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Ketones and the cardiovascular system

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Ketone bodies, the main one being β-hydroxybutyrate, have emerged as important regulators of the cardiovascular system. In healthy individuals, as well as in individuals with heart failure or post-myocardial infarction, ketones provide a supplemental energy source for both the heart and the vasculature. In the failing heart, this additional energy may contribute to improved cardiac performance, whereas increasing ketone oxidation in vascular smooth muscle and endothelial cells enhances cell proliferation and prevents blood vessel rarefication. Ketones also have important actions in signaling pathways, posttranslational modification pathways and gene transcription; many of which modify cell proliferation, inflammation, oxidative stress, endothelial function and cardiac remodeling. Attempts to therapeutically increase ketone delivery to the cardiovascular system are numerous and have shown mixed results in terms of effectiveness. Here we review the bioenergetic and signaling effects of ketones on the cardiovascular system, and we discuss how ketones can potentially be used to treat cardiovascular diseases.

Cardiovascular (CV) disease is the leading cause of morbidity and mortality in the world, which creates a major burden on societies, as well as health care systems and economies. Heart failure, ischemic heart disease, arrhythmias, cardiomyopathies, stroke, atherosclerosis and hypertension, are all common forms of CV disease. Because of the prevalence and adverse consequences of CV disease, major efforts and advances have been made in treating these CV diseases. This includes treatments for diabetes, obesity and dyslipidemias, which are major comorbidities that contribute to the incidence and severity of CV disease. Alterations in heart and vascular energy metabolism are also hallmarks of CV disease[1–3]; therefore, therapies targeting energy metabolism are also potential viable approaches to treating CV diseases.

The heart has a very high energy (ATP) demand that is primarily met by the mitochondrial metabolism of various energy substrates such as fatty acids, glucose, lactate, amino acids and ketones. Mitochondrial metabolism of these energy substrates also occurs in the vasculature, although glycolysis is a more prominent source of ATP production[2]. Of the different energy substrates, recent interest has focused on ketone bodies as a source of energy for the heart and vasculature[2,4–10]. Ketone bodies are an important alternative source of fuel, particularly during times of nutrient deprivation[2,9–12]. Ketone bodies consist of β-hydroxybutyrate (βOHB), acetoacetate and acetone, with βOHB being the most abundant of the ketones. Ketones are best recognized as an important alternative source of energy for the brain during periods of fasting and carbohydrate deprivation[12]. However, the heart and vasculature can also readily metabolize ketones, particularly βOHB[2,9,10]. As will be discussed, the mitochondrial oxidation of ketones can have beneficial effects in a number of CV diseases. This includes providing an important additional source of ATP production for an 'energy-starved' heart[1,6,9,10,13]. In addition to being an important source of energy for the heart and vasculature, emerging evidence has shown that ketones are important signaling molecules[2,4,14–23] that can influence CV function. Through many of these signaling pathways, ketones also influence posttranslational modifications and gene transcription, which have profound effects on both the heart and vasculature.

There have been many recent attempts to increase circulating ketone levels as an approach to treat CV disease. Unfortunately, ketones are not palatable and are not easily ingested. However, various strategies have been used to promote endogenous liver production of ketones, including intermittent fasting, ketogenic diets and administration of sodium-glucose cotransporter-2 (SGLT2) inhibitors (reviewed in ref. 24). The intravenous administration of ketones or ingestion of ketones in the form of ketone precursors and/or ketone ester drinks has also been examined in CV diseases[6,25,26].

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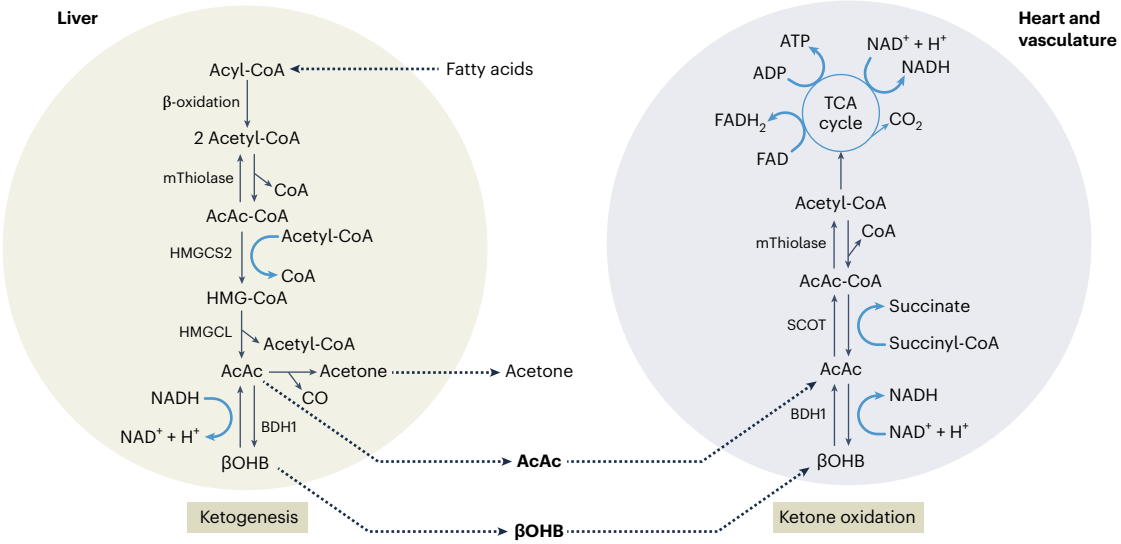

Fig. 1 | Ketogenesis and ketone oxidation in the liver, heart and vasculature. AcAc, acetoacetate; HMGCS2, hydroxy-3-methylglutaryl-CoA synthase 2; HMG-CoA, hydroxy-3-methylglutaryl-CoA; BDH1, β-hydroxybutyrate dehydrogenase 1; TCA, tricarboxylic acid.

The aim of this Review is to discuss the actions of ketones on the CV system, and to discuss the pros and cons of using ketone supplementation to treat CV disease.

Ketone metabolism

Ketone synthesis occurs primarily in the liver[27] (Fig. 1), although the potential for ketogenesis in the heart has been proposed[28,29]. Circulating ketone levels increase under many physiological states including during fasting, during the neonatal period, after exercise and during a low-carbohydrate diet[27]. This rise in ketones can be dramatic, rising from normal levels of 0.1–0.2 mM to 0.5–1 mM with carbohydrate restriction and 5–10 mM with sustained fasting[30,31]. These ketones are derived primarily from increased hepatic fatty acid β-oxidation, with a smaller amount arising from ketogenic amino acids such as leucine and lysine (Fig. 1). Increased release of fatty acids from adipose tissue triacylglycerols undergo hepatic fatty acid β-oxidation to produce acetyl-CoA, a substrate for ketogenesis.

