Circulation

FRONTIERS

Critical Reanalysis of the Mechanisms Underlying the Cardiorenal Benefits of SGLT2 Inhibitors and Reaffirmation of the Nutrient Deprivation Signaling/Autophagy Hypothesis

Milton Packer, MD

ABSTRACT: SGLT2 (sodium-glucose cotransporter 2) inhibitors produce a distinctive pattern of benefits on the evolution and progression of cardiomyopathy and nephropathy, which is characterized by a reduction in oxidative and endoplasmic reticulum stress, restoration of mitochondrial health and enhanced mitochondrial biogenesis, a decrease in proinflammatory and profibrotic pathways, and preservation of cellular and organ integrity and viability. A substantial body of evidence indicates that this characteristic pattern of responses can be explained by the action of SGLT2 inhibitors to promote cellular housekeeping by enhancing autophagic flux, an effect that may be related to the action of these drugs to produce simultaneous upregulation of nutrient deprivation signaling and downregulation of nutrient surplus signaling, as manifested by an increase in the expression and activity of AMPK (adenosine monophosphate–activated protein kinase), SIRT1 (sirtuin 1), SIRT3 (sirtuin 3), SIRT6 (sirtuin 6), and PGC1-α (peroxisome proliferator–activated receptor γ coactivator 1-α) and decreased activation of mTOR (mammalian target of rapamycin). The distinctive pattern of cardioprotective and renoprotective effects of SGLT2 inhibitors is abolished by specific inhibition or knockdown of autophagy, AMPK, and sirtuins. In the clinical setting, the pattern of differentially increased proteins identified in proteomics analyses of blood collected in randomized trials is consistent with these findings. Clinical studies have also shown that SGLT2 inhibitors promote gluconeogenesis, ketogenesis, and erythrocytosis and reduce uricemia, the hallmarks of nutrient deprivation signaling and the principal statistical mediators of the ability of SGLT2 inhibitors to reduce the risk of heart failure and serious renal events. The action of SGLT2 inhibitors to augment autophagic flux is seen in isolated cells and tissues that do not express SGLT2 and are not exposed to changes in environmental glucose or ketones and may be related to an ability of these drugs to bind directly to sirtuins or mTOR. Changes in renal or cardiovascular physiology or metabolism cannot explain the benefits of SGLT2 inhibitors either experimentally or clinically. The direct molecular effects of SGLT2 inhibitors in isolated cells are consistent with the concept that SGLT2 acts as a nutrient surplus sensor, and thus, its inhibition causes enhanced nutrient deprivation signaling and its attendant cytoprotective effects, which can be abolished by specific inhibition or knockdown of AMPK, sirtuins, and autophagic flux.

Key Words: autophagy ■ heart failure ■ sirtuins ■ sodium-glucose transporter 2 inhibitors ■ TOR serine-threonine kinases

SGLT2 (sodium-glucose cotransporter 2) inhibitors slow the evolution and progression of heart failure (HF), whether they are administered to high-risk patients without symptoms or to symptomatic patients across a broad range of ejection fractions. In 12 large-scale trials involving >70 000 patients, long-term SGLT2 inhibition produced a consistent 20% to 25% reduction in the combined risk of cardiovascular death or hospitalizations for HF.[1] SGLT2 inhibitors are now considered 1 of the 4 foundational drugs for the management of HF in patients with a reduced ejection fraction.

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

Correspondence to: Milton Packer, MD, Baylor Heart and Vascular Institute, 621 North Hall Street, Dallas, TX 75226. Email milton.packer@baylorhealth.edu

For Sources of Funding and Disclosures, see page 1398.

Circulation is available at www.ahajournals.org/journal/circ

Nonstandard Abbreviations and Acronyms

AFABP	adipocyte fatty acid binding protein 4
Akt	protein kinase B
AMP	adenosine monophosphate
AMPK	adenosine monophosphate–activated protein kinase
ATP	adenosine triphosphate
GLUT1	glucose transporter 1
GLUT4	glucose transporter 4
HF	heart failure
HIF-2α	hypoxia-inducible factor 2α
IGF-1	insulin-like growth factor binding protein 1
mTOR	mammalian target of rapamycin
mTORC1	mammalian target of rapamycin complex 1
NAD	nicotinamide adenine dinucleotide
NF-κB	nuclear factor kappa B
NH_4+	ammonium ion
NHE1	sodium-hydrogen exchanger isoform 1
NHE3	sodium-hydrogen exchanger isoform 3
NLRP3	NLR family pyrin domain containing 3
PGC-1α	peroxisome proliferator–activated receptor γ coactivator 1α
PI3KC3	class III phosphatidylinositol 3-kinase
SGLT1	sodium-glucose cotransporter 1
SGLT2	sodium-glucose cotransporter 2
SIRT1	sirtuin 1
SIRT3	sirtuin 3
SIRT6	sirtuin 6
TfR1	transferrin receptor protein 1
TLR9	Toll-like receptor 9
ULK1	Unc-51-like kinase 1

POTENTIAL ACTIONS OF SGLT2 INHIBITORS AS NEPHROCENTRIC NEUROHORMONAL ANTAGONISTS AND AS OSMOTIC DIURETICS

The other foundational drugs for HF—angiotensin receptor neprilysin inhibitors, β-blockers, and mineralocorticoid receptor antagonists—interfere with a neurohormonal mechanism whose activation leads to deleterious effects on the myocardium (ie, angiotensin II, catecholamines, aldosterone, and neprilysin). However, an important neurohormonal mechanism that is not fully antagonized by current foundational drugs is the marked increase in sympathetic nerve traffic to the kidneys. Renal sympathetic tone is strikingly enhanced in experimental and clinical HF and adversely influences prognosis.[2,3] An increase in renal sympathetic nerve traffic adversely affects the heart by increasing the release of angiotensin II and neprilysin from the kidneys, both acting to promote adverse cardiac remodeling and fibrosis as well as sodium retention and volume expansion.[4,5] Enhanced renal sympathetic nerve traffic has also been implicated in the progression of chronic kidney disease, in part related to the stimulation of intrarenal angiotensin II and in part related to the activation of α-2 adrenoreceptors, which may play a role in the pathogenesis of renal interstitial inflammation and fibrosis.[6,7] Renal denervation produces favorable effects in experimental HF and renal disease and improves cardiac function in the clinical setting.[4–9] Of note, although they are generally characterized as inhibitors of the sympathetic nervous system, β-blockers do not reduce renal sympathetic nerve traffic.[10]

Potential Antagonism of Renal Sympathetic Hyperactivity by SGLT2 Inhibitors

Enhanced renal sympathetic nerve traffic would be expected to promote sodium retention by the kidney. The antinatriuretic effect of renal sympathetic activation is particularly pronounced in the proximal renal tubule[11] and proximal tubular hyperreabsorption of sodium is abrogated by renal denervation.[12] The interplay of NHE3 (sodium-hydrogen exchanger isoform 3) and SGLT2 plays a key role in the reabsorption of sodium in the proximal tubule, and increases in renal sympathetic nerve traffic augments the expression of both NHE3 and SGLT2,[13,14] accounting for their increased activity in HF.[14–16]

Most diuretics do not address sodium reabsorption in the proximal renal tubule. It is therefore noteworthy that SGLT2 inhibitors interfere with both SGLT2 and NHE3 in the proximal tubule[16,17] and this action yields short-term increases in the fractional excretion of sodium.[18] HF potentiates the natriuretic effects of SGLT2 inhibition (as would be expected by upregulation of SGLT2 and NHE3 in this disorder)[14,16] and renal NHE3 inhibition by SGLT2 inhibitors promotes euvolemia in rats with HF.[16] In addition, SGLT2 inhibition attenuates renal sympathetic activity and reduces renal norepinephrine content in states of experimental nutrient excess.[19,20] Renal denervation attenuates the magnitude of the responses to SGLT2 inhibition in the kidney in animals with (but not without) HF.[14] Taken collectively, these observations position SGLT2 inhibitors as functional antagonists of renal sympathetic nerve hyperactivity in HF. However, any postulated sympatholytic action is likely to be highly selective for the renal nerves, because SGLT2 inhibition does not reduce cardiac sympathetic activity[21] and has not been shown to decrease central sympathetic outflow.

Potential Benefits of SGLT2 Inhibitors Acting as Osmotic Diuretics

The possibility that SGLT2 inhibitors might treat HF by functioning as antagonists of renal sympathetic nerve

hyperactivity or by ameliorating sodium retention is appealing (Figure 1). However, it has been difficult to show that SGLT2 inhibitors exert important natriuretic effects in patients with HF. In this setting, SGLT2 inhibition exerts a short-term osmotic diuretic effect with little increase in urinary sodium excretion,[22,23] suggesting that any increase in urine volume is related to augmented glucose (and not sodium) excretion. However, early increases in urinary water excretion are not sustained because of the activation of adaptive mechanisms that decrease free water clearance,[24] explaining why long-term SGLT2 inhibition does not change serum sodium concentration.[25]

Additional lines of evidence raise further doubts that a sustained diuretic effect can account for the benefits of SGLT2 inhibitors in HF. SGLT2 inhibitors are not more effective in patients with HF who have volume overload, as compared with those who are euvolemic.[25] Although these drugs produce a modest decrease in body weight, this appears to be related to the urinary caloric loss and the shrinkage of fat depots rather than to changes in fluid status[26,27]; changes in body weight are poorly correlated with changes in natriuretic peptides.[25] The increase in hematocrit seen in clinical trials is a delayed effect that is related to erythrocytosis rather than hemoconcentration.[28] Although SGLT2 inhibitors can decrease plasma volume, this represents a compensatory mechanism that is triggered by the increase in red blood cell mass[29]; if plasma volume were not reduced, erythrocytosis would lead to intolerable hypervolemia.

It has been proposed that the osmotic diuretic effect of SGLT2 inhibitors leads primarily to decreases in interstitial fluid (rather than plasma volume), but this hypothesis has been on the basis of modeling rather than direct measurements.[30] Indeed, at a time when the diuretic effects of SGLT2 inhibitors are most prominent (ie, during the first weeks of treatment), changes in cardiac filling pressures and natriuretic peptides are very small.[31–33] A transient diuresis cannot explain the meaningful changes in cardiac geometry[34] or the striking reduction in the risk of major adverse renal events seen in patients with HF with a reduced ejection fraction.[35] Any improvement stemming from an osmotic diuretic action of these drugs would be expected to be potentiated in individuals with diabetes (who exhibit the most marked glycosuria), but patients with HF are not more likely to respond to SGLT2 inhibitors if they are diabetic.[35,36] Conversely, preexisting renal impairment would be expected to attenuate the glycosuric and osmotic diuretic actions of SGLT2 inhibitors; however, chronic kidney disease does not diminish the effect of SGLT2 inhibitors to reduce hospitalizations for HF.[26,37]

Enthusiasm for the hypothesis that SGLT2 inhibitors act primarily as nephrocentric neurohormonal antagonists or as diuretics has been driven by the belief that the efficacy of these drugs should be linked to their actions within the kidney, because SGLT2 is expressed primarily in the proximal renal tubule. A diuretic effect of SGLT2 inhibitors may underlie some of the short-term benefits of these drugs, and the enhanced distal delivery of sodium may also promote the exchange of sodium for potassium, thus enhancing kaliuresis and mitigating the risk of hyperkalemia.[38] Aside from these actions, renal sympatholysis or increases in urinary volume do not appear to contribute importantly to the long-term ability of SGLT2 inhibitors to reduce the risk of HF or renal events.

