Even is better than odd: One fat may conceal another!

Christophe BEAULOYE$^{1,2}$, Sandrine HORMAN$^1$ & Luc BERTRAND$^{1#}$

$^1$Université catholique de Louvain, Institut de Recherche Expérimentale et Clinique, Pole of Cardiovascular Research, Brussels, Belgium; $^2$Cliniques Universitaires Saint-Luc, Division of Cardiology, Brussels, Belgium

#Address for correspondence: Luc Bertrand

Pole of Cardiovascular Research
Institut de Recherche Expérimentale et Clinique
Université catholique de Louvain
Avenue Hippocrate, 55, B1.55.05
B-1200 Brussels, Belgium
Fax: +32 2 764 5536; Tel: +32 2 764 5552
E-mail: luc.bertrand@uclouvain.be

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Cardiac work efficiency is tightly interconnected to substrate metabolism. Indeed, the heart is the highest energy-consuming organ, producing and instantaneously consuming between 3.5 to 5 kg of ATP per day (in human) in order to sustain its contractile function (21). In other words, the daily amount of ATP turned over in the heart is equivalent to 15-20 times its own weight (15). Actually, the heart utilizes its entire ATP pool in only 10 seconds (19). Continuous and substantial ATP production is then an absolute requirement for efficient heart contraction. Almost all (>95%) ATP production is provided from the oxidation of carbon substrates into the mitochondria via the citric acid cycle (CAC). Inasmuch as the intracellular bioenergetic reserve capacity (mainly constituted by glycogen and intracellular lipid pools) of the cardiomyocytes is relatively limited, the heart needs to purchase available substrates from the circulation.

The heart can use a variety of different types of energy-producing substrates depending on their availability and hormonal status. These include carbohydrates (glucose, pyruvate and lactate), lipids, ketone bodies and even certain amino acids like leucine, all providing acetyl-CoA to feed the CAC. Under resting condition, 60-70% of ATP generation in mitochondrion comes from β-oxidation of fatty acids and 30-40% from carbohydrates. This preference for fatty acids as energetic substrate is due to the fact that fatty acid oxidation inhibits glucose utilization by a mechanism named Randle cycle (10). The most abundant fatty acid that can be found into the circulation is the long-chain monounsaturated oleate (19). Even if requiring more oxygen than carbohydrates, such long-chain fatty acid is efficiently oxidized by the heart giving around 6.6 molecules of ATP per fatty acid carbon (in comparison to 5.2 ATP/carbon for glucose) (6). One of the main rate-limiting steps of the oxidation of long-chain fatty acids is their import into the mitochondria, which is mediated by the carnitoyl palmitoyltransferase I (CPT1). Long-chain fatty acids, which are initially esterified to Coenzyme A (CoA), are used by CPT1 to produce long-chain acylcarnitines able to shuttle
into the mitochondria (Figure 1). CPT1 is allosterically negatively regulated by malonyl-CoA, reducing long-chain fatty acid oxidation when the concentration of this molecule rises in the cell.

Besides long-chain fatty acids, we can also find medium-chain fatty acids (MCFAs), which appear to be a promising metabolic therapy in cardiac diseases (11, 16). In contrast to long-chain fatty acids, MCFAs are minor components of usual human diet but can be found in large amount in coconut oil, which contains over 50% MCFAs. MCFAs also possess particular metabolic properties, distinct from those of long-chain fatty acids. Indeed, MCFAs do not depend on transporters for their uptake through sarcolemmal and mitochondrial membranes, bypassing the rate-limiting membrane transporting system CPT1 (Figure 1). Consequently, MCFAs are promptly taken up by mitochondria, where they are easily and preferentially oxidized, the rate of their oxidation being exclusively determined by their blood concentrations.

