Cardiac anaplerosis in health and disease: food for thought

Christine Des Rosiers1*, François Labarthe2, Steven G. Lloyd3, and John C. Chatham3

1Department of Nutrition, Montreal Heart Institute and Université de Montréal, Montreal, QC, Canada H3C 3J7; 2Centre Hospitalier Régional et Universitaire de Tours, Institut National de la Santé et de la Recherche Médicale U921, Université François Rabelais, Tours, France; and 3Division of Metabolic and Cellular Pathology, Department of Pathology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

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Abstract
There has been a resurgence of interest for the field of cardiac metabolism catalysed by the increased need for new therapeutic targets for patients with heart failure. The primary focus of research in this area to date has been on the impact of substrate selection for oxidative energy metabolism; however, anaplerotic metabolism also has significant interest for its potential cardioprotective role. Anaplerosis refers to metabolic pathways that replenish the citric acid cycle intermediates, which are essential to energy metabolism; however, our understanding of the role and regulation of this process in the heart, particularly under pathophysiological conditions, is very limited. Therefore, the goal of this article is to provide a foundation for future directions of research on cardiac anaplerosis and heart disease. We include an overview of anaplerotic metabolism, a critical evaluation of current methods available for its quantitation in the intact heart, and a discussion of its role and regulation both in health and disease as it is currently understood based mostly on animal studies. We also consider genetic diseases affecting anaplerotic pathways in humans and acute intervention studies with anaplerotic substrates in the clinics. Finally, as future perspectives, we will share our thoughts about potential benefits and practical considerations on modalities of interventions targeting anaplerosis in heart disease, including heart failure.

Keywords
Heart failure • Energy metabolism • Mitochondria • NMR • GCMS

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1. Introduction
Heart disease remains a leading cause of morbidity and mortality in industrialized societies, despite significant advances in therapies in the past decades. A commonly identified feature of cardiac dysfunction and susceptibility to injury, as well as progression of hypertrophy to failure, is dysregulation of cardiac energy metabolism (for reviews, see refs1–6). Although uncertainty remains regarding the precise cause and effect relationships between metabolic dysfunction and heart disease, ‘metabolic therapies’ aiming to optimize cardiac energy metabolism are currently being considered as adjuncts or alternatives to the currently used treatments,7,8 since they potentially act additively while not exerting any adverse haemodynamic effects.

To date, the primary focus of this metabolic therapy has been on the modulation of substrate selection for energy producing pathways (for reviews, see refs1,2,4,9–11). Specifically, a common target of currently available drugs, which are in clinical use (e.g. trimetazidine), is to favour carbohydrate (CHO) over long-chain fatty acid (LCFA) β-oxidation for energy production through partial inhibition of the latter pathway. However, we believe that the ultimate success of metabolic-based interventions requires a better understanding of how major substrates for energy metabolism, namely CHOls and LCFAls, are diverted away from their major fate into collateral metabolic pathways, such as the conversion of LCFAls to various lipid metabolites, ceramides, and diacylglycerides, and the funneling of glucose into the hexosamine biosynthetic pathway (for reviews, see refs12–14).

Another collateral metabolic pathway is ‘anaplerosis’, a term that was first used by Kornberg15 in 1966 to describe pathways or reactions that replenish the pool of intermediates of a metabolic cycle, such as the citric acid cycle (CAC). The role of anaplerosis is well recognized as a major biosynthetic pathway in the liver,16 but in the...
heart, it is still not widely appreciated, despite the fact that it has been the subject of a number of studies (for reviews, see refs 17–19). The prevailing view is that anaplerosis is essential for normal function of the heart and increasing anaplerotic flux particularly in the setting of cardiac disease has typically been considered beneficial. In reality, however, this is likely to be oversimplification of the situation since pathways and regulation of cardiac anaplerosis and the techniques for quantifying anaplerotic flux remain poorly understood.

2. Cardiac anaplerosis: basic concepts

The levels of CAC intermediates are crucial for the normal functioning and regulation of the cycle, whose principal role is the oxidation of acetyl-CoA to CO₂, a central process to the generation of NADH and FADH₂ for the electron transport chain. In theory, the reactions of the CAC recycle 100% of the catalytic intermediates; however, in reality as discussed below, most CAC intermediates are involved in other metabolic pathways in the mitochondria and in the cytosol following their efflux from the mitochondria through membrane-specific transporters. Consequently, the entry of catalytic carbons into the CAC is both variable and very rapid ranging from 5–10 nmol/g. Since the rate of acetyl-CoA oxidation in the CAC, usually transporters. Consequently, the entry of catalytic carbons into the CAC via anaplerotic reactions is essential to balance this efflux, often referred to as cataplerosis in the recent literature, thereby maintaining levels of CAC intermediates required for optimal cycle activity.