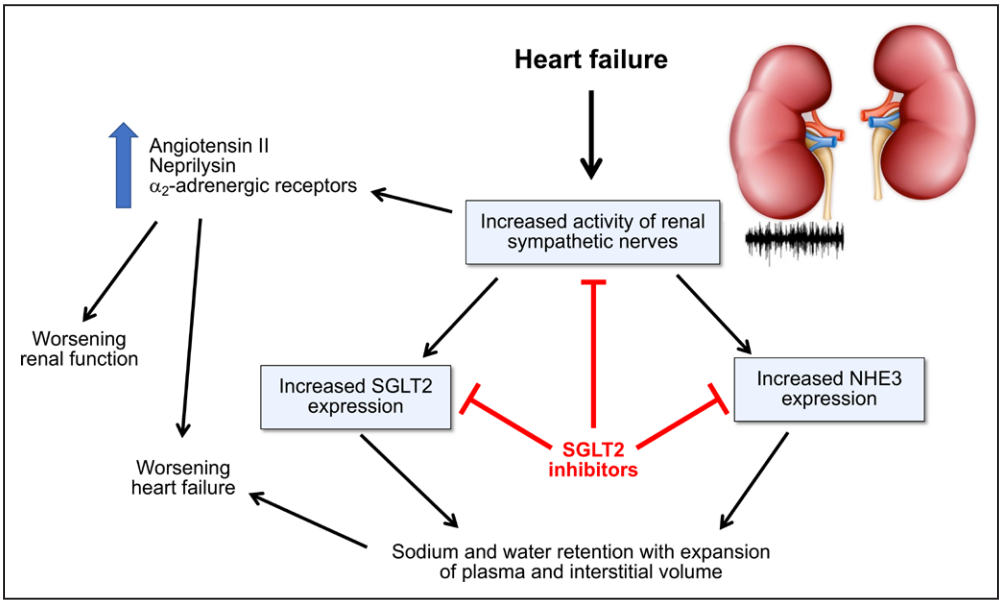

Figure 1. Proposed framework by which SGLT2 (sodium-glucose cotransporter 2) inhibitors might exert cardioprotective and nephroprotective effects by acting to mute renal sympathetic nerve activity and promote natriuresis and osmotic diuresis.
NHE3 indicates sodium-hydrogen exchanger isoform 3.

Potential Importance of Mitigation of Glomerular Hypertension by SGLT2 Inhibitors

Glomerular hyperfiltration has long been regarded as the mechanism driving the progression of kidney disease in patients with diabetes. The benefits of angiotensin receptor blockers and mineralocorticoid receptor antagonists on the evolution of diabetic nephropathy have been attributed to their effect to decrease intraglomerular pressures. However, the role of glomerular hyperfiltration in the progression of kidney disease in HF has been questioned. Angiotensin-converting enzyme inhibitors and mineralocorticoid receptor antagonists do not slow the loss of nephrons in patients with HF.[39,40] Furthermore, neprilysin inhibition reduces the risk of major renal events in patients with HF, even though the afferent arteriolar vasodilation produced by enhanced cyclic guanosine monophosphate signaling increases intraglomerular pressures and albuminuria.[41]

Doubts about the role of glomerular hyperfiltration in the progression of renal disease in patients with HF have clouded efforts to understand the nephroprotective effects of SGLT2 inhibitors in this disorder. By inhibiting proximal tubular sodium hyperreabsorption, SGLT2 inhibitors can rapidly reduce intraglomerular pressures, although this may not be related to enhanced tubuloglomerular feedback.[42] SGLT2 inhibition produces an immediate decrease in glomerular filtration rate in patients with HF.[35] The magnitude of the early decline in glomerular filtration is attenuated in patients with renal impairment[43]; however, the ability of SGLT2 inhibitors to slow the progression of renal disease is not diminished.[37] It is therefore noteworthy that experimental knockout of SGLT2 is sufficient to attenuate glomerular hyperfiltration, but it does not prevent renal injury, inflammation, or fibrosis in experimental diabetes or ischemia.[44,45] An action of SGLT2 inhibitors to reduce intraglomerular pressures may not be relevant to their renoprotective effects in HF.

POTENTIAL ACTIONS OF SGLT2 INHIBITORS TO PROMOTE ENERGY SUBSTRATE DELIVERY

In the absence of a nephrocentric explanation for the benefits of SGLT2 inhibitors, investigators have focused on 2 prominent physiologic features of these drugs: ketogenesis and erythrocytosis. Both ketonemia and an increased red blood cell mass might augment delivery of efficient fuels and oxygen, both of which may have favorable effects on the energy state of hearts and kidneys under stress (Figure 2).

Potential Role of Ketone Bodies Acting as a Fuel for the Heart and Kidneys

By promoting glycosuria, SGLT2 inhibitors trigger a state of starvation mimicry that is characterized by the production of ketone bodies—particularly β-hydroxybutyrate—in the liver.[46] Although the infusion of large doses of β-hydroxybutyrate exerts positive inotropic and chronotropic effects,[47] SGLT2 inhibition does not produce these hemodynamic effects in patients or in isolated cardiomyocytes.[33,34,48] Furthermore, circulating ketone bodies are increased in patients with HF,[49] and the failing heart already uses ketone bodies as a preferred fuel.[50] In patients without diabetes, the degree of ketonemia is muted,[36,51] but the HF benefits are not.[35]

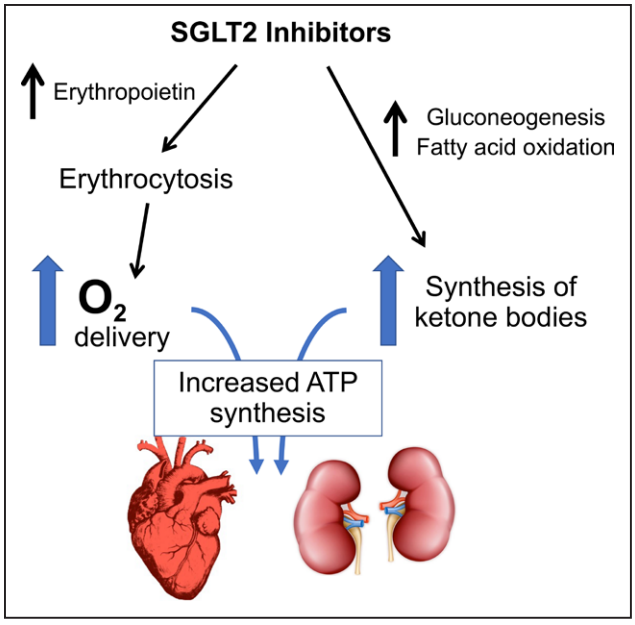

Figure 2. Proposed framework by which SGLT2 (sodium-glucose cotransporter 2) inhibitors might act to increase delivery of substrates that could lead to enhanced synthesis of ATP (adenosine triphosphate).

Furthermore, under experimental conditions, SGLT2 inhibitors have not consistently enhanced ketone body consumption by the myocardium,[52–54] and increases in ATP (adenosine triphosphate) production seen after SGLT2 inhibition are not related to increased ketone body metabolism.[52] Augmentation of ketone body oxidation is not beneficial in experimental HF,[55] and clinically, ketogenesis after SGLT2 inhibition does not consistently yield increases in myocardial ATP.[54,56] Similarly, the nephroprotective actions of SGLT2 inhibitors cannot be directly linked to ketogenesis-related increases in ATP.[57]

Potential Role of Erythrocytosis to Increase Tissue Oxygen Delivery

It has been hypothesized that the increased tubular workload related to increased distal sodium delivery after SGLT2 inhibition might produce renal medullary hypoxia,[58] thus triggering the synthesis of erythropoietin and an increase in hemoglobin. However, magnetic resonance imaging has not discerned evidence for the induction or alleviation of renal hypoxia in patients treated

with SGLT2 inhibitors,[59] suggesting that the increase in erythropoietin is likely related to an effect on hypoxia-inducible factors.[60]

Might the increase in red blood cell mass improve tissue oxygenation and thereby reduce HF or renal events? Oxygen utilization is not typically impaired in hemodynamically stressed hearts, but oxygen delivery still might be important in patients with coronary artery disease. However, the benefits of SGLT2 inhibitors in HF are not enhanced by the presence of ischemic heart disease.[35] Furthermore, when red blood cell mass is increased by means of erythropoietin-mimetic agents in large-scale trials, the risk of major HF events or of progression of renal disease is not diminished, even though patients were anemic at the start of treatment.[61,62] Therefore, the totality of evidence does not suggest that the cardioprotective or renoprotective effects of SGLT2 inhibitors can be explained by enhanced delivery of energy substrates.

POTENTIAL ACTIONS OF SGLT2 INHIBITORS TO INHIBIT SODIUM-HYDROGEN EXCHANGE

The function of sodium-glucose cotransporters and sodium-hydrogen exchangers are closely intertwined in several organs. The actions of SGLT1 (sodium-glucose cotransporter 1) and NHE3 are linked in the intestinal mucosa[63] and SGLT2 and NHE3 are colocalized in the proximal renal tubule,[64] such that inhibition of SGLT2 also impairs the actions of NHE3.[16,17] It is therefore possible that SGLT2 inhibitors might interfere with other sodium-hydrogen exchangers (eg, NHE1 [sodium-hydrogen exchanger isoform 1]). Experimental activation of NHE1 in the heart can promote hypertrophy, cell death, and the development of HF,[65] making it an attractive therapeutic target.

Baartscheer and colleagues[66,67] first proposed that SGLT2 inhibitors might function as NHE1 antagonists in the heart; they postulated that these drugs could exert direct benefits on cardiomyocytes, independent of renal glycosuria or any interaction with SGLT2, because SGLT2 is not expressed in the myocardium. Incubation of rat cardiomyocytes with SGLT2 inhibitors delayed pH recovery after an ammonium ion (NH_4+) pulse and reduced intracellular sodium concentrations, actions consistent with NHE1 inhibition, and the investigators reported a docking site for SGLT2 inhibitors on the NHE1 protein. The effects on cardiomyocytes were attenuated by cariporide, a canonical NHE1 antagonist.