The primary fate of hepatic-derived ketones, such as βOHB and acetoacetate, is their mitochondrial oxidation by extrahepatic tissues (acetone is primarily released during respiration) (Fig. 1). However, it should be recognized that ketones also have a number of non-oxidative fates, such as sterol and fatty acid biosynthesis, which will not be discussed here. Once these ketones enter the circulation, ketone oxidation becomes an important source of energy production by the brain during fasting[30]. Additionally, ketones can be readily oxidized by skeletal muscles, kidney, the heart and the vasculature[2,9,10].

The role of ketones in energy metabolism

Heart

The heart has a very high energy demand and must continually produce large amounts of ATP to sustain contractile function and ionic homeostasis. There are essentially no ATP reserves in the heart, and if not replenished cardiac ATP levels would be completely diminished within 6–10 heart beats[32]. To maintain ATP levels, the heart has a high mitochondrial oxidative capacity, which is responsible for approximately 95% of the heart's ATP production (the remainder originating from glycolysis). The heart is an omnivore and can oxidize a variety of energy substrates, including fatty acids, carbohydrates (glucose and lactate), amino acids and ketones (Fig. 2). Of these, fatty acids and glucose are the major energy substrates, providing 40–90% and 20–60% of the heart's ATP production, respectively[32]. Ketone oxidation is also a notable source of myocardial ATP production. However, although ketones are normally a minor source of energy (10–20% of ATP production), ketone oxidation contribution to ATP production can increase dramatically at higher ketone concentrations[9,10]. While fatty acid and glucose oxidation are highly regulated at allosteric and posttranslational levels, ketone oxidation is less regulated at these levels, with ketone oxidation rates in the heart being more dependent on ketone supply to the heart[9,10].

Transcriptional regulation of ketone oxidative enzymes is another important determinant of ketone oxidation rates. As will be discussed, in certain forms of heart failure, key enzymes of ketone oxidation (BDH1 and succinyl-CoA:3-ketoacid CoA transferase (SCOT)) are transcriptionally upregulated[5,7,9], leading to an increase in ketone oxidation. This upregulation of ketone oxidation enzymes is not, however, a universal finding. For instance, transgenic mice with cardiac-specific overexpression of the insulin-independent glucose transporter GLUT1 fed a high-fat diet develop contractile dysfunction, but SCOT expression was actually decreased[33]. Similarly, Nagao et al.[34] also showed that SCOT expression was decreased in mice subjected to an ascending aortic banding pressure overload. This latter study also suggests that accumulation of myocardial βOHB occurs in this model of heart failure due to a decline in its utilization. Regardless, in the heart failure model in which ketone oxidative enzymes are increased, the increases in ketone oxidation in the heart are not accompanied by significant substantial decreases in the oxidation of other energy substrates, such as fatty acids and glucose[9,10]. For instance, increasing βOHB exposure of hearts from 0.6 mM to 2 mM results in a dramatic increase in ketone oxidation rates, with no decrease in fatty acid oxidation rates, and only a minor decrease in glucose oxidation rates[9,14]. As a result, increasing ketone supply to the heart has the potential to increase overall cardiac ATP production.

It has been proposed that ketones hold an energetic advantage over carbohydrates and fatty acids and may be a 'superfuel' or 'thrifty' energy substrate[35–37]. However, while ATP produced per oxygen consumed (P/O ratio) may be greater for ketones versus fatty acids, it is actually lower than the P/O ratio for glucose[38]. Indeed, increasing ketone supply to the heart increases ATP production, but does not increase cardiac efficiency (cardiac work/O_2 consumed)[9]. Infusion of ketones into humans also does not increase cardiac efficiency, as measured by myocardial external efficiency[25]. As a result, increasing ketone supply to the heart may provide an extra

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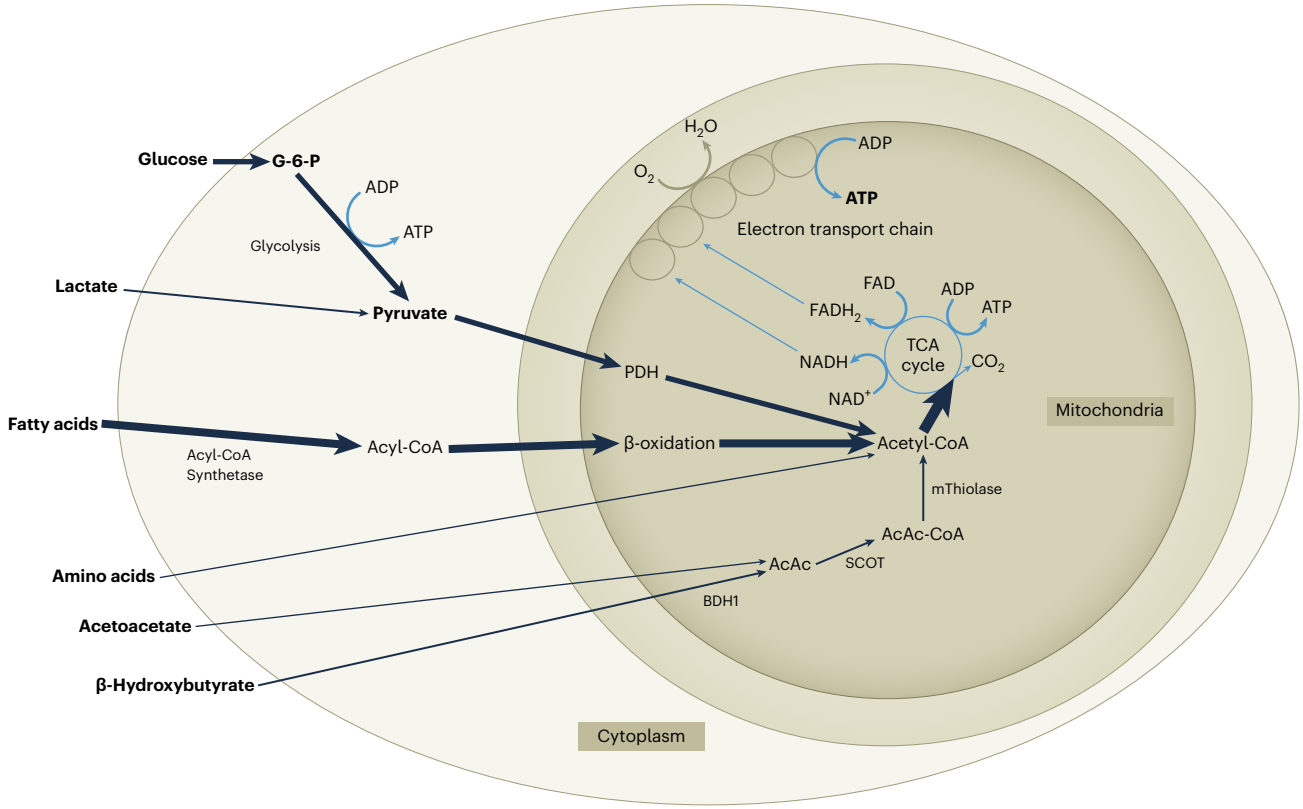

Fig. 2 | Contribution of different energy substrates to ATP production in the heart. PDH, pyruvate dehydrogenase.

source of energy for the heart, but is not necessarily a more efficient source of energy.