It is frequently underappreciated that the catalytic intermediates of the CAC in the heart are present in very low concentrations, usually <2 μmol of total intermediates per gram tissue; one key intermediate, oxaloacetate (OAA), is present in minuscule concentrations (5–10 nmol/g). Since the rate of acetyl-CoA oxidation in the CAC ranges from 0.1 to 4 μmol/min/g, the turnover time of individual CAC intermediates is both variable and very rapid ranging from <1 s to 1 min; the turnover rate for OAA is up to 800 times per minute. Therefore, adequate NADH/FADH₂ formation via the CAC requires not only a constant supply of acetyl-CoA, but also a constant pool of catalytic intermediates which carry the acetyl groups as they are being oxidized.

Figure 1 depicts reactions and pathways relevant to the CAC; it emphasizes reactions involved in anaplerosis and CAC efflux that will be discussed in this review article. Metabolic pathways that have long been recognized as potential sites for anaplerosis and CAC intermediate efflux include (cf. numbers in figure 1): (i) pyruvate carboxylation (PC) to OAA (#2) or malate (#3), (ii) malate decarboxylation to pyruvate (#3), (iii) transamination between OAA and α-ketoglutarate (αKG) and their corresponding amino acids, aspartate (#4) and glutamate (#5), respectively, (iv) formation of fumarate through the purine nucleotide cycle, and (v) formation of succinyl-CoA from propionyl-CoA precursors such as branched-chain amino acids, propionate, and odd-carbon ketone bodies and FAs. As discussed below, this scheme is incomplete and regulatory factors need to be integrated including specific roles of some CAC intermediates, reactions, or pathways. For example, succinyl-CoA facilitates ATP formation by substrate level phosphorylation via succinyl-CoA synthetase (#6), participates in ketone body metabolism via 3-oxoacid-CoA transferase (#7), and is also a substrate for haem biosynthesis. In addition, recent studies detailed below in Section 4 emphasize the importance of mitochondrial efflux of CAC intermediates such as citrate (#8) and αKG (indicated with an asterisk together with other co-substrates) through specific transporters and their role in the regulation of substrate selection for oxidation in the CAC or cytosolic NAD(P)+/NAD(P)H redox state, respectively. Interestingly, a role for NAD⁺ in transcriptional reprogramming has also emerged recently (for review, see ref 20).

3. Methodological considerations

A critical limitation in our understanding of anaplerosis has been its quantification, particularly in the intact heart in situ. Since the metabolic network involved is highly complex, measurements of steady-state concentrations of CAC intermediates provide little if any information about either the magnitude or the site of anaplerotic reactions. In the early 1980s, experiments using isolated hearts perfused with 13C-labelled pyruvate demonstrated that anaplerotic flux into the CAC through PC is a normal part of metabolism. However, detailed characterization of reactions feeding into and out of the CAC with radioactive or stable isotopes is complicated by label recycling due to metabolism via the CAC, as well as by exchange reactions between CAC intermediates and other metabolites such as aspartate and glutamate. Consequently, anaplerotic substrates can label CAC intermediates without net anaplerotic flux. Furthermore, except for the carbon 1 of glutamine and glutamate, the formation of labelled CO₂ from anaplerotic substrates does not reflect net oxidation unless additional reactions label mitochondrial acetyl-CoA from the anaplerotic substrate, e.g. malic enzyme, followed by pyruvate dehydrogenase for propionate. Consequently, the complexity of the metabolic networks involved typically requires sophisticated mathematical models for detailed analysis of isotopic labelling; such models are frequently underdetermined, thereby limiting their utility and reliability.

3.1. Use of nuclear magnetic resonance- and gas chromatography–mass spectrometry-based techniques for quantification of anaplerosis

Over the past 10 years, our understanding of cardiac anaplerosis has improved in part due to the application of 13C-isotopomer analysis by nuclear magnetic resonance (NMR) and gas chromatography–mass spectrometry (GCMS). We have previously provided an overview of the theory behind these techniques as well as their critical evaluation and comparison as applied to CAC metabolism in the ex vivo perfused heart. Therefore, here, we focus on methodological considerations regarding the interpretation and reliability of data obtained by NMR and GCMS in quantifying cardiac anaplerosis.

The primary difference between NMR and GCMS is their sensitivity. Thus, while GCMS is able to directly detect 13C-labelling of CAC intermediates, 13C-NMR spectroscopy uses 13C-labelling of glutamate as a proxy for αKG. Analyses of the 13C-glutamate isotopomer patterns have been greatly facilitated by the availability of the program tcaCALC (developed and provided by Dr Jeffrey; http://www4.utsouthwestern.edu/rogersnmr/software/index.html), which uses algebraic equations that describe the 13C-NMR spectrum in terms of metabolic (pathways fluxes) and experimental (substrate labelling patterns and enrichment) parameters.