Despite these observations, a role for NHE1 inhibition in mediating the cardiovascular benefits of SGLT2 inhibitors remains in doubt. Chung et al.[69] reported no effect of SGLT2 inhibitors to delay pH recovery after a NH_4+ pulse or to reduce intracellular sodium concentrations, thus challenging the findings of Baartscheer and colleagues.[66,67] The published interchange between the 2 groups revealed that the data of Chung et al.[69] were derived from a larger sample size.[68,69] Osaka et al.[70] claimed that SGLT2 inhibition inhibited NHE1, but the effect was substantially less than that seen with cariporide. Li et al.[48] failed to show meaningful docking of empagliflozin to NHE1 and reported that cariporide did not mimic the action of the SGLT2 inhibitor on isolated cardiomyocytes. Therefore, if there is an effect of SGLT2 inhibitors to blunt increases in intracellular sodium, it may be secondary to their effects to reduce cellular stress or to an action on the late sodium current.[60,71]

ACTIONS OF SGLT2 INHIBITORS TO PROMOTE NUTRIENT DEPRIVATION SIGNALING AND AUTOPHAGY TO REDUCE CELLULAR STRESS AND PROMOTE CELLULAR SURVIVAL

The key clinical biomarkers of the action of SGLT2 inhibitors—ketogenesis and erythrocytosis—reflect the typical responses to nutrient and oxygen deprivation.[60] SGLT2 inhibitors also reduce serum uric acid, an indicator of oxidative stress in patients with HF.[72] How are these 3 biomarkers related to each other? Enhanced nutrient and oxygen deprivation signaling can promote erythropoietin synthesis and reduces oxidative stress in cardiomyocytes and renal parenchymal cells.[60] It is therefore noteworthy that mediation analyses of large-scale outcomes trials in patients with type 2 diabetes have consistently identified increases in hemoglobin and decreases in uric acid as statistical determinants of the ability of SGLT2 inhibitors to reduce the risk of HF hospitalizations and major adverse renal events.[73–75] These clinical findings, taken together with a wealth of data from experimental studies, have led to the hypothesis that the cardiorenal benefits of SGLT2 inhibitors are related to the activation of nutrient deprivation signaling, with its attendant effects to promote autophagy and mitochondrial health, reduce the generation of reactive oxygen species, mute inflammation and fibrosis, and enhance the viability of cardiomyocytes and renal parenchymal cells.[60] Although this response may be triggered by an action of SGLT2 inhibitors to induce a state of starvation mimicry secondary to the urinary loss of calories,[76] numerous studies published during the past 2 years have shown that the adaptive cellular reprogramming produced by these drugs is seen in isolated cell cultures, indicating that SGLT2 inhibitors have direct glycosuria-independent actions to reduce cellular stress and promote cellular survival.

Nutrient Sensors and Cellular Signaling in the Heart and Kidney in Health and Disease

SGLT2 protein functions as an energy sensor, discerning an excess nutrient state when there is excessive

glucose in the proximal renal tubules, and changes in SGLT2 parallel changes in other energy sensors in response to shifts in environmental nutrients.[77] When faced with changes in extracellular glucose and amino acids, the interplay of several master switches adapts the cell to promote its growth or survival. These include mTOR (mammalian target of rapamycin); sirtuins (SIRT1 [sirtuin 1], SIRT3 [sirtuin 3], and SIRT6 [sirtuin 6]); and AMPK (adenosine monophosphate–activated protein kinase; Figure 3).

Mammalian Target of Rapamycin

mTOR is a serine/threonine protein kinase that is activated by a surplus of environmental amino acids and functions to promote cell growth and proliferation. mTOR exists in 2 complexes—mTOR complex 1 (mTORC1) and mTOR complex 2—and Akt (protein kinase B) potentiates the activation of mTORC1, which is preferentially inhibited by rapamycin. Akt/mTORC1 signaling influences hundreds of downstream effectors to promote anabolic pathways, driving the mitochondrial production of reactive oxygen species to facilitate cellular replication, proinflammatory pathways, and innate immunity, and enhancing the expression of the senescence-associated secretory phenotype that is essential to the cellular disposal required for effective organ growth.[78] The action of mTOR activity to promote anabolic pathways is required for cardiomyocyte replication during fetal development and adaptive hypertrophy during acute massive pressure overload,[79,80] but it leads to maladaptive cardiac remodeling when activated in hearts that are injured in adulthood. In experimental models, cardiac-specific overactivation of the Akt/mTOR pathway induces HF,[81] whereas suppression of Akt or mTOR signaling ameliorates the development of cardiomyopathy.[82,83] Similarly, Akt/mTOR signaling is hyperactivated in the kidney in nutrient surplus states, thus triggering proinflammatory pathways and promoting renal injury,[84–86] whereas inhibition of mTOR can ameliorate fibrosis and the development of chronic kidney disease.[86,87]

In the clinical setting, patients with dilated cardiomyopathy show aberrant myocardial activation of mTORC1, the intensity of which is associated with the severity of cardiac fibrosis and a poor prognosis.[88] Activation of Akt in the human myocardium characterizes the transition from well-compensated left ventricular hypertrophy to decompensated HF.[89] mTOR-activating sequence variations can cause clinical cardiomyopathy and kidney tubulopathy[90] and renal mTORC1 activation is associated with disease activity and prognosis in patients with nondiabetic kidney disease.[91]

Sirtuin 1 and Other Sirtuins

Sirtuins are a family of redox-sensitive nicotinamide adenine dinucleotide (NAD)–dependent deacetylases that catalyze the posttranslational modification of hundreds of proteins that are involved in metabolism and cellular homeostasis. They serve as a redox rheostat and

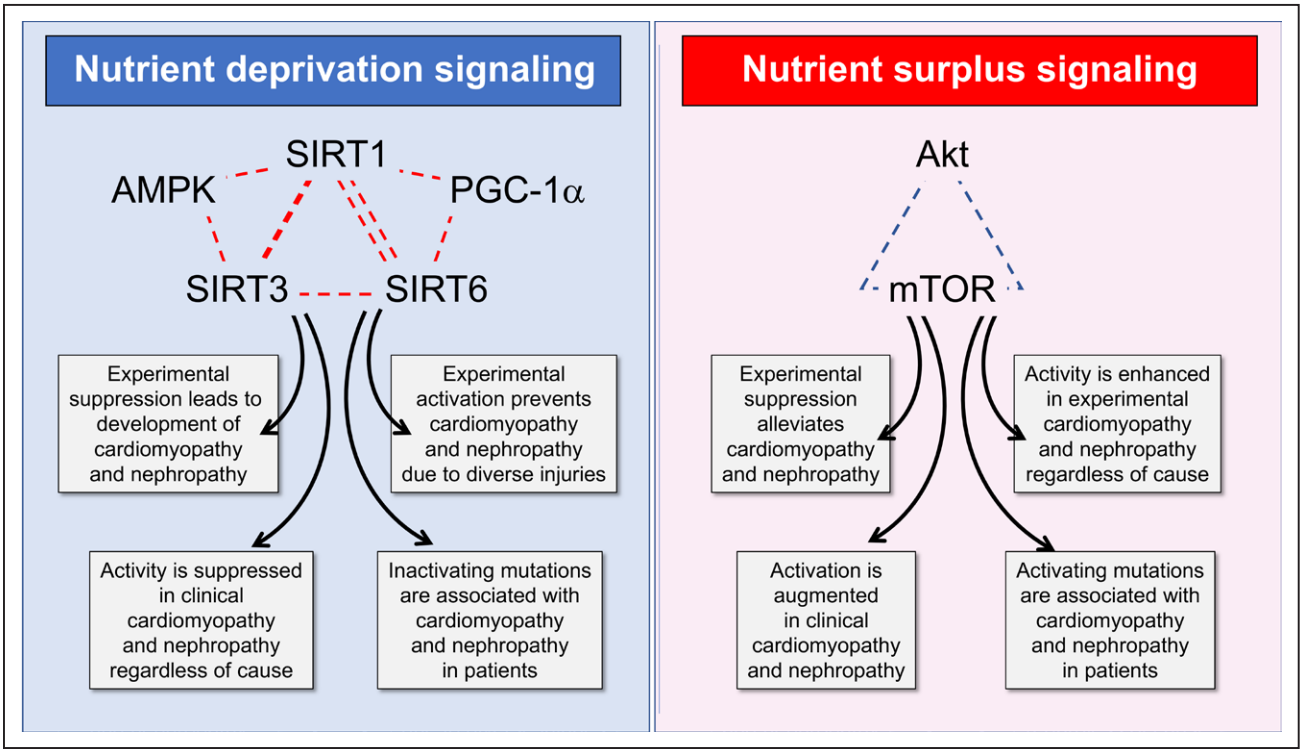

Figure 3. Effect of nutrient deprivation and nutrient surplus signaling on the evolution and progression of cardiomyopathy and nephropathy in experimental and clinical settings.
Akt indicates protein kinase B; AMPK, adenosine monophosphate–activated protein kinase; mTOR, mammalian target of rapamycin; PGC-1α, peroxisome proliferator–activated receptor γ coactivator 1-α; SIRT1, sirtuin 1; SIRT3, sirtuin 3; and SIRT6, sirtuin 6.

represent the primary cellular response to glucose deprivation. SIRT1, SIRT3, and SIRT6 share similar functions, with SIRT1 and SIRT6 being localized to the nucleus and SIRT3 to the mitochondria. Cardiac-specific deletion or suppression of SIRT1, SIRT3, and SIRT6 augments production of reactive oxygen species, enhances endoplasmic reticulum stress, and sensitizes the heart to injury, leading to cardiomyopathy.[92–94] Conversely, SIRT1, SIRT3, and SIRT6 enrichment or activation alleviates oxidative and endoplasmic reticulum stress, disposes of dysfunctional mitochondria and promotes mitochondrial biogenesis, mitigates cell senescence and death, ameliorates fibrosis and remodeling, and preserves cardiac function.[95–98] Deficiencies in SIRT1, SIRT3, and SIRT6 exacerbate glomerular injury and glomerulosclerosis after renal stress,[99–101] and SIRT1, SIRT3, and SIRT6 activation is accompanied by reduction in oxidative stress, tubulointerstitial inflammation, and fibrosis; amelioration of glomerular and tubular damage; and maintenance of renal function.[102–104] The adaptive effects of sirtuins on organellar health and cellular stress are potentiated by its downstream effector (eg, PGC-1α [peroxisome proliferator–activated receptor γ coactivator 1-α]), which plays a key role in mitochondrial biogenesis, and PGC-1α has been implicated in the pathogenesis of experimental cardiomyopathy and nephropathy.[105,106]

In the clinical setting, patients with cardiomyopathy show suppressed expression of SIRT1, SIRT3, and PGC-1α[107,108] and point sequence variations in the genes for SIRT3 and PGC-1α.[109,110] Loss-of-function polymorphisms in the *SIRT1* gene increase the risk of chronic kidney disease in patients with type 2 diabetes.[111] In patients with diabetes, the glomerular expression of SIRT6 is suppressed[101]; serum SIRT1 levels are decreased, with a decline that parallels the development of albuminuria[112]; and there is an inverse relationship between the expression of SIRT1 and SGLT2 in the human diabetic kidney.[77]