Vasculature

Like the heart, the vasculature can also readily oxidize ketones. Using rabbit aortic rings, Chace and Odessey[10] demonstrated that vascular smooth muscle can use a spectrum of substrates as energy sources, including glucose, amino acids, fatty acids and ketone bodies. Ketones were readily oxidized at physiologically relevant concentrations (0.5 mM βOHB) and accounted for 8% of the total vascular smooth muscle O_2 consumption. Endothelial cells also oxidize ketones[2,27]. The metabolic state of quiescent endothelial cells is characterized by high levels of anaerobic glycolysis as well as fatty acid oxidation and ketone oxidation[2,39]. This use of ketones for fuel is not surprising given that SCOT and BDH1 are also expressed in endothelial cells[2]. Ketones are also an important source of biomass production in endothelial cells[2,40]. Activation of endothelial cells toward a proliferating phenotype increases ketone oxidation and rates of glycolysis, while fatty acid oxidation levels are decreased[2]. Ketone oxidation in endothelial cells fuels energy production, resulting in increased proliferation, migration and sprouting potential[2].

The role of ketones in cell signaling

In addition to being a source of fuel for the heart and vasculature, ketones also have a variety of additional effects that may be relevant in regulating the CV system. Ketones alter signaling mechanisms in the heart and vasculature and high levels of circulating ketones (specifically βOHB) can alter pathways including cardiac remodeling[21], G-protein-coupled receptor activation[21], anti-oxidative stress responses[15,41], chronic inflammation[42–44] and mitochondrial quality control[45–47] (Fig. 3). While βOHB interacts with these signaling pathways, acetoacetate is also involved in cellular signaling; some of which overlap with those of βOHB as well as others that appear to be distinct. However, the distinct signaling pathways that are regulated by acetoacetate, such as those involved in regulating fibroblast growth factor 21 (FGF21) expression in the liver[48], buffering methylglyoxal in diabetes[49] and in enhanced tumor growth[50], are arguably less relevant to the CV system than the pathways regulated by βOHB. That said, the ability of acetoacetate to activate G-protein-coupled receptor 43 (GPR43) in adipocytes to promote increased plasma lipoprotein lipase activation, may indirectly contribute to altered fuel metabolism in other organs such as the heart[16].

The role of ketones in inflammation

Arguably, one of the most important actions of βOHB in the CV system is the reduction of inflammation. For instance, at high concentrations, βOHB has been shown to activate the G-protein-coupled receptor 109A receptor (GPR109A)[51]. As this receptor is also found in microvascular endothelial cells[2], it is possible that reduced inflammation in the vasculature may also lead to improved CV health. Notwithstanding this, it is also likely that a reduction in vascular inflammation occurs as a consequence of ketones signaling through the GPR109A receptor found in M1 macrophages[52]. Moreover, the ability of βOHB to attenuate NLRP3 inflammasome-mediated release of pro-inflammatory cytokines also occurs in human monocytes[53], suggesting that this signaling axis may be responsible for the anti-inflammatory effects of ketogenic diets. However, this latter study suggested that the ability of ketones to inhibit the NLRP3 inflammasome in these cells occurred independently of the GPR109A receptor[53]. Thus, it is possible that βOHB can signal through at least two independent pathways to decrease the NLRP3 inflammasome. It should be recognized, however, that βOHB has also been shown to promote the expression of proinflammatory factors by activating the NLPR3 pathway[54].

The ability of βOHB to inhibit the NLRP3 inflammasome and reduce inflammation also appears to play an important role in the heart. The NLRP3 inflammasome is a significant contributor to chronic

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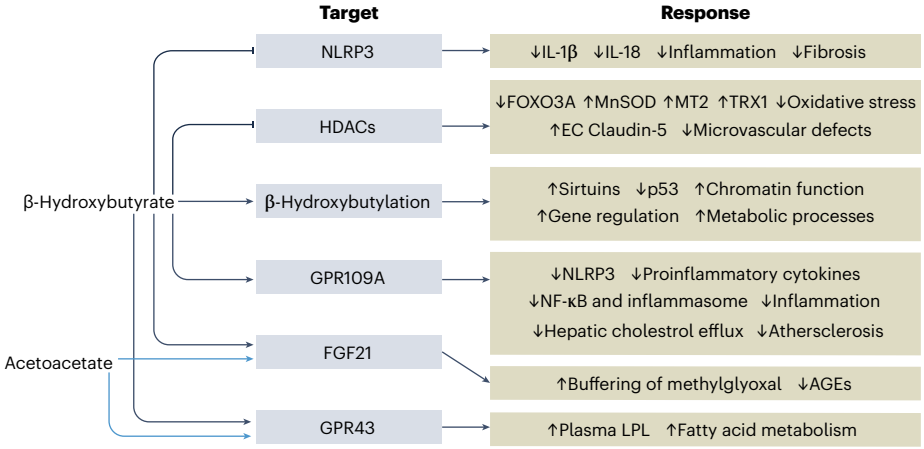

Fig. 3 | Ketone signaling in the heart and vasculature. NLRP3, NLR family pyrin domain containing 3; IL-1β, interleukin 1β; IL-18, interleukin 18; FOXO3A, forkhead box O3A; TRX2; thioredoxin 2; p53, tumor protein 53; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; AGEs, advanced glycation end products; LPL, lipoprotein lipase.

inflammation during heart failure and thus affects heart failure progression[41–43]. In addition, activation of the NLRP3 inflammasome in response to injury is involved in direct local effects such as cardiac remodeling, as well as contributing to systemic inflammation[41–43]. As such, it has been postulated that cardiac NLRP3 activation may contribute to heart failure pathogenesis, and conversely, that inhibition of NLRP3 may be a therapeutic approach to heart failure treatment[41]. In agreement with this, in rodent models of heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF), chronic elevation of βOHB resulted in the inhibition of NLRP3 inflammasome and reduced cardiac fibrosis as well as systolic and diastolic dysfunction, respectively[26,55]. Of importance, this protective effect of ketones was not restricted to inflammation only observed in heart failure because oral administration of a ketone ester to mice with sepsis significantly reduced systemic, renal and cardiac inflammation and protected the mice from cardiac dysfunction[56]. Thus, the ability of βOHB to reduce inflammation has implications in CV disease as well as other inflammatory states. For instance, elevated levels of βOHB via a ketogenic diet or supplementation of a ketone ester drink in mice can restore impaired production of βOHB in acute respiratory distress syndrome caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; ref. 57). By restoring βOHB in this situation, $CD4^+$ T cell metabolism and function are restored and can improve T cell response to infections caused by SARS-CoV-2 (ref. 57). Whether or not this regulatory circuit has a role in the CV system is currently unknown.