In the current issue of the American Journal of Physiology-Heart and Circulatory Physiology, Kajimoto et al. (12) nicely evaluated the impact of MCFA infusion on myocardial metabolism and energy state during extracorporeal membrane oxygenation (ECMO), a common mechanical circulatory support system of the failing heart in neonatal and pediatric population. ECMO, when prolonged, is characterized by a high level of mortality and morbidity (7, 20). The emergence of an imbalance between energy production and consumption under these conditions is assumed to delay left ventricular recovery and is responsible for ECMO circuit weaning failure. This is the reason why it is worthwhile to identify metabolic therapies able to increase CAC intermediate concentration and to intensify substrate oxidation in the goal to enhance ATP production and to improve function recovery after ECMO. Kajimoto and collaborators already previously showed that ECMO is characterized by a metabolic adaptation that promotes fatty acid oxidation (13, 14). Using an
infant swine ECMO model, they also showed that, when supplied at physiological concentrations (0.4 mM), octanoate, a typical dietary even-numbered MCFA, provides 60% of the acetyl-CoA molecules entering to the CAC, whereas long-chain fatty acids only contribute for 12% (14). In their article highlighted in the current issue, Kajimoto and colleagues went further and nicely compared the action of the even-numbered octanoate with that of heptanoate, an odd-numbered MCFA (12). The rational of this study resides in the theoretical different fate of odd- and even-numbered MCFAs (Figure 1). Even-numbered MCFAs are fully oxidized into acetyl-CoA (octanoate, containing 8 carbons gives 4 acetyl-CoAs) that can enter into the CAC. On the other hand, odd-numbered MCFAs are oxidized into both acetyl-CoA and propionyl-CoA (heptanoate, containing 7 carbons, provides 2 acetyl-CoAs and 1 propionyl-CoA). Subsequently, propionyl-CoA can be metabolized into succinyl-CoA, via an anaplerotic reaction which contributes to the formation of CAC intermediates (2, 5). Already suggested in the past (3, 17), the authors of the present study hypothesized that producing CAC intermediates in addition to feed CAC with acetyl-CoA would give a evident energetic advantage to the heart. Elegantly combining different $^{13}$C labeled substrates, $^{13}$C-Nuclear magnetic resonance and gas chromatography-mass spectrometry approaches, Kajimoto et al. (12) demonstrated that octanoate significantly increases cardiac energy state under ECMO whereas heptanoate fails to do so. This specific octanoate-mediated elevation in ATP/ADP ratio is accompanied by an increase in citrate concentration. The authors postulated that this might result from the increase in leucine oxidation eventually observed (Figure 1). By contrast, anaplerosis does not seem to play an important role because both MCFAs equally promote this metabolic pathway.

These results are puzzling because they contradict the usually accepted notions related to MCFA metabolism. First, odd-numbered but not even-numbered MCFAs, are supposed to promote anaplerosis and succinyl-CoA production. This seems clearly not so simple with an
efficient anaplerosis induced by even-numbered MCFAs via a mechanism that still needs to be determined (Figure 1). Second, the role of anaplerosis is to replenish CAC intermediates that are regularly extracted for other biosynthesis. Increasing anaplerosis is then supposed to maintain CAC intermediate pool promoting efficient ATP production. However, the present study reveals that anaplerotic rate is similar for heptanoate and octanoate, whereas only the latter increases energy state of the ECMO heart. Kajimoto and collaborators (12) proposed that the increase in anaplerosis could be linked to the increase in expression of propionyl-CoA carboxylase α, which converts propionyl-CoA to succinyl-CoA through methylmalonyl-CoA but this connection remains to be established. Similar surprising data has been obtained by Okere and colleagues who compared the impact of even- (hexanoate) and odd-numbered (heptanoate) MCFAs in an in vivo myocardial ischemia-reperfusion model in swine (22). Both MCFAs were able to increase CAC intermediate concentrations but the even-numbered MCFA was the sole giving a significant increase in succinyl-CoA concentration.