Adenosine Monophosphate–Activated Protein Kinase

AMPK is a serine/threonine kinase that discerns the balance between cytosolic levels of ATP and AMP (adenosine monophosphate). AMPK is activated when the ATP-to-AMP ratio is low and phosphorylates downstream proteins that promote increased catabolism and decrease anabolism, thereby augmenting ATP production.[199] Knockout or suppression of AMPK diminishes the heart's ability to respond to stress, promotes cardiac aging, and leads to cardiomyopathy.[113,114] Activation of AMPK ameliorates oxidative stress, mitochondrial dysfunction, proinflammatory pathways, fibrosis, and apoptosis and preserves ventricular function during cardiac stresses produced by ischemia, diabetes, pressure overload, or cardiotoxic agents.[115–117] AMPK activity is impaired in the diabetic kidney, which contributes to the development of nephropathy.[118] Activation of AMPK promotes glomerular health and podocyte survival and reduces tubular injury, apoptosis, and fibrosis triggered by metabolic stresses, ischemia, and nephrotoxic drugs.[119–121]

In the clinical setting, patients with sequence variations in the *PPKAG2* gene (which codes for an AMPK subunit) develop cardiomyopathy with atrial and ventricular arrhythmias.[122] Sequence variants of *PPKAG2* are also linked to accelerated decline in glomerular filtration rate and the development of kidney disease in the general population and in patients with diabetes.[123,124]

These 3 master regulators—mTOR, sirtuins, and AMPK—modulate changes in cellular biology that allow organisms to adapt to environmental opportunities and challenges. When nutrients are plentiful, organisms prioritize the use of fuels to expand cell mass and mTOR signaling is central to this process. In contrast, when nutrients are depleted, organisms mute anabolic pathways and adopt a sheltered set of biological conditions to preserve the structural and functional integrity of existing cells; the sirtuins, PGC-1α, and AMPK are critical to this response. The set point for the interplay of these master switches is determined by the level of nutrients and the redox state.[125]

Of note, SIRT1/PGC-1α and Akt/mTOR pathways are highly interconnected at a molecular level. SIRT1 and PGC-1α can negatively regulate the transcription of Akt and directly interfere with the actions of Akt and mTOR.[126,127] Conversely, upregulation of Akt leads to suppression of PGC-1α, whereas inhibition of mTOR by rapamycin or Akt downregulation leads to activation of SIRT1 and PGC-1α.[128,129] The effect of AMPK to promote NAD+ leads to SIRT1 activation[130] and AMPK can activate PGC-1α by phosphorylation.[131] Conversely, AMPK can inhibit mTOR by an action on Akt as well as through a direct effect on the mTORC1 complex.[132]

Action of Nutrient Sensors to Influence Cellular Stress by Modulation of Autophagy

The interplay of the actions of the mTOR, sirtuin, and AMPK master switches to reduce cellular stress and promote cellular survival may be primarily mediated by their actions to influence the cellular housekeeping process of autophagy. Autophagy is an evolutionarily conserved intracellular degradative pathway, which involves the encircling of unwanted or dangerous cellular components by a double-membrane vesicle (the autophagosome), whose fusion with the lysosome allows degradative enzymes to destroy the contents of the vesicle.[133,134] The primordial stimulus to autophagy is nutrient deprivation, and when activated nonselectively, autophagy allows for recycling of cellular constituents, which generates ATP for energy-starved cells.[135] However, autophagic flux also can be activated selectively in response to a broad range of cellular

stresses, including oxidative and endoplasmic reticulum stress. The most important sources of oxidative stress are dysfunctional mitochondria and peroxisomes and their clearance by autophagy is referred to as mitophagy and pexophagy. Endoplasmic reticulum stress is typically caused by the accumulation of misfolded proteins or glucose or fatty acid intermediates that may result from a variety of cellular injuries. Autophagic clearance of these damaged cellular constituents markedly reduces cellular stress and autophagic flux also directly acts to mitigate proinflammatory and profibrotic responses.[136] The overall effect of enhanced autophagic flux is to preserve cellular integrity and prevent apoptotic cell death, thus maintaining and restoring organ structure and function.

Autophagic flux is driven by the interplay of nutrient deprivation and surplus sensors. The formation of the autophagosome is initiated by the concerted interaction of the ULK1 (Unc-51-like kinase 1) complex with the Beclin 1–PI3KC3 (class III phosphatidylinositol 3-kinase) complex.[134,135] AMPK activates ULK1, but ULK1 phosphorylation by mTORC1 prevents its activation. Beclin 1 is activated when deacetylated by SIRT1[137] and TLR9 (Toll-like receptor 9) interacts with Beclin 1 to regulate the assembly of the PI3KC3 complex.[138] Elucidation of the numerous influences of mTOR, sirtuins, and AMPK on the components of the autophagic machinery is beyond the scope of this review.

The adaptive actions of autophagy are particularly important in the heart and kidneys. Both the heart and kidneys are replete with mitochondria and peroxisomes, which underlie their enormous capacity to consume oxygen and generate reactive oxygen species. Amelioration of oxidative and endoplasmic reticulum stress is particularly important to cardiomyocytes, because nonproliferating cells cannot replace cells that have died. Chronic HF and chronic kidney disease is characterized by the accumulation of intracellular debris and deleterious metabolic intermediates, a marked increase in oxidative stress, and the activation of proinflammatory and profibrotic signals.[60] Autophagic flux represents a major adaptive and restorative response; impairment of autophagy dramatically heightens the likelihood that an injurious event will lead to cardiomyopathy and nephropathy.[139,140] Although excessive stimulation of autophagy may exert deleterious effects on the heart (particularly after acutely imposed massive stresses in the absence of HF),[141,142] measured augmentation of selective autophagy protects the heart against pressure overload, hypoxia, and injury produced by cardiotoxic agents,[143,144] and shields podocytes and renal tubular cells from damage caused by diabetes, ischemia, and nephrotoxic drugs.[145,146]

Autophagic vacuoles are increased in patients who show reverse cardiac remodeling or receive mechanical unloading,[147,148] whereas persistent impairment in autophagy flux in the failing human heart is a poor prognostic sign.[149,150] Similarly, autophagic function is blunted in renal tubular cells of patients with diabetic nephropathy[151] and loss of autophagosomes is a marker of chronic graft dysfunction in renal transplant patients.[152] By contrast, increased autophagosome density in podocytes presages the preservation of glomerular function and a reduced risk of progression in patients with kidney disease.[153,154]

Effects of SGLT2 Inhibitors on Nutrient Deprivation and Surplus Signaling, Autophagic Flux, and Cellular Stress in Experimental Cardiomyopathy and Nephropathy

SGLT2 inhibitors produce a highly distinctive pattern of favorable effects on the evolution and progression of cardiomyopathy and nephropathy in experimental animals (Figure 4). First, SGLT2 inhibitors consistently enhance autophagic flux (including mitophagy) in the heart and kidney; the action to augment autophagy is seen in tissue harvested from chronically treated animals as well as in isolated cell cultures perfused with SGLT2 inhibitors (Table 1).[155–173] Second, SGLT2 inhibitors mute the generation of reactive oxygen species, enhance antioxidant mechanisms, and mitigate endoplasmic reticulum stress.[165,167,169,174–176] Third, SGLT2 inhibitors accelerate the disposal of injured mitochondria, restore healthy mitochondrial function, and promote mitochondrial biogenesis[167,169]; the increase in mitochondrial mass is a characteristic feature of the action of these drugs on electron microscopy.[156] Fourth, SGLT2 inhibitors reduce inflammation and fibrosis by interfering with the activation of NF-κB (nuclear factor kappa B) and the NLRP3 (NLR family pyrin domain containing 3) inflammasome, thus reducing the synthesis of proinflammatory cytokines; they also mute profibrotic pathways, fibroblast proliferation, and the deposition of collagen.[177,178] Fifth, SGLT2 inhibitors preserve cellular functions and integrity, prevent the loss of cells by apoptosis and senescence, and act to maintain normal tissue architecture, leading to the prevention of adverse structural remodeling and the restoration of organ function.[34,48,54,167,179] This characteristic pattern of cellular and tissue responses has been observed with diverse SGLT2 inhibitors in experimental cardiomyopathy and nephropathy, regardless of the triggering mechanism.

The distinctive pattern of cellular and tissue benefits of SGLT2 inhibitors in the heart and kidney is consistently accompanied by simultaneous upregulation of nutrient deprivation signaling and downregulation of nutrient surplus signaling. The effect of SGLT2 inhibitors to reduce oxidative stress, enhance mitochondrial integrity, mute inflammatory pathways, and preserve cell viability is paralleled by an increase in expression or activity of AMPK, SIRT1, SIRT3, SIRT6, and PGC-1α and decreased activation of mTOR in diverse tissues under stress, particularly in experimentally induced cardiomyopathy and

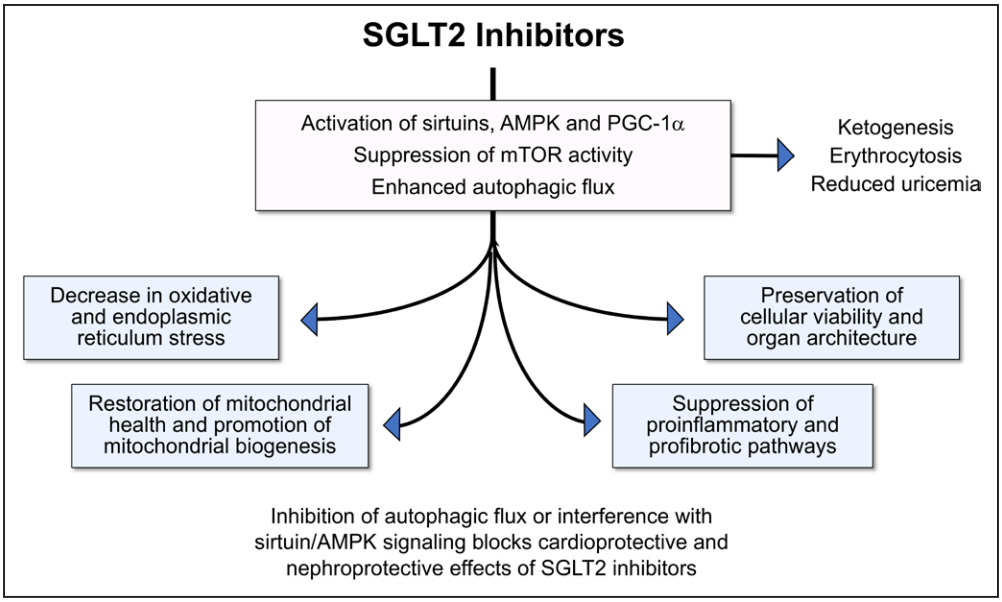

Figure 4. Proposed framework by which SGLT2 (sodium-glucose cotransporter 2) inhibitors can modulate nutrient deprivation signaling and thereby enhance autophagic flux and reduce cellular stress.
AMPK indicates adenosine monophosphate–activated protein kinase; mTOR, mammalian target of rapamycin; and PGC-1α, peroxisome proliferator–activated receptor γ coactivator 1-α.

