental hyperthyroidism [2,22]. Perfusions of hyperthyroid rat hearts with glucose and palmitate, but not with a combination of glucose, pyruvate, and lactate, resulted in reduced cardiac efficiency that was accompanied by increased expression of UCP2 and UCP3 and increased mitochondrial consumption of oxygen in the presence of the ATP synthase inhibitor, oligomycin, suggesting that mitochondrial uncoupling had occurred [2]. These results support the concept that, similar to diabetic hearts, hyperthyroid hearts possess a predisposition that makes them prone to fatty acid induced mitochondrial uncoupling. Heart failure is another condition in which cardiac efficiency can be impaired [23]. The extent to which cardiac efficiency is reduced in dilated cardiomyopathy may even have prognostic value as a predictor of mortality in heart failure [24]. However, the molecular basis of impaired cardiac efficiency in failing hearts remains to be elucidated.

Substrate preference and oxygen cost

Alterations in the pattern of substrate utilization per se may also contribute to increased oxygen consumption

and impaired cardiac efficiency in the heart. Theoretical calculations of the yield of ATP per atom of oxygen consumed show that fatty acids are a less efficient fuel than is glucose [25]. It is calculated that shifting from 100% palmitate to 100% glucose as substrate would increase the yield of ATP by 12–14%. Thus, if any heart, such as a diabetic heart, oxidizes relatively more fatty acids and less glucose, more oxygen is needed to generate the same amount of ATP compared with a healthy heart. As cardiac work would not change, the higher oxygen cost for ATP generation would also impair cardiac efficiency. In failing hearts, in which substrate preference seems to switch towards glucose, such an adaptation could be considered beneficial for the heart, because the amount of oxygen required to maintain ATP levels is reduced.

Futile cycling

Another mechanism that has been proposed to contribute to reduced cardiac efficiency in diabetes is “futile cycling” [26]. Cardiac expression of both cytosolic (cTE1) and mitochondrial (mTE1) thioesterase 1* is induced in diabetes [20,26]. Increased expression of cTE1 may increase hydrolysis of acyl coenzymes A (CoA) back to non esterified fatty acids before acyl CoAs can be utilized for oxidation or other pathways, thereby accumulating non esterified fatty acids in the cytosol. Increased expression of mTE1 may increase intramitochondrial hydrolysis of acyl CoAs and accumulation of nonesterified fatty acids, which may then be transported back into the cytosol via UCPs. In both cases, the acyl CoAs are reconverted to fatty acids instead of being utilized. Because esterification of fatty acids to acyl CoA requires ATP, the increase in esterification reactions that prepare the accumulated cytosolic fatty acids for subsequent utilization will consume more ATP than direct esterification and utilization of fatty acids after fatty acid uptake. Thus increased amounts of ATP are utilized for fatty acid re-esterification, instead of being used for oxidation and generation of ATP to support the energetic requirements of contraction in diabetic hearts, thereby potentially contributing to impaired cardiac efficiency.

Summary

We have discussed potential molecular mechanisms that may underlie impairment of cardiac efficiency. Good evidence exists to support the idea that fatty acid induced mitochondrial uncoupling may be an underlying mechanism for impaired cardiac efficiency in diabetic cardiomyopathy, thereby contributing to the development of contractile dysfunction.

Further studies will be required to identify additional molecular mechanisms that predispose diabetic hearts to fatty acid induced mitochondrial uncoupling. As both fatty acids and ROS appear to be required for mitochondrial uncoupling, lipid-decreasing strategies and antioxidant treatment may provide therapeutic options to prevent or delay the onset of contractile dysfunction. Partial fatty acid oxidation inhibitors such as trimetazidine and ranolazine have been shown to be efficacious in reducing symptomatic and asymptomatic ischemia in patients with diabetes, and to increase left ventricular function in diabetic individuals with ischemic cardiomyopathy [27–31]. As recently reviewed by Ashrafian and colleagues [32], trimetazidine has also been reported to increase left ventricular function in non diabetic individuals with heart failure. However, in some non diabetic patients with severe heart failure, significant inhibition of myocardial fatty acid oxidation as a result of the inhibition of substrate availability (by means of the lipolysis inhibitor, acipimox) might reduce left ventricular function further and may worsen cardiac efficiency [23]. These potentially conflicting results could reflect differences between the direct effects that partial fatty acid oxidation inhibition exerts on the heart and effects resulting from restriction of substrate availability. Thus the possibility remains that the beneficial effects of inhibitors of partial fatty acid oxidation in diabetic patients and nondiabetic individuals with angina or ischemic cardiomyopathy could result directly from an improvement in cardiac efficiency that is based on a relative shift in glucose utilization from fatty acid to glucose. However, this remains to be demonstrated definitively. In experimental and human studies [33], ranolazine has been shown to possess antiarrhythmic properties, and in animal studies [34,35], trimetazidine has been reported to alter membrane phospholipid metabolism and adrenergic receptor signaling. Thus additional studies are warranted to examine the mechanism of action of these compounds, particularly their contribution to changes in cardiac efficiency.

*See glossary for definition of these terms. ■

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Basic article

Molecular basis of cardiac efficiency

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