Related to the reduction in NLRP3 inflammasome activation in the presence of βOHB, the CV benefit observed by SGLT2 inhibition has also been suggested to be mediated via elevated βOHB levels. Indeed, it has been proposed that SGLT2 inhibition reduces NLRP3 inflammasome activation due to a rise in circulating ketones[58]. In agreement with this, the subsequent ability of the ketones to inhibit the NLRP3 inflammasome in macrophages isolated from humans treated with empagliflozin has already been shown[26]. Moreover, SGLT2 inhibitors also suppressed NLRP3 activation in hearts[59,60], which may be related to SLGT2 inhibitors having profound efficacy in individuals with heart failure[61]. However, studies have also shown that while SGLT2 inhibitors have benefit in heart failure, the ability to inhibit NLRP3 inflammasome activation and/or prevent worsening cardiac functional decline in heart failure occurs independent from elevated βOHB levels[60,62]. Thus, while the ability of SGLT2 inhibitors to elevate ketones may contribute to reduced NLRP3 inflammasome activation, it is also possible that off-target effects also play a role[63,64].

In addition to the key role of ketones in cell signaling and inflammation, ketones also have an important role in epigenetics[45,65–77] (Box 1) and mitochondrial quality control[45–47,78,79] (Box 2).

Ketones in cardiovascular disease

Heart

Marked alterations in energy metabolism occur in the heart in the presence of CV disease. This can include impaired mitochondrial ATP production, decreases in metabolic flexibility and decreases in the efficiency of ATP production[1,32]. Depending on the type of CV disease, mitochondrial oxidation of glucose and fatty acid oxidation can be compromised leading to an 'energy deficit' in the heart[1,32]. Ketones provide a potential alternative source of fuel for the energy-starved heart, and the mitochondrial oxidation of ketones may help the heart adapt to stress (Fig. 4).

Heart failure. In HFrEF, myocardial ketone oxidation rates are increased[5–7,9] (Fig. 4). In one study, patients with end-stage heart failure have increased levels of the ketogenic derivative β-hydroxybutyryl-CoA, along with decreased βOHB in myocardial tissue in association with increased expression of ketone oxidative enzymes such as BDH[5]. The same study showed increased expression of BDH1 and genes for proteins responsible for ketone body transport in mice subjected to transverse aortic constriction (TAC)-induced heart failure and distal coronary ligation. Direct measurements of ketone oxidation by us in a HFrEF mouse model of heart failure also showed increased ketone oxidation[9]. Assessment of arterial-venous differences in ketones also suggests that ketone oxidation is increased in patients with HFrEF[8]. This increase in ketone oxidation is thought to be an adaptive process that provides the energy-starved heart with an extra source of energy[6]. This is supported by studies in which BDH1 overexpression attenuates cardiac remodeling and DNA damage in mice subjected to TAC[80]. However, in individuals with acute myocardial infarction, plasma levels of βOHB are elevated[81,82] and these levels were negatively correlated to the percentage ejection fraction. In addition, exposure of human cardiomyocytes to increased βOHB levels exacerbated cardiomyocyte death and decreased glucose absorption and glycolysis under hypoxic conditions. Furthermore, in individuals with angina-like chest pain, myocardial use of ketone bodies is suppressed by the ischemic condition in the coronary circulation[83,84]. Lastly, plasma ketones have also been shown to be associated with an increased risk of myocardial infarction[82]. Together, these studies challenge the notion that increased ketone use is an adaptive process in the ischemic failing ischemic heart.

BOX 1

The role of ketones in epigenetics

In addition to βOHB reducing inflammation, research has shown that this ketone can also help to reduce oxidative stress. While numerous pathways may be involved in this process, it is clear that histone deacetylases (HDACs) are important targets of βOHB due to their downstream effects. For instance, βOHB increased histone acetylation in human embryonic kidney 293 (HEK293) cells, suggesting that this occurs via βOHB-mediated inhibition of HDACs[45]. Indeed, physiological concentrations of βOHB can inhibit HDAC1, HDAC3 and HDAC4 to increase histone acetylation[45]. Although histone acetylation can increase or decrease the expression of numerous genes[65], βOHB appears to significantly increase the expression of genes involved in the forkhead transcription factor 3A (FOXO3A) network[45]. Given that elevated FOXO3A can reduce oxidative stress via the upregulation of manganese superoxide dismutase (MnSOD) levels[66], it is likely that βOHB contributes to protection from reactive oxygen species via a HDAC–FOXO3A–MnSOD-mediated pathway, at least in the kidney. Although this may also be the case in CV tissues, other studies have shown that inhibition of HDACs by βOHB promotes increased FOXO3A-mediated expression of mammalian metallothionein-2 (MT2) in hearts of septic mice[67]. Therefore, because MT2 has been shown to be a potent free radical scavenger that assists with reducing oxidative stress in many tissues[68], βOHB may improve cardiac function in septic cardiomyopathy[67].

The ability of βOHB to inhibit HDACs and promote acetylation of certain proteins is not the only mechanism by which βOHB can regulate the posttranslational modification of proteins. In fact, β-hydroxybutyrylation is reported to be a new epigenetic regulatory mechanism that can modify histone activity to ultimately regulate gene expression[73]. Directly related to this, at least four sirtuins and HDAC3 possess de-β-hydroxybutyrylase activity that can hydrolyze histone β-hydroxybutyrylase[74]. Thus, it is likely that histone β-hydroxybutyrylation and acylation are linked by the activity of sirtuins[74]. In addition, while histone lysine β-hydroxybutyrylation can link metabolism and βOHB levels to chromatin function and gene regulation[75], more recent work has shown that other proteins can also undergo β-hydroxybutyrylation. Indeed, p53 has been shown to undergo β-hydroxybutyrylation during times of fasting in mice[76], suggesting that this tumor suppressor may be regulated by the metabolic status of cells and/or the organisms. Consistent with this, high levels of β-hydroxybutyrylation have been shown to occur on numerous proteins involved in metabolic processes such as the citrate cycle, pyruvate metabolism and glycolysis/gluconeogenesis in the liver[77], further strengthening the link between β-hydroxybutyrylation and the regulation of metabolism. Thus, although the role of β-hydroxybutyrylation appears to link metabolism and βOHB levels to the regulation of histones and other proteins, the importance of this regulatory mechanism in the CV system is largely unknown.