In principle, this provides a simple and fairly robust assessment of total anaplerosis; it does not, however, identify the specific anaplerotic pathway(s).
With GCMS, equations are used to calculate metabolic fluxes based on $^{13}$C-isotopomer balance and precursor-product relationship using the measured $^{13}$C-labelling of CAC intermediates. Anaplerotic flux through PC is determined using [U-$^{13}$C]-labelled pyruvate or its precursors and is expressed relative to flux through of citrate synthesis (CS). The PC/CS flux ratio obtained from this analysis represents a combination of pyruvate metabolism via both pyruvate carboxylase and NADP-linked malic enzyme. Contrary to reports suggesting otherwise, none of the currently available isotope methods can distinguish between these two pathways. Nevertheless, GCMS analysis of $^{13}$C-enrichment of other CAC intermediates can potentially provide additional information regarding the sites and extent of anaplerosis other than via PC, by determining the amount of $^{13}$C-dilution between tissue CAC intermediates. Alternatively, one can use other $^{13}$C-labelled substrates, such as (i) glutamate, (ii) aspartate, as well as (iii) valine, propionate, or odd-carbon fatty acids to assess directly the entry of $^{13}$C-labelling at the level of aKG, OAA, or succinyl-CoA, respectively.

3.2 Study models

Just as important as techniques used for the quantification of anaplerosis are the study models and experimental conditions used for these measurements. The most commonly used model for evaluating cardiac anaplerosis has been the isolated ex vivo rat and mouse heart perfused with $^{13}$C-labelled substrates. To the best of our knowledge, there are only two groups that have evaluated anaplerotic flux in the in situ pig heart. Many early perfusion studies used simple buffers containing high concentrations of one or two substrates such as 5 mM acetate or pyruvate. These studies demonstrated the constant need for anaplerotic substrates, as illustrated by the decrease in tissue levels of glutamate or aspartate, as well as the importance of these substrates for maintaining contractile function particularly for hearts perfused with the ketone body acetoacetate as a sole substrate. The broader conclusions that can be drawn from such studies are, however, inherently limited, since they lack many of the substrates that are physiologically relevant fuels for the in vivo heart. This issue is of even greater importance for studies of cardiomyopathy where cardiac efficiency is decreased, due to increased oxygen cost for non-contractile processes. For example, in the mdx mouse, a model of dystrophic cardiomyopathy, performance of hearts perfused ex vivo was improved using a physiological mixture of CHOs and an LCFA compared with glucose as the sole substrate.

Of particular importance for energy metabolism as well as for availability of substrates for anaplerosis are lactate and pyruvate, whose normal concentrations in plasma vary between 0.5 and 2, and 0.05 and 0.2 mM, respectively. Since PC appears to be a major anaplerotic pathway, experimental conditions in which glucose is the only source of pyruvate could be considered as ‘pyruvate-limited’, leading to consumption of endogenous anaplerotic amino acids. Of note, insulin...
Anaplerosis in heart failure

4. Cardiac anaplerosis in health and disease: role and regulation

Here, we provide a summary of findings from experimental animal models on cardiac anaplerosis, emphasizing studies (i) in which hearts were perfused in situ or ex vivo with an adequate supply of CHO and LCFA, and (ii) that included measurements of anaplerotic fluxes using tracer methodology as well as tissue CAC intermediate levels. This is followed by an overview of human genetic diseases indicating the potential importance of cardiac anaplerosis.

4.1 Lessons from animal studies

4.1.1 Anaplerosis and its link to mitochondrial citrate efflux

Over the past 10 years, 13C-labelled substrates and isotopomer analysis by GCMS experiments have been conducted in various study models, over a range of (patho)physiological conditions to further understand the role and regulation of pyruvate partitioning between decarboxylation (energy) and carboxylation (anaplerosis), CAC pool size, and mitochondrial citrate efflux. These studies were prompted by earlier studies reporting: (i) cardioprotective effects of anaplerotic substrates, particularly pyruvate (for reviews, see refs 17,18), as well as (ii) myocardial citrate efflux in humans, which was increased with coronary artery disease while correlated with nutrient uptake (positively with LCFA, but negatively with glucose) in normal subjects, but not in patients with coronary artery disease suggesting dysregulation.55–57 Specifically, partly based on studies in β-cells and the skeletal muscle,58,59 we formulated the following working hypothesis for the heart whereby pyruvate anaplerosis and mitochondrial citrate efflux would contribute to metabolic signal transmission from mitochondria to the cytosol. This hypothesis is based on the regulatory role of cytosolic citrate in restricting both glucose (via inhibition of phosphofructokinase) and LCFA utilization (via inhibition of carnitine palmitoyl transferase-1 subsequent to its cleavage by ATP-citrate lyase to acetyl-CoA, a precursor of malonyl-CoA). Our initial studies provided support for the importance of mitochondrial citrate efflux as evidenced by a decrease in membrane integrity, observed when hearts were perfused with an inhibitor of the tricarboxylate transporter and its modulation by substrate abundance and energy demand.60 Furthermore, a link between pyruvate anaplerosis and regulation of cytosolic metabolic processes was supported by studies with the ATP-citrate lyase inhibitor hydroxycitrate; we estimated that malonyl-CoA synthesis by cytosolic cleavage of citrate may represent ~10% of mitochondrial citrate efflux (between 0.2 and 0.7 nmol/min/g).61,62