nephropathy (Figure 4 and Table 2).[48,54,57,76,77,87,110,157,158,161–171,174–219] The increase in AMPK and autophagic flux also explains the increase in ATP production that is seen in the heart and kidneys, both experimentally and clinically, after SGLT2 inhibition.[52,54,57,181,220]

When the concept of SGLT2-enhanced nutrient deprivation signaling and autophagic flux was first proposed,[60] there were only scattered supportive observations. However, during the past 2 years, nearly 60 additional studies have been published, using a broad range of experimental conditions and methods, and all have confirmed the original hypothesis (Tables 1 and 2). Changes in nutrient deprivation and surplus signaling produced by SGLT2 inhibitors have been noted both in harvested whole organs and in isolated cell cultures and have been seen in tissues that express SGLT2 (eg, proximal renal tubules) and in those that do not express SGLT2 (eg, heart). They also have been demonstrated in the endothelium, liver, and adipose tissue. Most importantly, in a large proportion of these reports, when the effects of SGLT2 inhibitors to promote activation of AMPK, SIRT1, SIRT3, and SIRT6 were attenuated by specific pharmacologic inhibition or knockdown of nutrient deprivation signaling pathways or when their ability to promote autophagy has been genetically or pharmacologically blocked, the beneficial effects of SGLT2 inhibitors to mute oxidative stress, promote mitochondrial health, attenuate inflammation and fibrosis, and maintain cell viability and organ integrity have been abolished (Tables 1 and 2). These experimental observations, taken together, provide compelling evidence that the cardioprotective and nephroprotective effects of SGLT2 inhibitors can be explained by their actions to modulate nutrient and energy sensors and their downstream effect to promote autophagy.

Are nutrient deprivation pathways activated when SGLT2 inhibitors are used in the clinical setting? AMPK, SIRT1, SIRT3, and PGC-1α are the cornerstone of the body's response to starvation, and thus, their synthesis in the liver is enhanced when there is substantial loss of calories in the urine (as with SGLT2 inhibition).[76,210] After the induction of glycosuria, the activation of SIRT1, SIRT6, and PGC-1α supports blood glucose by promoting gluconeogenesis[76,221]—a distinctive metabolic signature of SGLT2 inhibitors (demonstrated clinically), which limits their antihyperglycemic response.[201,222] Activation of AMPK and PGC-1α promotes fatty acid oxidation and increased synthesis of SIRT1 and SIRT3 stimulates 3-hydroxy-3-methylglutaryl CoA synthase, the rate-limiting enzyme for ketone body formation.[223] As a result, AMPK, SIRT1, SIRT3, and PGC-1α act in concert to promote ketogenesis in the liver and the clinical finding of ketogenesis during starvation and after SGLT2 inhibition reflects enhanced nutrient deprivation signaling. In addition, SIRT1 activates HIF-2α (hypoxia-inducible factor 2α),[224] the master regulator of the production of erythropoietin and stimulus to erythrocytosis. The action of SIRT1 to reduce oxidative stress can also contribute to the decrease in uric acid seen in patients treated with SGLT2 inhibitors[72] and SIRT1 can directly promote uric acid secretion.[225] The simultaneous finding of ketogenesis, erythrocytosis, and reduced uricemia in clinical trials with SGLT2 inhibitors suggests that nutrient deprivation signaling is upregulated in patients who are treated with these drugs. Increased AMPK and SIRT1 activity may

Table 1. Experimental Studies on the Effect of SGLT2 Inhibitors on Autophagic Flux

Study	SGLT2 inhibitor	Experimental setting	Direct in vitro or systemic effect	Evidence for increased autophagic flux after SGLT2 inhibition	Confirmatory inhibition or knockdown studies
Xu et al. (2018)[155]	Canagliflozin	RAW264.7 macrophages and THP-1 monocytes exposed to lipopolysaccharide	Direct cellular effect	Increased biomarkers of autophagic flux (increased LC3-II to LC3-I ratio, LC3 punctae), lysosomal activation before and after 3-methyladenine	Effect of SGLT2i to mute proinflammatory signaling abolished by autophagy inhibition with 3-methyladenine and by AMPK inhibition
Mizuno et al. (2018)[156]	Empagliflozin	Myocardial tissue in noninfarcted zone after myocardial infarction in obese rats	Effect seen after systemic administration	Increased autophagic vacuoles; increased evidence for mitophagy; increased expression of mitophagy-promoting protein (BNIP3)	–
Aragón-Herrera et al. (2019)[157]	Empagliflozin	Myocardium of Zucker diabetic fatty rats	Effect seen after systemic administration	Increased biomarkers of autophagic flux (decreased p62, increased LC3B-II to LC3B-1 ratio)	–
Fukushima et al. (2020)[158]	Empagliflozin	Kidney tissue from high-calorie diet–fed mice; LLC-PK1 proximal tubular cells exposed to high glucose	Direct cellular effect	Increased evidence for mitophagy in kidney sections and LLC-PK1 cells; increased biomarker of autophagic flux (decreased p62)	–
Korbut et al. (2020)[159]	Empagliflozin	Kidney tissue from diabetic db/db mice	Effect seen after systemic administration	Increased biomarkers of autophagic flux (increased LAMP-1 and Beclin 1); increased density of autophagosomes	–
Wang et al. (2020)[110]	Empagliflozin	Mouse hearts exposed to doxorubicin; isolated neonatal rat cardiomyocytes	Direct effect on cardiomyocytes, not mediated through SGLT2	Increased biomarker of autophagic flux (increased LC3-II to LC3-I ratio); increased autophagosomes and autolysosomes (before and after bafilomycin A1); increased formation of Beclin 1-TLR9-SIRT3 complex	Effects of SGLT2i abolished by knockdown of SIRT3 or TLR9
Arab et al. (2021)[160]	Dapagliflozin	Colon tissues from 2,4,6-trinitrobenzene sulfonic acid–induced rat colitis	Effect seen after systemic administration	Increased biomarkers of autophagic flux (increased Beclin 1and decreased p62)	–
Jaikumkao et al. (2021)[161]	Dapagliflozin	Rat kidney from high-fat diet–induced obese rats	Effect seen after systemic administration	Increased biomarkers of autophagic flux (decreased p62, increased LC3B-II, Beclin 1 and Atg5)	–
Li et al. (2021)[162]	Dapagliflozin	Diabetic Zucker rats; human LO2 hepatocytes exposed to palmitic acid	Direct effect on hepatocytes expressing SGLT2	Increased biomarkers of autophagic flux (increased LC3B-II and Beclin1 and decreased p62)	–
Meng et al. (2021)[163]	Empagliflozin	Diabetic mice with nonalcoholic fatty liver disease; primary hepatic macrophages	Effect seen after systemic administration and in isolated cell cultures	Increased expression of Beclin 1, Atg5, and LC3BII and decreased p62	Effects of SGLT2i to prevent liver injury and mute proinflammatory pathways were abolished by inhibition of autophagy or AMPK
Nasiri-Ansari et al. (2021)[164]	Empagliflozin	Liver tissue from apoE (–/–) mice fed high-fat diet	Effect seen after systemic administration; minimal expression of SGLT2 in liver	Increased biomarkers of autophagic flux (decreased p62, increased LC3B-II and Beclin 1)	–
Ren et al. (2021)[165]	Empagliflozin	Sunitinib cardiotoxicity in mice; H9c2 cardiomyocytes exposed to sunitinib	Direct effect on cardiomyocytes, not mediated through SGLT2	Increased biomarkers of autophagic flux (decreased p62 and changes in LC3-II to LC3-I ratio); increased autolysosomes	Protective effect of SGLT2i on cardiomyocyte viability was blocked after autophagy inhibition with bafilomycin A1
Xu et al. (2021)[166]	Dapagliflozin	HK2 proximal renal tubular cells exposed to hyperglycemia	Direct cellular effect	Increased biomarker of autophagic flux (increased LC3-II to LC3-I ratio), assessed before and after autophagy inhibition with chloroquine	Effect of SGLT2i on autophagic flux abolished by autophagy inhibition with chloroquine and by AMPK knockdown
Ala et al. (2022)[167]	Empagliflozin	Rats subject to renal ischemia and reperfusion	Effect seen after systemic administration	Increased biomarker of autophagic flux (increased LC3-II to LC3-I ratio)	–
Cai et al. (2022)[168]	Empagliflozin	Mouse cardiac microvascular endothelial cells	Direct cellular effect	Increased biomarkers of autophagic flux (decreased p62 and increased LC3B-II)	Protection of mitochondria by SGLT2i abolished by knockout of AMPK and FUNDC1 (a mitophagy receptor)
Li et al. (2022)[169]	Empagliflozin	Transverse aortic constriction pressure overload in nondiabetic mouse; primary cardiomyocytes	Effect seen after systemic administration	Increased autophagosomes; increased autophagic flux (increased Ulk phosphorylation; increased expression of Beclin1 and Atg7; increased LC3 II to LC3I ratio)	–

(*Continued*)

Table 1. Continued

Study	SGLT2 inhibitor	Experimental setting	Direct in vitro or systemic effect	Evidence for increased autophagic flux after SGLT2 inhibition	Confirmatory inhibition or knockdown studies
Park et al. (2022)[170]	Canagliflozin	Whole kidneys and human HK2 proximal tubular cells	Direct cellular effect	Increased biomarker of autophagic flux (increased LC3-II to LC3-I ratio), assessed before and after autophagy inhibition with chloroquine	Effects of SGLT2i to enhance cell viability and prevent renal tubular injury were abolished by autophagy inhibition
Yang et al. (2022)[171]	Dapagliflozin	Cultured podocytes exposed to advanced glycation end products	Direct cellular effect; confirmed SGLT2 expression	Increased biomarkers of autophagic flux (ratio of LC3II-LC3I, increase LC3 puncta; decreased p62; increased expression of Beclin-1) before and after autophagy inhibition with 3-methyadenine	Effects of SGLT2i to decrease proinflammatory signaling and increase biomarkers of autophagic flux were abolished by autophagy inhibition and by AMPK inhibition
Xu et al. (2022)[172]	Canagliflozin	High-fat diet–induced nonalcoholic fatty liver disease; primary AML-12 hepatocytes	Direct cellular effect; no expression of SGLT2 in whole liver or in isolated hepatocytes	Increased biomarkers of autophagic flux (increased autophagosomes and autolysosomes, LC3-II to LC3-I ratio, and Atg7) before and after autophagy inhibition with 3-methyadenine	Effects of SGLT2i to mute proinflammatory signaling and enhance autophagic flux were abolished by autophagy inhibition
Yu et al. (2022)[173]	Dapagliflozin	Ischemia-reperfusion mouse model; primary cardiomyocytes	Direct effect on cardiomyocytes, not mediated through SGLT2	Increased biomarkers of autophagy (increased autophagosomes, LC3B-II, Atg5, and Beclin 1, decreased p62) before and after autophagy inhibition by chloroquine	Effects of SGLT2i to attenuate myocardial and cardiomyocyte injury and inflammation and promote autophagic flux were abolished by autophagy inhibition