BOX 2

The role of ketones in mitochondrial quality control

Ketones have also been shown to be important regulators of mitochondrial quality control. For instance, early work has shown that mice fed a ketone ester diet consisting of D-β-hydroxybutyrate-(*R*)-1,3-butanediol monoester displayed evidence of increased mitochondrial size and number in brown adipose tissue along with elevations in the expression of important mitochondrial proteins that contribute to mitochondrial function and biogenesis[45]. More recently, the importance of ketone signaling in the mitochondria has been expanded to include enhanced mitochondrial repair in the aging heart[46]. Specifically, the increase in mitochondrial damage that is observed in hearts during the aging process can be lessened via a βOHB-dependent restoration of mitophagy[46]. Thus, it appears likely that ketones can improve mitophagy flux to improve mitochondrial repair and overall mitochondrial function in the cardiomyocyte. Consistent with these findings in the cardiomyocyte as well as those found in brown adipose tissue, additional work has shown that *db/db* mice administered D-β-hydroxybutyrate-(*R*)-1,3-butanediol monoester had improved cardiomyocyte mitochondrial quality control likely resulting from increased mitophagy[47]. Furthermore, mitochondrial biogenesis was shown to be increased in hearts from *db/db* mice as a result of ketone ester administration[47]. Importantly, all of these improvements in mitochondrial quality/function resulted in an improvement in cardiac function in the *db/db* mice[47], providing evidence that increases in ketones seen in diabetes and fasting[78,79] may play an important role in mitochondrial quality control in the heart.

While myocardial ketone oxidation rates are increased in HFrEF, this is not the case in HFpEF (Sun, Q. Y. et al., unpublished observations). In patients with HFpEF, ketone uptake by the heart is not increased[27]. Furthermore, in diabetic mice with HFpEF, myocardial ketone oxidation rates are decreased[13]. In mice where HFpEF is induced by increasing blood pressure and inducing obesity, we also did not observe any increase in myocardial ketone oxidation[85], while Deng et al.[55] showed a decreased contribution of ketones to mitochondrial oxidative metabolism.

After myocardial infarction. It is less clear what happens to myocardial ketone oxidation after myocardial infarction. In mice subjected to a permanent left anterior descending coronary artery ligation, we observed a significant decrease in myocardial ketone oxidation 4 weeks after myocardial infarction. By contrast, in mice subjected to a TAC and distal coronary ligation, an increased expression of BDH1 and genes encoding ketone body transport were observed[5]. Notably, in this same mouse model of TAC/distal ligation, cardiac-specific *Bdh1* knockout results in a more severe ventricular remodeling and dysfunction after TAC/myocardial infarction[80]. This suggests that low ketone oxidation rates after myocardial infarction may contribute to contractile dysfunction.

Vasculature

Heart failure is associated with microvascular endothelial inflammation and microvascular rarefaction. This results in increased arterial and myocardial stiffness and decreased left ventricle relaxation, resulting in increased left ventricle end-diastolic pressure with impaired left ventricle filling[86]. Coronary microvascular rarefaction (reduced myocardial capillary density) and endothelial cell rarefaction, with reduced coronary flow reserve, contributes to the severity of heart failure. Although the microvasculature and endothelial cells can oxidize ketones[2,10], it is not clear if this is altered in heart failure.

In mice subjected to TAC hypertrophy, vascular rarefaction occurs[2]. However, if mice are fed a ketogenic diet, higher rates

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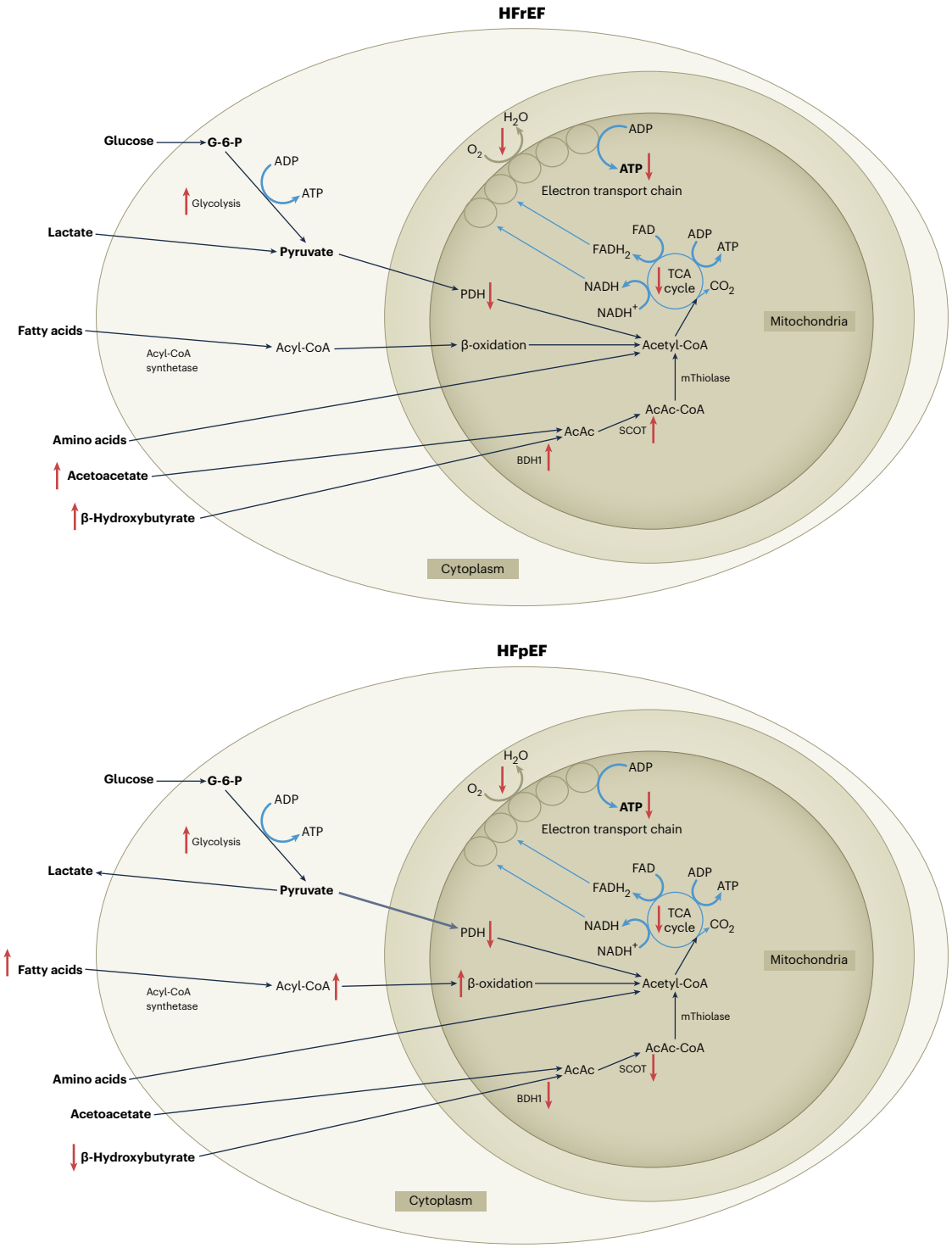

Fig. 4 | Ketone metabolic changes in heart failure. Red arrows indicate an increase or decrease in heart failure.

of endothelial cell proliferation occur, and blood vessel density is maintained[2]. This suggests that ketones can increase the angiogenic potential of cardiac endothelial cells, which might help to maintain a dense blood vessel network in the heart. Whether this is due to increased ketone oxidation is not clear. Support for a role of ketone oxidation in this process is that lymphatic endothelial cell proliferation is associated with increased ketone oxidation, and this promotes formation of new lymphatic vessels in mice[40].