The experimental models used to investigate the aforementioned pathways included ex vivo work-performing perfused rat and mouse hearts and in situ pig hearts27,28,37,38,63,64,65 and results obtained are summarized in Tables 1 and 2. We found that all study models exhibited remarkably similar values for the PC/CS flux ratio (2.5–10%), absolute PC (0.05–0.2 µmol/min/g), CAC flux rates (1.6–2.2 µmol/min/g), and citrate efflux rates (8–17 µmol/min/g equivalent to between 5 and 21% of PC flux). Consistent with our basic understanding of the regulation of enzymes catalysing pyruvate decarboxylation and carboxylation, the PC/CS flux ratio was also found to be affected in a way that differs from that of the PDC/CS flux ratio. This is illustrated by changes in the PC/PDC flux ratio, which was significantly increased at a higher FA concentration in perfused hearts from normal mice, Wistar rats, and spontaneously hypertensive rat (SHRs; Tables 1 and 2), during hibernation in the pig heart in situ,28,37,38,63,64 whereas it was decreased in the mdx and PPARα-null mouse heart (Table 2C).66,64 Furthermore, the myocardial citrate efflux rate was increased in various models of cardiomyopathy (Table 2C), consistent with earlier reports of an increase in the myocardial release of citrate in patients with coronary artery disease.67 However, there were also some unexpected findings, an issue that has been previously discussed.18 For example, as depicted in Table 2, results from the various studies do not support a direct relationship between anaplerosis (PC/CS), total CAC pool size, and CAC flux rate. This is best illustrated by studies on the supplementation with the medium-chain fatty acid (MCFA) octanoate (Table 2A). In all studies, including ex vivo perfused hearts and the in situ pig heart,28,37,64 octanoate consistently increased CAC intermediate levels, with no change in CAC flux rate, whereas the PC/CS ratio was decreased (Wistar), increased (SHR), or unchanged (pig; Table 2A). Furthermore, findings of a selective increase in CAC intermediates of the first span (i.e. citrate and αKG) by increasing substrate supply66 or of the second span of the CAC (i.e. succinate and beyond) under low-flow ischaemia or by increasing workload29,64 emphasize the importance of individual CAC intermediates—rather than the total pool size—as the primary factor for the operation of the
however, it decreased infarct size. The latter observations suggest hibernation, or reperfusion in the pig heart did it affect citrate efflux or CAC intermediate level, under normoxia, overload up to 8 mM did not further stimulate anaplerosis, nor did it affect citrate efflux or CAC intermediate level, under normoxia, hibernation, or reperfusion in the pig heart in situ (Table 2B); however, it decreased infarct size. The latter observations suggest that the cardioprotective effects of pyruvate observed in various models in vivo (for review, see ref. 69) are not mediated by increased anaplerosis.

Taken together, all these results emphasize the complex network of regulatory mechanisms governing flux through anaplerotic PC, citrate efflux, and maintaining CAC pool size in the heart as evidenced by the lack of direct relationship between these metabolic parameters.

### Table 1 | Flux values assessed using [U-13C3]pyruvate and isotopomer analysis by GCMS in various heart study models

<table>
<thead>
<tr>
<th>Parameters measured</th>
<th>Working hearts perfused ex vivo</th>
<th>Hearts in situ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wistar rat&lt;sup&gt;28&lt;/sup&gt;</td>
<td>BL10 mouse&lt;sup&gt;63&lt;/sup&gt;</td>
</tr>
<tr>
<td>[LCFA] (mM)</td>
<td>0.4 mM oleate</td>
<td>1.0 mM oleate</td>
</tr>
<tr>
<td>PC/CS</td>
<td>0.098 ± 0.013</td>
<td>0.041 ± 0.011</td>
</tr>
<tr>
<td>PDC/CS</td>
<td>0.66 ± 0.06</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>PC/PDC</td>
<td>0.16 ± 0.02</td>
<td>0.24 ± 0.034</td>
</tr>
<tr>
<td>[Total CAC intermediates] (µmol/gww)</td>
<td>0.54 ± 0.02</td>
<td>0.65 ± 0.06</td>
</tr>
<tr>
<td>Absolute CAC flux (µmol/min gww&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>1.9 ± 0.2</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Absolute PC flux (µmol/min gww&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.187 ± 0.002</td>
<td>0.071 ± 0.001</td>
</tr>
<tr>
<td>Citrate release (nmol/min gww&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>8.4 ± 1.2</td>
<td>8.4 ± 1.2</td>
</tr>
<tr>
<td>Citrate release (%PC flux)</td>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>