It has to be noted that none of the MCFAs tested in Okere study (22) conferred a significant improvement in cardiac function after reperfusion. By contrast, another study performed in an ex vivo perfused ischemia/reperfusion model of diabetic rat heart (8), showed that octanoate increases long-chain fatty-acid oxidation, reduces glucose oxidation and improve post-ischemic cardiac function recovery. Similarly, Labarthe et al. (17) showed in a model of ex vivo working hypertensive hearts subjected to acute adrenergic stimulation that octanoate improves cardiac function. Kajimoto and colleagues, in their highlighted paper (12), did not see any beneficial action of even- and odd-numbered MCFAs on cardiac function parameters of unloaded ECMO hearts but they did not evaluate them after pressure and volume reloading. Taken into account all these apparent conflicting results, which have been obtained in different pathological models and/or following distinct experimental procedures, it is
difficult to definitively conclude on the putative cardioprotective action of even- and odd-numbered MCFAs.

Another intriguing result obtained by Kajimoto and collaborators (12) is the fact that the sole CAC intermediate really increased by MCFA treatment is citrate, which is elevated by octanoate but not by heptanoate. The increase in leucine oxidation found in octanoate treatment is the element proposed by the authors to explain such rise in citrate concentration via a modification in acetyl-CoA production.

The same research team recently published another interesting study in American Journal of Physiology-Heart and Circulatory Physiology (18). Using the same ECMO swine model, they showed that pyruvate supplementation promotes anaplerosis and increased CAC intermediates without any significant increase in pyruvate oxidation. This is accompanied by the increase of the AMP-activated protein kinase (AMPK) and O-GlcNAcylation pathways, two mechanisms playing an important role in the regulation of metabolism and cardiac function (1, 4, 9).

To conclude, numerous questions remain. How even-numbered promotes anaplerosis? Does the increase in leucine oxidation really explain the increase in citrate concentration found with octanoate treatment? Is there any gain of increasing energetic state using even-numbered MCFA treatment during ECMO weaning? Does pyruvate act similarly to octanoate? Do AMPK and O-GlcNAcylation play a role in the action of MCFAs on metabolic parameters? Are these metabolic alterations induced by MCFAs also present in other pathological situations? Do they differentially give any real benefits in term of cardiac function maintenance or recovery? This article by Kajimoto et al. (12) represents a good start but long is the road for the full comprehension of myocardial metabolism of MCFAs and their impact on cardiac function under both physiological and pathological conditions!
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17. Labarthe F, Khairallah M, Bouchard B, Stanley WC, Des Rosiers C. Fatty acid oxidation and its impact on response of spontaneously hypertensive rat hearts to an adrenergic


Figure 1. Mitochondrial metabolism of energy-providing substrates in cardiomyocyte and particular action of even- and odd-numbered MCFAs under ECMO. Black, white and grey circles represent substrate carbons generating acetyl-CoA (black and white) or propionyl-CoA (grey). Dashed blue lines represent the stimulatory action of even-numbered MCFAs on leucine oxidation and anaplerosis. The exact molecular mechanisms involved in these effects are not yet fully identified. Abbreviations: αKG, α-ketoglutarate; βOx, fatty-acid β-oxidation; CAC, citric acid cycle; CPT, carnitine palmitoyltransferase; GOx, glucose oxidation; LCFAs, long-chain fatty acids; OAA, oxaloacetate. This cartoon is largely inspired from (12).
Cytosol

Mitochondrial matrix

Odd-numbered MCFAs (Heptanoate)

Even-numbered MCFAs (Octanoate)

Leucine

LCFAs

Glucose

Propionyl-CoA

Succinyl-CoA

Succinate

Citrate

CAC

Fumarate

Malate

OAA

Acetyl-CoA

βOx

LCFAs

CPT1

CPT2

βOx

GOx

ATP

Anaplerosis

αKG

ATP

Odd-numbered MCFAs

βOx

Even-numbered MCFAs

βOx

Leucine

βOx

LCFAs

βOx

Glucose

βOx