Targeting ketone oxidation and signaling to treat cardiovascular disease

Heart

Increased cardiac ketone oxidation in HFrEF is thought to be an adaptive process that benefits the failing heart[5–7]. Partly for this reason, increasing ketone oxidation has been proposed as an approach to treat heart failure. For instance, acute infusion of βOHB into patients with chronic heart failure (left ventricular ejection fraction,

37% ± 3%) resulted in beneficial hemodynamic effects[25]. Notably, this was not accompanied by an increase in cardiac efficiency, suggesting that the beneficial effects of βOHB are not due to the heart oxidizing a more efficient substrate, but instead caused by an increased energy (ketone) supply to the heart. Chronic infusion of ketones into failing dog hearts is also associated with an improvement in cardiac function[6]. Some of this benefit may occur secondary to afterload, as ketones decrease systemic vascular resistance, suppress sympathetic nervous system activity and reduce total energy expenditure and heart rate[87]. In patients with heart failure, the benefits of ketone supplementation on percentage ejection fraction and cardiac output were accompanied by decreases in systemic and pulmonary vascular resistances[25].

Ketone ester administration is another potential approach to increasing circulating ketone levels (and therefore presumably increasing cardiac ketone oxidation). Administration of ketones to healthy fasting individuals results in an increase in systolic blood pressure, heart rate, biventricular function, and left ventricular and left atrial strain, similar to several effects observed in the failing heart[88]. Administration of the ketone ester (*R*)-3-hydroxybutyl-(*R*)-3-hydroxybutyrate to patients with HFrEF, increases circulating ketones, and myocardial ketone uptake. This acute nutritional ketosis correlates with the degree of cardiac dysfunction and remodeling[89]. Chronic administration of the ketone ester (*R*)-3-hydroxybutyl-(*R*)-3-hydroxybutyrate to mice with heart failure due to TAC surgery significantly elevates circulating ketones. The decline in percentage ejection fraction in TAC mice is also absent in ketone ester-treated mice, which is associated with a decrease in cardiomyocyte hypertrophy[90]. In two rodent heart failure models (TAC/myocardial infarction in mice and post-myocardial infarction remodeling in rats) chronic administration of the ketone ester hexanoyl-hexyl-3-hydroxybutyrate prevents the development of left ventricular dysfunction and remodeling[91]. This treatment normalizes myocardial ATP production following myocardial infarction, consistent with ketones providing an additional source of fuel for the failing heart.

The ketogenic diet has also been used as an approach to increase ketone supply to the failing heart. However, this approach has been less successful than other approaches of increasing circulating ketones. For example, in mouse models of heart failure, implementation of a ketogenic diet has shown only minor benefit[6,92,93], no benefit[6,91] or negative effects[94] on improving heart function. Consistent with this, we recently observed no benefit of a ketogenic diet in preventing TAC-induced heart failure in mice[95]. This appears to be due to excessive cardiac fatty acid oxidation rates and low glucose oxidation rates seen after the administration of a high-fat ketogenic diet. In addition, the ketogenic diet protocol appears to downregulate ketone oxidation in the failing heart, negating any potential benefits of increasing ketone delivery to the heart with a ketogenic diet. This is supported by previous studies by Wentz et al.[12] showing that a ketogenic diet engages mechanisms that curtail ketolytic capacity of the heart. It should be recognized that although a ketogenic diet does raise blood ketones, it also raises circulating fatty acid levels that can contribute to cardiac lipotoxicity and adversely modify cardiac muscle energy metabolism. In addition, ketogenic diets have been shown to inhibit mitochondrial biogenesis and induce cardiac fibrosis[96]. However, in contrast to these studies, in mice in which heart failure was specifically induced by a cardiac-specific deletion of the mitochondrial pyruvate carrier, the progressively developing cardiac dilation and contractile dysfunction was completely reversed by a high-fat, low-carbohydrate ketogenic diet[93]. Attempts have been made to improve the effects of a ketogenic diet on heart failure by using alternate-day ketogenic diet feeding, in which alternate-day ketogenic diet feeding to TAC mice exerted cardioprotective effects and increased hepatic ketogenesis[93]. Administering a medium-chain fatty acid ketogenic diet also partially recovered age-related decreases in succinate dehydrogenase activity and metabolically active mitochondria seen with aging[97].

An alternative approach to increase ketone supply to the heart is with the use of SGLT2 inhibitors. SGLT2 inhibitors increase urinary glucose excretion, resulting in a 'perceived fasting state' that promotes hepatic ketogenesis and increases circulating ketone levels[98]. While originally developed to treat diabetes, SGLT2 inhibitors have generated considerable excitement in the CV community due to their beneficial effects in reducing CV morbidity and mortality in HFrEF and HFpEF[61,99–101]. These beneficial effects occur regardless of the presence or absence of diabetes. While many potential mechanisms for the beneficial effects of SGLT2 inhibitors have been proposed[24], it has also been suggested that these beneficial effects may be mediated by an increase in circulating ketone bodies[24,36,37]. Although it has been proposed that ketones are an energy-efficient and oxygen-sparing fuel for the heart[36,37], other data do not support this[9], and we suggest that increasing circulating ketones may simply provide the heart with an extra source of energy. In TAC-induced heart failure in mice and in post-myocardial infarction mice, SGLT2 inhibition improves heart function[26,91]. In diabetic cardiomyopathic mice (which display symptoms of HFpEF), SGLT2 inhibition with empagliflozin increases cardiac ketone oxidation, improves cardiac energy production and improves cardiac function[13]. This increase in ketone oxidation may be important in HFpEF, as unlike HFrEF, ketone oxidation rates are impaired in HFpEF hearts, contributing to a cardiac energy deficiency. As a result, increasing ketone supply to the heart with SGLT2 inhibition increases overall cardiac energy production[13].