Data are means ± SE. Working hearts were perfused under normoxia with the following mixtures of substrates: rat: 5.5 mM glucose, 1.0 mM lactate, 0.2 mM pyruvate, 0.4 or 1 mM oleate bound to 3% albumin; 8 mM insulin, and 50 µM l-carnitine; and mouse: 11 mM glucose, 1.5 mM lactate, 0.2 mM pyruvate, 0.4 or 0.7 mM oleate bound to 3% albumin, 0.8 mM insulin, and 50 µM l-carnitine. For details, refer to indicated references. PC, pyruvate carboxylation; PDC, pyruvate decarboxylation; CS, citrate synthesis; CAC, citric acid cycle; FFA, free fatty acid; LCFA, long-chain fatty acid; n.a., not available; gww, gram wet weight. For additional experimental details, refer to indicated references.

* P < 0.05 high vs. low concentration of oleate.
** P < 0.01 high vs. low concentration of oleate.
*** P < 0.001 high vs. low concentration of oleate.

### Table 2 | Overview of results obtained in various conditions or animal models illustrating the complex network of regulatory mechanisms governing flux through anaplerotic PC, CAC, citrate efflux, and maintaining CAC pool size in the heart as evidenced by the lack of direct relationship between these metabolic parameters

<table>
<thead>
<tr>
<th>Conditions or models</th>
<th>PC/CS</th>
<th>PDC/CS</th>
<th>PC/PDC</th>
<th>[Sum CAC intermediates]</th>
<th>CAC flux</th>
<th>Citrate release</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of acute octanoate supplementation (0.2–1 mM)</td>
<td></td>
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<tr>
<td>Wistar rat hearts perfused with 0.4 mM oleate</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR hearts perfused at 95 mmHg with 0.4 mM oleate and 10 µM Epi</td>
<td>58%</td>
<td>82%</td>
<td>119%</td>
<td>73%</td>
<td>NS</td>
<td>NS</td>
<td>28</td>
</tr>
<tr>
<td>Normoxic pig heart in situ</td>
<td>NS</td>
<td>93%</td>
<td>868%</td>
<td>95%</td>
<td>NS</td>
<td>NS</td>
<td>37</td>
</tr>
</tbody>
</table>

Effect of acute pyruvate supplementation (1 mM)

| Normoxic pig heart in situ | NS | NS | NS | NS | NS | NS | 37 |
| Hibernating heart in situ | NS | NS | NS | NS | NS | NS | 38 |

Models of cardiomyopathy or fatty acid oxidation defect

| SHR vs. Wistar rat hearts perfused at with 0.4 mM oleate and 10 µM Epi | 38% | NS | 19% | NS | 27% | 88% | 64 |
| mdx vs. BL10 mouse hearts perfused with 0.7 mM oleate | 19% | 110% | 62% | 35% | NS | NS | 46 |
| PPARα-null vs. BL6 mouse hearts perfused with 0.4 mM oleate | NS | 54% | NS | NS | 206% | 54 |

Data are shown as per cent changes in metabolic parameters relevant to anaplerosis and the citric acid cycle assessed using [U-13C3]pyruvate and GCMS in various conditions or animal models. Working rat and mouse hearts were perfused under normoxia with the mixtures of substrates as described in Table 1. For details, refer to indicated references. PC, pyruvate carboxylation; PDC, pyruvate decarboxylation; CS, citrate synthesis; CAC, citric acid cycle; Epi, epinephrine; SHR, spontaneously hypertensive rat; NS, non-significant.

* P < 0.05 vs. control.
** P < 0.01 vs. control.
*** P < 0.001 vs. control.
4.1.2 Other potential sites and roles for anaplerosis and mitochondrial CAC efflux

As discussed above, removal or efflux of CAC intermediates other than citrate must also occur as a counterbalance to the rates of PC. Among other potential sites for CAC intermediate removal, we have demonstrated the presence of succinate efflux which was increased by oxygen deprivation; however, its magnitude is in a similar range of that for citrate efflux, thus accounting for, at most, another 20% of PC flux rate. In the presence of exogenous [U-13C3]-labelled pyruvate, malate decarboxylation to pyruvate by malic enzyme would result in the formation of M1 or M2 labelling of tissue pyruvate; however, under all conditions studied to date, we have found no evidence for this pathway in the heart. This is consistent with the known regulation of this reaction, which is unfavourable towards decarboxylation.