The following refer to specialized cell lines or mutant mice: AML-12 hepatocytes, db/db mice, H9c2 cardiomyocytes, HK2 proximal renal tubular cells, HL-1 cardiomyocytes, LLC-PK1 proximal tubular cells, LO2 hepatocytes, RAW264.7 macrophages, and THP-1 monocytes. AMPK indicates adenosine monophosphate–activated protein kinase; Atg5, autophagy related gene 5; BNIP3, B-cell lymphoma 2 interacting protein 3; FUNDC1, FUN14 domain containing 1; LAMP-1, lysosomal-associated membrane protein 1; LC3, microtubule-associated protein 1A/1B-light chain 3; SGLT2, sodium-glucose cotransporter 2; SGLT2 p62, sequestosome 1 (ubiquitin-binding protein); SGLT2i, sodium-glucose cotransporter 2 inhibitor; SIRT3, sirtuin-3; TLR9, Toll-like receptor 9; and Ulk,Unc-51-like kinase.

also directly enhance tubuloglomerular feedback,[226,227] thereby linking changes in these energy sensors to the effect of SGLT2 inhibitors to rapidly reduce glomerular filtration rate in the clinical setting.[35]

A recent proteomics analysis of blood samples collected from >1100 patients with HF before and after treatment with placebo or empagliflozin identified a limited number of proteins whose serum levels were significantly changed as a result of SGLT2 inhibition.[228] Of the 21 differentially increased proteins with known effects on the heart and kidneys, 6 were linked to the promotion of autophagy in the heart, kidneys, or endothelium, and 4 proteins (IGF-1 [insulin-like growth factor binding protein 1], TfR1 [transferrin receptor protein 1], erythropoietin, and AFABP [adipocyte fatty acid binding protein 4]) are known to be upregulated by enhanced SIRT1 signaling.[224,229–231] The latter proteins were also noted to be differentially increased by Ferrannini et al.[232] in a second proteomics analysis of blood collected from patients with type 2 diabetes. Neither of the 2 proteomics analyses performed direct measurements of SIRT1, SIRT3, SIRT6, PGC-1α, or phosphorylated AMPK, but it is not clear that changes in blood levels of these proteins have the capacity to reflect the importance of their intracellular actions accurately.

Mechanism of Cellular Benefits of SGLT2 Inhibitors in Organs Without SGLT2 Expression

How can SGLT2 inhibitors promote nutrient deprivation signaling and autophagy with their attendant favorable effects on cellular stress, homeostasis, and survival in tissues that do not express SGLT2? There are 3 possibilities: glycosuric caloric loss, ketogenesis-induced autophagic flux, and direct nutrient-independent cellular effects.

Glycosuric Caloric Loss

SGLT2 inhibitors induce a substantial loss of calories in the urine and the resulting glucose depletion can stimulate a system-wide upregulation of nutrient deprivation signaling in organs throughout the body, a shift that does not depend on the presence or absence of SGLT2 in a specific tissue.[76,210] Because the action of SGLT2 inhibitors to promote gluconeogenesis through SIRT1 limits the reduction in blood glucose, glycosuria serves to shift the set point between SIRT1 signaling and glycemia, and as a result, the upregulation of nutrient deprivation signaling with SGLT2 inhibitors can occur even when blood glucose is only modestly diminished.[76] This hypothesis may explain the reported relationship between the magnitude of glycosuria and increases in erythropoietin in patients treated with these drugs.[233] However, this hypothesis is difficult to reconcile with the fact that chronic kidney disease meaningfully impairs the glycosuric response to SGLT2 inhibitors, but it does not diminish the ability of these drugs to reduce HF and major renal events.[26,37]

Ketone Body–Induced Nutrient Deprivation Signaling and Autophagic Flux

SGLT2 inhibitors induce a state of starvation mimicry characterized by ketogenesis[46,51] and the delivery of ketone bodies to organs can exert a direct effect to increase AMPK and decrease mTOR phosphorylation, to increase the expression of PGC-1α, and to promote autophagic flux.[57,234–238] Potentially acting through these mediators,

Table 2. Experimental Studies on the Effect of SGLT2 Inhibitors on Nutrient Deprivation and Surplus Signaling

Study	SGLT2 inhibitor	Experimental conditions	Effect on nutrient sensor signaling	Biological effects of SGLT2 inhibition
Studies of cardiac tissue and cardiomyocytes				
Ye et al. (2018)[180]	Dapagliflozin	Mouse cardiofibroblasts exposed to lipopolysaccharide	Increased phosphorylation of AMPK	Phosphorylation of AMPK by SGLT2i was blocked by AMPK inhibition
Aragón-Herrera et al. (2019)[157]	Empagliflozin	Atrial myocardium of diabetic rats	Increased phosphorylation of AMPK	Decreased proinflammatory signaling in atrial myocardium
Oshima et al. (2019)[174]	Empagliflozin	Myocardial infarction induced in diabetic rats	Increased expression of SIRT3	Preservation of myocardial ATP; increased antioxidant activity
Santos-Gallego et al. (2019)[54]	Empagliflozin	Myocardial infarction induced in pigs	Increased phosphorylation of AMPK and expression of PGC-1α	Favorable effects on left ventricular remodeling and systolic function
Shao et al. (2019)[175]	Empagliflozin	Atrial tissue derived from diabetic rats	Increased expression of PGC-1α	Increased antioxidant activity; decreased atrial fibrosis
Koyani et al. (2020)[181]	Empagliflozin	HL-1 cardiomyocytes exposed to and mice administered lipopolysaccharide	Increased phosphorylation of AMPK	Preservation of cardiac and vascular function; effects of SLGT2i to prevent proinflammatory responses were abolished by AMPK inhibition and enhanced by AMPK activation
Lu et al. (2020)[182]	Empagliflozin	Ex vivo isolated mouse heart; temporary coronary artery ligation in mice; isolated cardiomyocytes subjected to hypoxia/reoxygenation	Increased phosphorylation of AMPK; increased expression of PGC-1α	Effects of SGLT2i to mitigate oxidative stress and inflammation and improve systolic function of cardiomyocytes and myocardium were abolished by inhibition of AMPK
Radlinger et al. (2020)[183]	Empagliflozin	Diabetic db/db mice	Decreased phosphorylation of Akt without change in PGC-1α	Decreased proinflammatory and profibrotic signaling and increased mitochondrial size
Wang et al. (2020)[110]	Empagliflozin	Doxorubicin cardiotoxicity in mice; isolated neonatal rat cardiomyocytes	Increased SIRT3 expression; enhanced formation of Beclin-TLR9-SIRT3 complex	Prevention of cardiomyopathy; effects of SGLT2i to reduce oxidative stress, apoptosis, and cardiac fibrosis were abolished by SIRT3 knockdown
Kondo et al. (2021)[184]	Canagliflozin	Human atrial myocardium; primary human cardiomyocytes exposed to high glucose	Increased phosphorylation and activity of AMPK	Effects of SGLT2i to reduce oxidative stress and apoptosis were abolished by AMPK inhibition
Lee et al. (2021)[185]	Dapagliflozin	Borderzone sampling of myocardial infarction in rats	Increased phosphorylation of AMPK	Reduction in cardiac arrhythmias; effect of SGLT2i to reduce oxidative stress was abolished by AMPK inhibitor
Li et al. (2021)[48]	Empagliflozin	Transverse aortic constriction pressure overload in nondiabetic mouse; primary cardiomyocytes	Increased AMPK phosphorylation and decreased mTOR phosphorylation, both because of GLUT1 docking and inhibition	Prevention of adverse ventricular remodeling and improvement in cardiomyocyte contractility
Liu et al. (2021)[186]	Empagliflozin	Myocardial infarction model in rats; isolated H9c2 cardiomyocytes	Increased phosphorylation of AMPK	Improvement of cardiac function; inhibition of apoptosis, oxidative stress, and fibrosis
Ren et al. (2021)[176]	Dapagliflozin	Transverse aortic constriction pressure overload model in mice; HL-1 cardiomyocytes exposed to angiotensin II	Increased expression of SIRT1	Inhibition of hypertrophy by pressure overload or angiotensin II; effects of SGLT2i to decrease ER stress and apoptosis were abolished by SIRT1 inhibitor
Ren et al. (2021)[165]	Empagliflozin	Sunitinib cardiotoxicity in mice; H9c2 cardiomyocytes exposed to sunitinib	Increased AMPK phosphorylation, decreased mTOR phosphorylation	Prevention of cardiotoxicity; effects of SGLT2i to enhance cell viability were abolished by AMPK inhibition
Sun et al. (2021)[187]	Canagliflozin	Diabetic mouse hearts, HL-1 cardiomyocyte cell line exposed to palmitic acid	Inhibition of mTOR phosphorylation by direct binding of canagliflozin to FRB domain of mTOR	Inhibition of oxidative stress, apoptosis, inflammation, and fibrosis
Tian et al. (2021)[179]	Empagliflozin	Ethanol-induced myocardial injury in H9c2 cardiomyocytes	Increased expression of SIRT1	Effects of SGLT2i to prolong cardiomyocyte survival and reduce cardiomyocyte and mitochondrial apoptosis were abolished by SIRT1 inhibitor
Tsai et al. (2021)[188]	Dapagliflozin	H9c2 cardiomyocytes and primary rat cardiomyocytes exposed to hypoxia/reoxygenation	Increased AMPK phosphorylation and PGC-1α expression	Reduction of oxidative stress and apoptosis, increase in mitochondrial biogenesis, and preservation of cardiac function
He et al. (2022)[189]	Canagliflozin	Hypertensive HFpEF by high-salt diet in Dahl-sensitive rats	Increased AMPK phosphorylation; increased expression of SIRT1 and PGC-1α in myocardium	Decreased LV hypertrophy, oxidative stress, and fibrosis and improvement in diastolic function

(*Continued*)