Vasculature

Ketones also have the potential of improving vascular function in disease. For instance, βOHB and acetoacetate can induce angiogenesis in cultured cardiac endothelial cells, and a ketogenic diet can reduce vascular rarefaction in mice after TAC[2]. Although the mechanisms responsible for this have not been clearly established, these findings highlight the beneficial effects of ketones in pathological hypertrophy potentially via improved blood flow to the myocardium. In agreement with this, when mice with either corneal injury or myocardial infarction are administered βOHB, lymphangiogenesis is increased, suggesting improvements in lymph vessel growth[49]. However, it is important to highlight that these beneficial βOHB-mediated vascular effects are not restricted to vascular angiogenesis. Indeed, βOHB improves endothelial function to cause vasodilation in Dahl salt-sensitive rats via an autophagy-dependent mechanism[102]. In the same rat model, βOHB improved endothelium-dependent relaxation in mesenteric arteries via the restoration of nitric oxide synthase activity[103]. Thus, the improvements in vascular function in the presence of βOHB appear to occur because of increased angiogenesis[2] and vasodilation[104]. In agreement with this, hypertensive rats supplemented with 1,3-butanediol to increase βOHB concentrations in the circulation, resulted in improved kidney function and lowered blood pressure following high salt intake[102,103]. This protective effect was also mediated via the inhibition of the NLRP3 inflammasome in the kidney. Together, βOHB appears to reduce hypertension in rodents by promoting endothelial cell proliferation and angiogenesis as well as reducing inflammation in the kidney.

Approaches to increasing ketone levels therapeutically

Although the popularity of therapeutically increasing ketone levels has had a resurgence of late, this approach is not new. Elevating ketone levels in the blood via fasting was first attempted in the late nineteenth century as an approach to help treat epilepsy[105]. However, only more recently has this approach been used to treat CV disease (see ref. 74 for a review). Numerous approaches have been used to elevate circulating levels of ketones, and they can broadly be grouped into two main categories: (1) dietary modifications such as fasting and/or ketogenic

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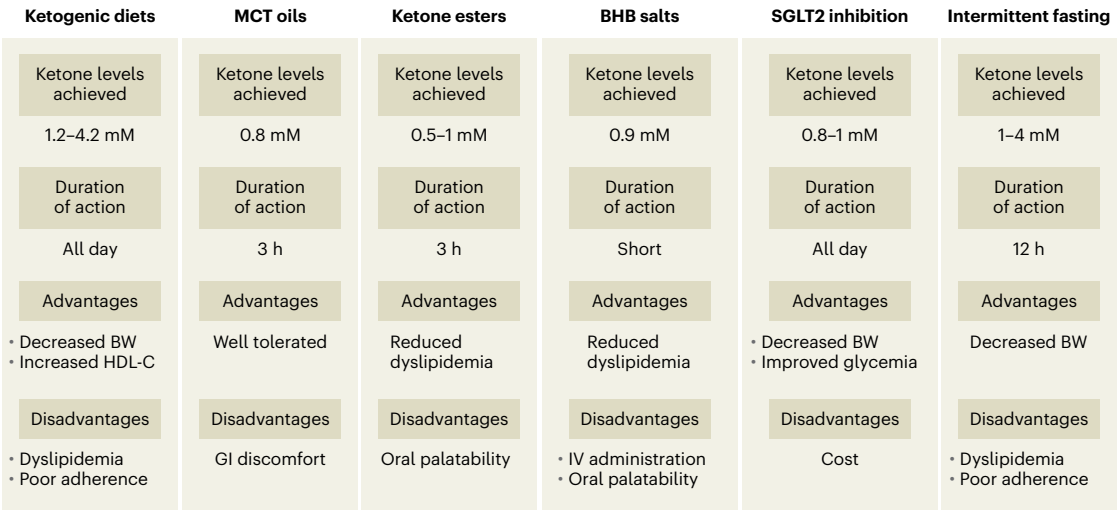

Fig. 5 | Approaches to increase ketone delivery to the heart and vasculature. BW, body weight; HDL-C, high-density lipoprotein cholesterol; GI, gastrointestinal; IV, intravenous.

diets; and (2) ingesting agents that either promote ketogenesis and/or increase ketones directly, including medium-chain triglyceride (MCT) oil, sodium-β-hydroxybutyric acid (BHB) or ketone esters (Fig. 5). Given that the approach of therapeutically increasing ketone levels to treat CV disease is relatively new, the risks and benefits associated with these approaches are not clearly established. However, the existing evidence indicates that there may be some CV benefits of many of these approaches. For example, ketogenic diets can promote weight loss, which should indirectly contribute to improving overall CV health. In addition, following 3 months of a ketogenic diet, circulating levels of acetoacetate and βOHB have been shown to increase to approximately 1.2 mM and 4.2 mM, respectively, in humans. These levels of ketones are within the physiological range of ketones in the blood and thus do not reach concentrations of ketones that can be observed in humans with diabetes[78], suggesting a safe level of ketones that may have CV benefit. That said, the preclinical data using a ketogenic diet to treat CV disease are mixed, with some studies showing benefit[106–110], whereas other studies show neutral or detrimental effects[5,111]. These inconsistencies also occur in human studies that were designed to investigate the effects of a ketogenic diet on CV risk factors such as dyslipidemia and hypertension. In some studies, a shorter duration (3 months) of a ketogenic diet reduced blood pressure, but this effect was lost after 1 year of the diet[112]. This observation at 1 year is consistent with two randomized clinical trials that do not observe any changes in blood pressure[99,100]. Similar discrepancies have also been reported in other clinical trials designed to determine if a ketogenic diet improved[112,113] or worsened[96,114] symptoms of diabetes. These included examining levels of HbA1c[114] or high-density lipoprotein cholesterol and triglyceride levels[105,111–113], where both beneficial and neutral effects were observed. In addition, ketogenic diets have been shown to inhibit mitochondrial biogenesis and induce cardiac fibrosis[96]. The circadian clock has also been shown to control ketogenesis and contribute to changes in the blood βOHB levels[115], so it will be important to consider time of day measurements of βOHB concentrations in subsequent studies. Together, these studies suggest that while a ketogenic diet may have some benefit for treating CV disease, much more research is needed before definitive conclusions can be drawn.

Given that maintaining a ketogenic diet is challenging and often leads to poor patient adherence[113], it is not surprising that other approaches to elevating circulating ketones have increased in popularity. Of these, the approaches that have been more extensively studied are consuming MCT oil, βOHB salts or ketone esters. While adherence to consuming these supplements is significantly higher than implementing a ketogenic diet, these approaches are not without their limitations. For instance, the dietary intake of these ketone 'promoters' does not produce consistently elevated levels of ketones throughout the day, and ketone concentrations in the blood often return to baseline levels within hours of ingestion[116–118]. Thus, the extremely pulsatile nature of this approach to therapeutically increase ketone levels makes interpreting the potential benefits of these approaches somewhat challenging as frequent administration is likely necessary to maintain elevated ketone levels throughout the day.