Another potential site for anaplerosis and/or CAC intermediate efflux, whose role remains to be better understood, is at αKG. Clearly, there is a rapid isotopic exchange between αKG and glutamate, as evidenced from the rapid labelling of 13C-glutamate, which makes any attempts of determining the net direction of this flux inherently very difficult. Using in vivo 13C NMR spectroscopy in the rat, the exchange rate between αKG and glutamate in the overnight-fasted Sprague–Dawley rat myocardium was similar to that of CAC flux (∼1.2 μmol/min/g) and the rate of myocardial glutamine synthesis was found to be substantial at 0.14 μmol/min/g, which is in the range of PC flux. Interestingly, αKG efflux has recently been proposed to balance that of anaplerosis based on the finding that the magnitude of both of these rates was increased in parallel in hypertrophied hearts, when assessed using ex vivo perfusion with 5 mM glucose and 0.4 mM palmitate in the absence of insulin, and 13C-NMR.25,72 The increased anaplerosis in the hypertrophied heart was attributed to increased cytosolic malic enzyme activity, based on the finding of a greater transcript level in these hearts; however, flux through this pathway can be boosted if one administers propionyl-CoA precursors. In fact, the intravenous administration of a novel synthetic precursor, namely dipropionylcysteine ethyl ester, was reported to compensate for loss of CAC intermediates during post-reperfusion in the pig heart in situ, although this did not affect mechanical function.73 Other precursors include propionyl-L-carnitine, which had first been shown beneficial in the isolated rat heart in the 1980s, as well as odd-carbon MCFA or ketone bodies. The latter substrates have recently generated much interest, particularly in the treatment of cardiomyopathies associated with LCFA β-oxidation defects as will be further discussed below.

In summary, numerous factors need to be considered in the overall regulation of reactions governing anaplerosis and mitochondrial CAC efflux. These include their role in metabolic signal transmission in the heart, specifically for pyruvate and citrate, as well as in regulation of the cytosolic redox status, for αKG and malate. It is noteworthy that these roles bear some similarity to the situation in pancreatic β-cells, where it has been proposed that the transferred molecules act as secretagogue or exporters of equivalents of NAD(P)H, acetyl-CoA, and malonyl-CoA and thereby regulate glucose-stimulated insulin secretion.

4.2 Lessons from human genetic diseases

There are only a few genetic disorders involving anaplerotic pathways that have been documented in humans. First, deficiency of pyruvate carboxylase is a very rare autosomal recessive disease, characterized by impairment of gluconeogenesis and lactate metabolism, leading to severe lactic acidosis and profound energy deficiency.77,78 Although the central nervous system and liver are affected, cardiac symptoms have not been reported in these patients. This suggests that either pyruvate carboxylase plays only a minor role in the heart, or perhaps more likely, that its deficiency may be easily compensated by malic enzyme. To the best of our knowledge, a genetic defect of malic enzyme has never been reported in humans. There are also some reported cases of genetic defects in the conversion of propionyl-CoA into succinyl-CoA, either at the level of propionyl-CoA carboxylase or methylmalonyl-CoA mutase. The clinical phenotype for these patients is dominated by metabolic acidosis and neurological symptoms; however, those surviving the first years of age frequently develop additional symptoms, including dilated cardiomyopathy with contractile dysfunction and electrophysiological changes.79,80 The molecular mechanisms underlying the cardiac symptoms remained unclear, but have been suggested to include depletion
of CAC intermediates and/or accumulation of various toxic metabolites that inhibit oxidative phosphorylation.⁴⁰,⁴¹

An imbalance between anaplerosis and CAC intermediate efflux has been suspected in the physiopathology of other genetic disorders such as those affecting LCFA β-oxidation.⁴²,⁴³ Clinical consequences of these diseases range from no symptoms to severe manifestations, which include acute and chronic cardiomyopathy with contractile dysfunction, cardiac rhythm disorders, myopathy with recurrent rhabdomyolysis, and sudden death. Currently, the nutritional treatment of these patients aims at providing alternative substrates to LCFA for energy metabolism, to block lipolysis, and reduce accumulation of potentially toxic LCFA derivatives, such as long-chain acylcarnitines or acyl-CoAs. This can be achieved with diets enriched in CHO and MCFAs, but restricted in LCFA, and also by avoiding any conditions that increase LCFA β-oxidation such as fasting, excessive exercise, or acute infection.