Table 2. Continued

Study	SGLT2 inhibitor	Experimental conditions	Effect on nutrient sensor signaling	Biological effects of SGLT2 inhibition
Li et al. (2022)[169]	Empagliflozin	Transverse aortic constriction pressure overload in nondiabetic mouse; primary cardiomyocytes	Increased AMPK phosphorylation in cardiac cells	Decrease in oxidative stress, apoptosis, ventricular remodeling, and improved mitochondrial biogenesis
Moellmann et al. (2022)[190]	Ertugliflozin	Transverse aortic constriction pressure overload in nondiabetic mouse	Increased AMPK phosphorylation and decreased Akt phosphorylation and mTOR signaling	Decrease in ventricular remodeling, hypertrophy, and fibrosis; enhanced systolic function; decrease in endoplasmic reticulum stress and apoptosis
Studies of endothelial cells				
Mancini et al. (2018)[191]	Canagliflozin, dapagliflozin, and empagliflozin	Human umbilical vein and human aortic endothelial cells	Only canagliflozin increased activity of AMPK	Effect of canagliflozin to reduce proinflammatory signaling was attenuated by AMPK knockout
Zhou et al. (2018)[192]	Empagliflozin	Cardiac microvascular endothelial cells from diabetic mice	Increased AMPK phosphorylation	Reduction in cardiac fibrosis, microcirculatory function, mitochondrial fragmentation, and oxidative stress
D'Onofrio et al. (2021)[193]	Canagliflozin	Human aortic endothelial cells exposed to high glucose demonstrated expression of SGLT2	Increased SIRT6 expression	Effects of SGLT2i to reduce oxidative stress and proinflammatory signaling was abolished by SIRT6 silencing
Tian et al. (2021)[194]	Dapagliflozin	Diabetes rat model, isolated cardiac fibroblasts, and human umbilical vein endothelial cells exposed to high glucose	Increased AMPK phosphorylation in endothelial cells	Effects of SGLT2i to reduce oxidative stress and profibrotic signaling in endothelial cells were abolished by AMPK silencing and inhibition
Uthman et al. (2021)[195]	Canagliflozin, dapagliflozin, empagliflozin	Human coronary artery endothelial cells exposed to lipopolysaccharide	Only canagliflozin increased AMPK phosphorylation	Reduction in proinflammatory signaling
Cai et al. (2022)[168]	Empagliflozin	Cardiac microvascular endothelial cells from mice with ischemia– reperfusion injury	Increased AMPK phosphorylation	Effects of SGLT2i to reduce microvascular injury, oxidative stress, and mitochondrial fission were abolished by AMPK knockout
Faridvand et al. (2022)[196]	Dapagliflozin	Human umbilical vein endothelial cells exposed to high glucose	Increased AMPK phosphorylation; increased SIRT1 and PGC-1α expression	Effects of SGLT2i to decrease inflammation, oxidative stress, and apoptosis were abolished with SIRT1 inhibitor or AMPK inhibitor
He et al. (2022)[197]	Dapagliflozin	Endothelial cells from high-fat diet–induced obesity in mice; human umbilical endothelial cells exposed to palmitic acid	Increased expression of SIRT1 and PGC-1α	Preservation of vascular endothelial function with decrease in apoptosis; effect of SGLT2i to inhibit apoptosis and preserve cellular viability was abolished by SIRT1 inhibitor
Studies of kidney tissue or glomerular or tubular cells				
Chang et al. (2016)[198]	Dapagliflozin	HK2 proximal tubular cells subject to hypoxia	Increased AMPK phosphorylation	Decrease in apoptosis and augmentation in cell viability
Birnbaum et al. (2018)[199]	Dapagliflozin	Mouse model of diabetic nephropathy	Increased AMPK phosphorylation	Reduction in renal levels of proinflammatory signals and collagen
Umino et al. (2018)[77]	Canagliflozin	Diabetic and nondiabetic mice; LLK-PK1 proximal tubular cells cultured in high-glucose medium; kidney biopsies of patients with diabetic nephropathy	Increased SIRT1expression	Inverse relationship between SGLT2 and SIRT1 in proximal tubular cells and in human kidney
Inoue et al. (2019)[200]	Canagliflozin	Kidneys of diabetic mice, CRL1927 mesangial cells	Increased AMPK phosphorylation	Reduction in peritubular fibrosis; effect of SGLT2i to reduce mesangial cell proliferation was attenuated by AMPK inhibition
Fukushima et al. (2020)[158]	Empagliflozin	Diet-induced obese mice; LLC-PK1 proximal tubular cells exposed to high glucose/palmitic acid	Decreased mTOR phosphorylation and activation	Decreased lipid accumulation in proximal tubular cells
Kogot-Levin et al. (2020)[87]	Dapagliflozin	Mouse model of diabetic nephropathy, LLC-PK1, and HK2 proximal tubular cells	Inhibition of mTORC1 activity in renal proximal tubular cells	Constitutive activation of mTORC1 abolished effects of SGLT2i to reduce peritubular fibrosis and preserve glomerular function
Kim et al. (2020)[201]	Dapagliflozin	Primary mouse renal tubular cells, HK2 proximal tubular cells	Increased expression of PGC-1α	Gluconeogenesis

(*Continued*)

Table 2. Continued

Study	SGLT2 inhibitor	Experimental conditions	Effect on nutrient sensor signaling	Biological effects of SGLT2 inhibition
Li et al. (2020)[202]	Empagliflozin	CD-1 diabetic mice	Increased SIRT3 in diabetic kidney	Prevention of renal profibrotic signaling
Liu et al. (2020)[203]	Empagliflozin	Diabetic mouse model, HK2 proximal tubular cells exposed to high glucose	Increased AMPK phosphorylation, but not if SGLT2 silenced	Reduced oxidative stress, mitochondrial fission, apoptosis in diabetic kidney and HK2 cells; effect of SGLT2i to preserve mitochondria in HK2 cells was abolished by AMPK inhibition
Mohamed et al. (2020)[204]	Empagliflozin	Kidney tissue from high-fructose diet–induced insulin resistance in rats	Increased SIRT1expression	Mitigation of proinflammatory and profibrotic signaling
Tomita et al. (2020)[57]	Empagliflozin	Kidney tissue from apoE (−/−) mice fed high-fat diet and from nondiabetic 5/6 nephrectomized mice	Suppression of mTORC1 hyperactivation in proximal tubular cells and podocytes	Attenuation of renal injury; amelioration of glomerular and interstitial fibrosis and renal hypertrophy and dysfunction
Jaikumkao et al. (2021)[161]	Dapagliflozin	Kidney tissue from high-fat diet–induced obesity in rats	Increased AMPK phosphorylation, decreased mTOR phosphorylation	Prevention of glomerular lesions and interstitial inflammation
Xu et al. (2021)[166]	Dapagliflozin	HK2 proximal renal tubular cells exposed to hyperglycemia	Increased AMPK phosphorylation, decreased mTOR downstream activation	Effect of SGLT2i to reduce proinflammatory signaling was abolished by AMPK inhibition
Zhang et al. (2021)[205]	Empagliflozin	Mouse models of diabetic nephropathy	Increased AMPK phosphorylation	Effect of SGLT2i to reduce interstitial fibrosis was abolished by AMPK inhibition
Ala et al. (2022)[167]	Empagliflozin	Rats subject to renal ischemia and reperfusion	Increased PGC-1α expression without increase in AMPK phosphorylation	Prevention of renal injury, apoptosis, and proinflammatory signaling
Chi et al. (2022)[206]	Dapagliflozin	NRK-52E proximal tubular cells exposed to lipopolysaccharide	Increased messenger RNA of AMPK and downstream effectors	Decreased proinflammatory signaling
Ke et al. (2022)[178]	Dapagliflozin	Ischemia–reperfusion fibrosis mouse model; primary proximal tubular cells	Inhibition of mTOR activity	Attenuation of injury and mitochondrial depletion; inhibition of proinflammatory/profibrotic signaling
Park et al. (2022)[170]	Canagliflozin	Mice and HK2 proximal tubular cells exposed to cisplatin	Increased AMPK phosphorylation; inhibition of mTOR phosphorylation	Effects of SGLT2i to decrease cell viability and prevent renal injury were abolished by AMPK inhibition
Yang et al. (2022)[171]	Dapagliflozin	Cultured podocytes exposed to advanced glycation end products	Increased AMPK phosphorylation; inhibition of mTOR phosphorylation	Effects of SGLT2i to reduce proinflammatory signaling and apoptosis were abolished by AMPK inhibition
Zhai et al. (2022)[207]	Empagliflozin	Podocytes from murine experimental preeclampsia	Increased AMPK phosphorylation and SIRT1 expression	Reduced podocyte injury and oxidative stress
Studies of liver, adipose tissue, and skeletal muscle				
Hawley et al. (2016)[208]	Canagliflozin, dapagliflozin, empagliflozin	Primary mouse hepatocytes; HEK-293 kidney cells; mouse embryo fibroblasts	Only canagliflozin increased AMPK phosphorylation, attributed to GLUT1 inhibition	Effect of canagliflozin to inhibit lipogenesis was abolished by AMPK knockout
Xu et al. (2017)[209]	Empagliflozin	High-fat diet–induced obese mice	Increased AMPK phosphorylation in skeletal muscle and FGF21 expression in liver	Decrease in proinflammatory signaling
Osataphan et al. (2019)[210]	Canagliflozin	Liver tissue from high-fat diet–induced obese mice	Increased AMPK and decreased mTOR phosphorylation; increased FGF21 expression	Reduction in hepatic steatosis
Swe et al. (2019)[76]	Dapagliflozin	Liver tissue from high-fat diet–induced obese rats	Increased expression of SIRT1 and PGC-1α	Reduction in hepatic oxidative stress, inflammation, and steatosis
Chiang et al. (2020)[211]	NGI001, dapagliflozin	High-fat diet–induced obese mice; human HuS-E/2 hepatocytes	Increased AMPK phosphorylation	Reduction in hepatic steatosis and hepatocyte lipid deposition
Suga et al. (2020)[212]	Ipragliflozin	Liver of ob/ob obese mice	Increased SIRT1, PGC-1α, and FGF21 expression and AMPK phosphorylation	Reduction in hepatic inflammation and steatosis
Wei et al. (2020)[213]	Canagliflozin	High-fat diet–induced obesity in mice; 3T3-L1 adipocytes	Increased PGC-1α expression	Reduction in adipocyte hypertrophy; promotion of mitochondrial biogenesis

(*Continued*)

Table 2. Continued

Study	SGLT2 inhibitor	Experimental conditions	Effect on nutrient sensor signaling	Biological effects of SGLT2 inhibition
Yang et al. (2020)[214]	Cana-gliflozin	Primary differentiated adipocytes from mice fed enriched diet	Increased expression of SIRT1 and PGC-1α and phosphorylation of AMPK	Effect of SGLT2i on mitochondrial biogenesis and thermogenesis was abolished by PGC-1α knockdown
Chen et al. (2021)[215]	Dapa-gliflozin	Hepatic tissue from type 1 diabetes mouse model	Increased AMPK phosphorylation	Reduction in proinflammatory signaling in the liver
Lee et al. (2021)[216]	Ipragliflozin	Adipocytes from high-fat diet–induced obese mice	Increased AMPK phosphorylation and SIRT1 expression	Enhancement of fat use and increase in mitochondria number
Li et al. (2021)[162]	Dapa-gliflozin	Diabetic Zucker rats and human LO2 hepatocytes exposed to palmitic acid	Increased AMPK and decreased mTOR phosphorylation	Effect of SGLT2i to reduce lipid deposition in liver and hepatocytes was abolished by AMPK inhibition
Luo et al. (2021)[217]	Dapa-gliflozin	Liver tissue from high-fat diet–fed mice and human LO2 hepatocytes exposed to oleic acid	Increased AMPK and decreased mTOR phosphorylation	Effect of SGLT2i to suppress lipid accumulation was abolished by AMPK inhibition
Meng et al. (2021)[163]	Empa-gliflozin	Liver tissue and hepatic macrophages harvested from murine model of diabetes and fatty liver disease	Increased AMPK and decreased mTOR phosphorylation	Effect of SGLT2i to prevent hepatic steatosis and inflammation was abolished by AMPK inhibition
Nasiri-Ansari et al. (2021)[164]	Empa-gliflozin	Liver tissue from apoE-deficient mice fed high-fat diet	Increased AMPK phosphorylation and decreased mTOR mRNA	Amelioration of hepatic steatosis, inflammation, ER stress, and apoptosis
Xu et al. (2021)[218]	Empa-gliflozin	KK Cg-Ay/J mice fed high-fat diet; 3T3-L1 adipocytes	Increased AMPK phosphorylation and PGC-1α expression blocked by AMPK inhibition with compound C	Browning of adipose tissue and adipocytes and improvement in mitochondria structure and function
Yang et al. (2021)[219]	Cana-gliflozin	Adipose tissue from mice fed high-fat diet	Increased AMPK phosphorylation and PGC-1α expression	Amelioration of hepatic steatosis and inflammation and stimulation of mitochondrial biogenesis