Consuming MCT oil elevates circulating ketones as a consequence of the metabolic conversion of the 6-carbon to 12-carbon saturated fatty acids to ketones in the liver[119]. While a variety of MCT doses have been used in human studies, many of these doses show that plasma concentrations of acetoacetate and BHB can be increased 3 h after ingestion[120]. More detailed pharmacokinetic studies in humans indicate that 10–20 g of MCT oil can increase βOHB concentrations in the blood as early as 30–60 min after ingestion and that elevated levels can last for 2–3 h[116]. Long-term dosing studies of 30 g MCT oil per day for 6 months show that this dose is reasonably well tolerated, and no severe adverse events were reported[121]. However, the use of MCT oil as a therapy has been hampered because many patients reported gastrointestinal discomfort[116,120,121], and the levels of ketones in the blood following MCT oil ingestion were far less than those observed using the ketogenic diet (that is, 0.8 mM versus 4 mM, respectively)[105,120]. While the benefit of humans consuming MCT oil for the treatment of CV disease has not been studied, the use of MCT oil for therapeutic benefit has been extensively investigated as a treatment for neurological diseases[121], indicating that there is some benefit to this approach in certain diseases. For instance, preclinical studies have shown that ingestion of MCT oil can improve glucose tolerance and insulin sensitivity in rats and that this is also associated with reduced fat mass[122]. Thus, it is possible that these effects are also observed in humans and that these changes can contribute secondarily to treat CV disease. However, future research in the area is necessary to test the effectiveness of MCT oil ingestion in the treatment of CV disease.

As a consequence of the limitations of a ketogenic diet and MCT oil ingestion, arguably the most promising approach to therapeutically increase ketone levels to treat CV disease may be the supplementation of ketone derivatives such as a βOHB salt or ketone esters. For the former, βOHB is conjugated either to a mixture of sodium, magnesium

and calcium or to sodium alone (Na-βOHB). There are certainly some advantages of administration of Na-βOHB over MCT oil or the implementation of the ketogenic diet, including: (1) the flexibility of administration of the βOHB salt via ingestion, injection and infusion; (2) the ketone salts causing low adverse gastrointestinal symptoms[123]; (3) the potential to lessen the risk of metabolic acidosis[25]; and (4) the potential for reduced dyslipidemia. Importantly, human studies have already demonstrated that supplementation of a βOHB salt can elevate circulating βOHB concentrations[25,124] to as high as 0.9 mM in as little as 30 min following ingestion[124]. Other studies using similar doses of Na-BHB (0.5 g per kg body weight) have also shown that peak concentrations of ketones can occur as long 2.5 h following ingestion and don't return to normal levels until approximately 4 h[118,125]. Thus, based on these numerous advantages, it is likely that the implementation of ketone salts as a therapy for CV disease may have some benefits compared to the other approaches described. One important example of this is the 40% increase in cardiac output and a 30% decrease in systemic vascular resistance observed in patients with HFrEF during an acute infusion of Na-βOHB[25], demonstrating the therapeutic potential of Na-βOHB in heart failure. That said, the use of Na-βOHB in heart failure increases sodium levels, potentially leading to additional fluid retention. Thus, this approach may not be ideal for this population, especially in the long term[126].

As a result of the potential for dyslipidemia, fluid retention and/or only modest and transient elevations in circulating ketone concentrations associated with some of the ketone therapies discussed above, another strategy to therapeutically increase ketone levels to treat CV disease is the use of ketone esters. Generally speaking, the ketone esters that have been studied in humans have a neutral pH and are sodium-free precursors of physiological ketones. Once metabolized, these precursors provide physiological ketones including βOHB and, in some instances, acetoacetate[127,128]. Owing to these increases, use of ketone esters to elevate circulating ketones to treat CV disease has been relatively successful in preclinical models. For instance, chronic oral supplementation with two different ketone esters (hexanoyl-hexyl-3-hydroxybutyrate and (*R*)-3-hydroxybutyl-(*R*)-3-hydroxybutyrate) showed benefit in heart failure as well as following an myocardial infarction[91]. This ability of chronic exogenous ketone ester supplementation to blunt the decline of cardiac function in the failing mouse heart induced by TAC was also observed in conjunction with a reduction in activated fibroblasts and cardiac fibrosis[88], but not in a TAC/myocardial infarction model of heart failure[91], suggesting the potential for variable responses depending on the type of heart failure-inducing insult. Interestingly, the benefits of ketone esters in heart failure have also been observed in mice with a skeletal muscle deletion of SCOT, which also results in chronic elevation of circulating ketones[129,130], thus strengthening the ketone ester supplementation results using a genetic approach. Thus, based on the preclinical evidence, it is possible that some of these ketone esters may have therapeutic benefit in humans with heart failure. Indeed, although ketones esters have not yet been tested in patients with heart failure, ingestion of the ketone ester, (*R*)-3-hydroxybutyl-(*R*)-3-hydroxybutyrate, by healthy individuals results in the modification of parameters that are relevant to patients with heart failure such as improved left ventricle function and increased stroke volume, elevated heart rate and increased left ventricle global longitudinal strain[88]. Thus, these echocardiographic and hemodynamic effects of ketone ester supplementation indicate therapeutic effects of ketone esters in a variety of CV diseases.

The nontoxic alcohol 1,3-butanediol is a precursor of βOHB. Oral administration of 1,3-butanediol increases blood BHB concentrations from 0.3 to 0.8 mM[130,131]. Administration of D-β-hydroxybutyrate-(*R*)-1,3-butanediol to *db/db* mice for 4 weeks improved systolic and diastolic function[47]. It also increased the expression of ketone oxidation enzymes in the heart (SCOT and BDH1) suggesting a restoration of myocardial ketone oxidation. In rats subjected to left anterior descending coronary artery occlusion, D-β-hydroxybutyrate-(*R*)-1,3-butanediol treatment attenuated the development of left ventricular dysfunction and remodeling after myocardial infarction[91]. In addition, cardiac hypertrophy was significantly attenuated, but there was no influence of 1,3-butanediol treatment on cardiac fibrosis after myocardial infarction[91]. Although these animal studies suggest benefits in humans, like other ketone esters and salts, side effects include an unpleasant taste, nausea and gastrointestinal distress. Euphoria and dizziness may also occur, which could be related to alcohol intoxication.

Intermittent fasting has generated recent interest as a potential therapeutic approach to treat heart failure[131,132]. Intermittent fasting increases ketogenesis and circulating ketone levels. Whether this increase in ketones contributes to any potential therapeutic effect of intermittent fasting remains to be determined. As discussed, SGLT2 inhibitors also increase ketogenesis and circulating ketone levels[24,36,37]. As a result, SGLT2 inhibition is another potential approach to therapeutically increase ketone levels in heart failure.

Conclusions

Ketones have an important protective role in CV disease, in part by providing an important fuel source for both the heart and vasculature in the failing heart. Ketones also influence several pathways involved in signaling, posttranslational modification and gene transcription, many of which have positive effects on cell proliferation, inflammation, oxidative stress, endothelial function and cardiac remodeling. A number of approaches have been used to increase ketone delivery to the CV system in heart failure, including ketone infusions or intermittent fasting, as well as administration of ketogenic diets, ketone esters or SGLT2 inhibitors. These ketone therapies have considerable potential in the treatment of CV diseases such as heart failure.

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Competing interests

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Additional information

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