However, despite marked improvements in the clinical status of most patients treated in this manner, cardiac and muscle dysfunctions still persist.⁴⁴ In 2002, Roe et al.⁵ reported a major improvement of cardiomyopathy in patients with very long-chain acyl-CoA dehydrogenase deficiency when dietary even-carbon medium-chain triglycerides were substituted by an odd-carbon MCFAs (i.e. triheptanoin). Triheptanoin ingestion induced a rapid appearance of both C4- and C5-ketone bodies in the plasma, the latter being precursors of propionyl-CoA, which is then converted to succinyl-CoA; thus, a key feature of triheptanoin is that it increases availability of anaplerotic substrates. These authors therefore concluded that the constant stimulation of anaplerosis in all tissues compensated for the excessive loss of CAC intermediates attributed to membrane ‘leakage’, thereby improving CAC activity and energy metabolism. Other mechanisms that may also be considered include compensation for decreased CAC flux due to post-translational modification of CAC enzymes by acylation subsequent to the accumulation of LCFA derivatives.³⁰,⁴⁴

The safety and beneficial effects of triheptanoin with LCFA oxidation defects were further confirmed; this diet reduced muscle pain and rhabdomyolysis and improved exercise tolerance and quality of life.⁵⁵ However, the benefit of anaplerotic therapy on cardiac symptoms in patients with LCFA β-oxidation defects remains to be substantiated in a controlled clinical trial. Nevertheless, studies by Roe and colleagues demonstrate the feasibility of anaplerotic interventions in vivo and raise the intriguing possibility of a cardio-protective effect linked to anaplerotic interventions in vivo under conditions of low LCFA β-oxidation, as occurs in the hypertrophied and failing heart.

5. Anaplerosis in heart failure: challenges and practical considerations

Our understanding of anaplerosis in the failing heart remains limited, particularly in humans. This can be attributed to the lack of sensitive methods for the direct assessment of these processes in vivo in patients, the absence of animal models that fully reproduce the complexity of the overall metabolic conditions encountered in heart failure (HF), as well as the need to account for multiple medications, which also likely affect cardiac metabolism. The recent emergence of hyperpolarized ¹³C-NMR raises the possibility of being able to assess pyruvate partitioning in the heart non-invasively; however, a number of important technical challenges remain.⁸⁶,⁸⁷ Beyond these considerations, given the metabolic dysfunction that characterizes the failing heart, there are many factors that have the potential to affect anaplerosis. This includes a compromised energy and redox status and metabolism in the midst of substrate abundance; mitochondrial dysfunction, loss of mitochondrial membrane integrity, substantial oxidative stress and oxygen-wastage attributed to high-circulating LCFA, sympathetic overdrive and/or insulin resistance, and ketosis (for reviews, see refs ¹⁻⁶,⁹⁻¹¹). Many of these factors have the potential to decrease flux through one or many CAC reactions and, thereby, enhance the need for anaplerosis in general and/or at a specific site (Figure 1). For example, decreased flux through αKG dehydrogenase (αKGDH; #10) may be limited by CoA availability in the presence of ketone bodies; furthermore, both aconitase and αKGDH are inactivated by oxidative stress-related molecules.⁸⁹,⁹⁰ The situation is likely to be amplified by multiple micronutrient deficiencies that are common in HF, such as biotin, coenzyme Q10, l-carnitine, and thiamine, which are cofactors for anaplerotic and CAC reactions.⁹¹,⁹²

Because the heart has a remarkable capacity to adapt to its substrate environment to meet its energy requirement, one would anticipate that restricted flux at any given CAC reaction(s), such as aconitase (reaction #9 in Figure 1) and αKGDH, may increase the entry of substrates downstream of these reactions, e.g. at the level of αKG or succinyl-CoA. The participation of endogenous amino acids to oxidation, such as glutamate or glutamine, would also increase the participation of anaplerotic and CAC intermediate efflux pathways as illustrated by studies in the intestine, kidney, or proliferating cells (for reviews, see refs ¹⁶,⁹³,⁹⁴). In cancer cells, the metabolic reprogramming towards glutamine catabolism, which is used to sustain anapleresis and cellular viability, depends on the expression level of Myc.⁹⁵ Interestingly, in the heart, the expression of this oncogene was shown to be increased in response to numerous pathological stressors, including hypertrophy and ischaemia, and to regulate glucose metabolism and mitochondrial biogenesis.⁹⁶ Hence, it appears worth investigating glutamine metabolism in this context.

Additional factors that may enhance mitochondrial CAC intermediates efflux or divert them away from their normal metabolic fate are mitochondrial alterations, particularly an enhanced opening of the permeability transition pore,⁹⁷ and increased haem synthesis from succinyl-CoA due to the activation of haem oxygenase in response to oxidative stress.⁹⁸ Finally, there is also the question as to whether in HF, FA β-oxidation is decreased due to PPARα deactivation and whether this may lead to increased demand for anaplerosis similar to that proposed for patients with LCFA β-oxidation defects.⁷⁵

The results discussed above in Section 4 demonstrate the presence of alterations in the magnitude and/or regulation of anaplerosis, CAC intermediate efflux, and/or CAC intermediate pool size in various pathophysiologically relevant conditions such as ischaemia and several models of cardiomyopathies. More recently, ageing was also found to impair insulin-stimulated anaplerosis in the pig heart in situ.⁴⁰ However, despite these observations, the potential benefit of specifically increasing anaplerosis in hypertrophied or failing heart is not supported by most of the currently available data in animal models, albeit these are acute models of HF. For example, levels of CAC intermediates in 15-week-old hypertrophied SHR hearts freeze-clamped in situ are 30% lower than those of age-matched Wistar rat hearts (unpublished observations); however, in a pig model of...
compensated HF, there was no change in CAC intermediates, despite the presence of severe mitochondrial dysfunction. Furthermore, studies on hearts from SHR rats using acute administration of even- (octanoate) vs. odd-carbon (heptanoate) MCFA (for review, see ref.30) and in PPARα-null mouse54 suggest that impaired energy metabolism rather than reduced anaplerosis contributed to contractile dysfunction induced by increased workload. Similarly, in the in situ pig heart, subjected to ischaemia and reperfusion, heptanoate treatment had no effect on contractile function, despite increasing tissue levels of CAC intermediates.12

On the other hand, long-term treatment with propionyl-L-carnitine, which had been shown beneficial for cardiac function in experimental animal models,100,101 has also been shown to improve cardiac function and exercise tolerance in HF patients (for review, see ref.105), supporting the benefits of anaplerotic therapy. The study by Roe et al.75 has opened up new possibilities for testing the anaplerotic hypothesis in various cardiac diseases in humans and animals.30 Furthermore, there are alternative strategies beyond the specific use of anaplerotic substrates to impact on mitochondrial CAC intermediate levels. These include, for example, enhancing cGMP signalling, which resulted in major improvement in contractile function and mitochondrial metabolic status, including the CAC intermediate level, in the mdx mouse heart.97,102 This is of particular relevance given that phosphodiesterase 5 inhibitors are now being considered for the treatment of HF.103

Clearly, much remains to be elucidated regarding the role of altered anaplerotic metabolism in the pathogenesis of HF. Even with appropriate technologies, the unambiguous demonstration of the benefit of anaplerosis for the failing heart in vivo is challenging largely because most anaplerotic substrates have multiple metabolic fates and also affect other cellular processes. This is best illustrated for pyruvate; as discussed above, it is unclear as to whether its cardioprotective effects reported in many study models, including in patients with congestive HF,104 can be attributed to any extent to anaplerosis rather than its other effects on the redox and oxidative stress status.69 Similar considerations also apply to propionyl-L-carnitine, which has also been reported to have antioxidant effects.105 Other examples are the branched-chain amino acids and glutamine, which exert regulatory roles in mammalian target of rapamycin signalling106 and in the hexosamine biosynthetic pathway.14,182 Furthermore, recent studies reported additional, unexpected roles for CAC intermediates as agonists for Gq-receptors,107 modulators of transcription via hypoxia inducible factor-1,108,109 or enzyme activity through post-translational modification.110 Some of these observations have been linked to tumour formation (for review, see ref.105); however, the significance for the heart remains to be determined. Nevertheless, in the heart, there is net cardiac efflux of CAC intermediates from the mitochondria to the cytosolic milieu and across the sarcolemma into the extracellular space. Since anaplerosis is essential for the net synthesis of these intermediates, this raises the provocative notion that alterations in anaplerotic flux could have effects well beyond metabolic regulation, including modulation of protein function and transcription as well as receptor activation.

6. Conclusion

Cardiac anaplerosis is essential for the maintenance of CAC intermediate concentrations at levels required for optimal CAC cycle activity and energy metabolism. Importantly, the rate of anaplerosis has to balance mitochondrial efflux of CAC intermediates for use in biosynthetic pathways or in metabolic signal transmission between the mitochondria and cytosol and regulation of cellular redox status. Thus, given the low steady-state concentration and high turnover rates of all CAC intermediates, anaplerosis has to be under tight control, since a relatively small discrepancy between synthesis and efflux of CAC intermediates could readily impair CAC flux and hence energy metabolism. Since many of the factors involved in the regulation of CAC intermediate pool sizes are known to be altered in pathophysiological states such as cardiac hypertrophy and failure, this may lead to an increased demand for anaplerotic substrates, thus setting the stage for an imbalance between anaplerosis and CAC cycle flux. Although the current evidence does not support a direct role for anaplerosis in HF, we postulate that dysregulation of anaplerosis, particularly at the level of glutamine and succinyl-CoA, is an under explored and yet potentially critical factor underlying the metabolic basis for cardiac disease in humans. The emergence of new clinically applicable modulators of anaplerosis, for the first time, provides the opportunity of improving our understanding of the regulation of anaplerotic pathways in the heart and of developing new approaches to reliably quantifying anaplerosis in vivo.

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References


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