The following refer to specialized cell lines or mutant mice: CD-1 diabetic mice, CRL1927 mesangial cells, HK2 proximal tubular cells, HuS-E/2 hepatocytes, KK Cg-Ay/J mice, LLK-PK1 proximal tubular cells, LO2 hepatocytes, and NRK-52E proximal tubular cells. Akt indicates protein kinase B; AMPK, adenosine monophosphate–activated protein kinase; ATP, adenosine triphosphate; FGF21, fibroblast growth factor 21; FRB, FK506 binding protein–rapamycin binding; GLUT1, glucose transporter 1; HFpEF, heart failure with preserved ejection fraction; mTOR, mammalian target of rapamycin; PGC-1α, peroxisome proliferator–activated receptor γ coactivator 1-α; SGLT2i, sodium glucose cotransporter 2 inhibitor; SIRT1, sirtuin-1; SIRT3, sirtuin-3; and SIRT6, sirtuin-6.

ketonemia has been shown to reduce oxidative stress, mute cellular inflammation, enhance mitochondrial biogenesis, and preserve cardiac and renal function after diverse stresses.[57,234–240] Ketone body supplementation inhibits phenylephrine-induced hypertrophy in experiments[238] and interruption of ketogenesis negates the benefits of SGLT2 inhibitors on the kidney.[57] These observations suggest that the ketonemia seen after SGLT2 inhibitors use may exert cardioprotective and nephroprotective effects as a result of a direct action to stimulate nutrient deprivation signaling rather than because of an ability to serve as an efficient fuel for the generation of ATP. However, the presence of diabetes attenuates the magnitude of ketonemia, but it does not influence the cardiorenal benefits of SGLT2 inhibitors.[35,36,51] Furthermore, as noted in Tables 1 and 2, SGLT2 inhibitors exert direct benefits on the heart and kidney in isolated cell preparations, which are not subject to fluctuations in environmental ketone bodies.

Direct Glucose- and Ketone-Independent Cellular Effects

SGLT2 inhibitors exert direct cellular effects that are independent of changes in glucose induced by loss of glucose by the kidney or ketone body production in the liver. Numerous studies (Tables 1 and 2) have shown that SGLT2 inhibitors can enhance nutrient deprivation signaling, promote autophagy, maintain mitochondrial health, mute cellular stresses, and mitigate proinflammatory and profibrotic pathways in isolated organs and in cell cultures—experimental conditions where the level of environmental glucose or ketone bodies is held constant. Studies demonstrating the direct effects of these drugs on isolated cardiomyocytes, podocytes, and proximal renal tubular cells have also established that the cardioprotective and renoprotective benefits of these drugs are not dependent on changes in cardiovascular physiology (eg, changes in blood pressure or renal sympathetic tone); on changes proximal renal tubular function (glycosuria or natriuresis); or on changes in the level of a blood or plasma constituent (eg, hemoglobin, glucose, uric acid, or ketones).

How do SGLT2 inhibitors exert direct effects on cardiac and renal parenchymal cells to preserve homeostasis and survival? Many studies have been performed in cells that express SGLT2, either clinically or experimentally (eg, proximal renal tubular cells, endothelial cells, and hepatocytes), but SGLT2 inhibitors exert direct cytoprotective effects in cardiomyocytes that do not express SGLT2 (Tables 1 and 2). It is possible that the concentrations of SGLT2 inhibitors used in certain experiments might interfere with other glucose transporters. Kondo et al.[184] suggested that the cardioprotection produced

by canagliflozin was related to SGLT1 inhibition,[241] but empagliflozin (which does not inhibit SGLT1) produces cytoprotective effects on isolated cardiomyocytes. Li et al.[48] have proposed that empagliflozin can dock with GLUT1 (glucose transporter 1) and GLUT4 (glucose transporter 4) receptors to inhibit the cellular uptake of glucose, which can trigger the nutrient deprivation signaling and autophagy. An inhibitory effect of SGLT2 inhibitors on GLUT receptors has been proposed by others,[208,242] but has yet to be confirmed. Potential effects of SGLT2 inhibitors on ion transport[67,71] or Ca/calmodulin-dependent kinase[243] cannot explain their effects on autophagy or nutrient deprivation signaling.[244]

Because SGLT2 functions as a nutrient surplus sensor, it is noteworthy that there exists an inverse relationship between SGLT2 and nutrient deprivation signaling in individual cells.[77] This observation raises the possibility that SGLT2 inhibitors might bind directly to nutrient sensors to influence their function. Sun et al.[187] reported that canagliflozin binds to mTOR in the same structural domain used by rapamycin. Ying et al.[245] demonstrated that phloretin (an inhibitor of SGLT1 and SGLT2) ameliorates cardiomyocyte injury by upregulating SIRT1 and showed that phloretin docks directly with SIRT1 to form a stable complex. Wang et al.[110] proposed that empagliflozin acts directly on SIRT3 to promote the formation of a complex of SIRT3, Beclin 1, and TLR9, which enhances autophagic flux. In that study, experimental knockout of either SIRT3 or TLR9 abolished the ability of SGLT2 inhibition to protect the heart from injury by doxorubicin. Wang et al.[110] identified 3 patients with dilated cardiomyopathy who underwent myocardial biopsy before and after treatment with empagliflozin for 28 days. Two patients without a *SIRT3* variation exhibited the expected enhancement of mitochondrial respiration and expression of TLR9 after treatment with the SGLT2 inhibitor; however, these changes were not seen in 1 patient who had a loss-of-function variation in *SIRT3* in the myocardium.[110]

CONCLUSIONS

SGLT2 inhibitors produce a distinctive pattern of favorable effects on the evolution and progression of cardiomyopathy and nephropathy, which is characterized by a reduction in oxidative and endoplasmic reticulum stress, restoration of healthy mitochondrial function and enhanced mitochondrial biogenesis, a decrease in proinflammatory and profibrotic pathways, preservation of cellular integrity and viability, and maintenance of normal organ structure and function. A substantial body of evidence indicates that this characteristic pattern of responses can be explained by the action of SGLT2 inhibitors to promote cellular housekeeping by autophagic flux, a process that is triggered by the effect of these drugs to produce simultaneous upregulation of nutrient deprivation signaling and downregulation of nutrient surplus signaling, as manifested by an increase in the expression and activity of AMPK, SIRT1, SIRT3, SIRT6, and PGC-1α and decreased activation of mTOR in diverse tissues under stress. The distinctive pattern of cardioprotective and renoprotective effects of SGLT2 inhibitors is abolished by specific inhibition or knockdown of autophagy, AMPK, and sirtuins. In the clinical setting, proteomics analyses of blood collected from participants in randomized trials show a pattern of differentially increased proteins that is consistent with enhanced autophagy and SIRT1 activity. Patients treated with SGLT2 inhibitors exhibit augmented gluconeogenesis, ketogenesis, and erythrocytosis and reduced uricemia, the hallmarks of nutrient deprivation signaling and the principal statistical mediators of the ability of SGLT2 inhibitors to reduce the risk of HF hospitalizations and serious renal events in large-scale trials. The action of SGLT2 inhibitor to promote autophagic flux is seen in isolated cells and tissues that do not express SGLT2 and are not exposed to changes in environmental glucose or ketones and may be related to an ability of these drugs to bind directly to sirtuins or mTOR. Changes in renal or cardiovascular physiology, energy use, or metabolism cannot explain the benefits of SGLT2 inhibitors. Substantial experimental work on the molecular and cellular actions of these drugs, when taken together with highly supportive observations in the clinical setting, has provided critical insights into the mechanisms that underlie their remarkable ability to preserve cardiac and renal function in patients whose hearts and kidneys are under stress.

ARTICLE INFORMATION

Received July 18, 2022; accepted August 10, 2022.

Affiliations

Baylor Heart and Vascular Institute, Dallas, TX. Imperial College, London, United Kingdom.

Sources of Funding

None.

Disclosures

Dr Packer has served as a consultant for AbbVie, Altimmune, Amarin, Amgen, Ardelyx, AstraZeneca, Boehringer Ingelheim, Caladrius, Casana, CSL Behring, Cytokinetics, Imara, Lilly, Moderna, Novartis, Reata, Relypsa, and Salamandra. The author reports no current or planned financial relationships related to SGLT2 inhibitors or neprilysin inhibition.

REFERENCES

1. Giugliano D, Longo M, Scappaticcio L, Bellastella G, Maiorino MI, Esposito K. SGLT-2 inhibitors and cardiorenal outcomes in patients with or without type 2 diabetes: a meta-analysis of 11 CVOTs. *Cardiovasc Diabetol.* 2021;20:236. doi: 10.1186/s12933-021-01430-3
2. Dunlap ME, Kinugawa T, Sica DA, Thames MD. Cardiopulmonary baroreflex control of renal sympathetic nerve activity is impaired in dogs with left ventricular dysfunction. *J Card Fail.* 2019;25:819–827. doi: 10.1016/j.cardfail.2019.08.012
3. Petersson M, Friberg P, Eisenhofer G, Lambert G, Rundqvist B. Long-term outcome in relation to renal sympathetic activity in patients with chronic heart failure. *Eur Heart J.* 2005;26:906–913. doi: 10.1093/eurheartj/ehi184

4. Sharp TE, Polhemus DJ, Li Z, Spaletra P, Jenkins JS, Reilly JP, White CJ, Kapusta DR, Lefer DJ, Goodchild TT. Renal denervation prevents heart failure progression via inhibition of the renin-angiotensin system. *J Am Coll Cardiol.* 2018;72:2609–2621. doi: 10.1016/j.jacc.2018.08.2186
5. Polhemus DJ, Trivedi RK, Gao J, Li Z, Scarborough AL, Goodchild TT, Varner KJ, Xia H, Smart FW, Kapusta DR, et al. Renal sympathetic denervation protects the failing heart via inhibition of neprilysin activity in the kidney. *J Am Coll Cardiol.* 2017;70:2139–2153. doi: 10.1016/j.jacc.2017.08.056
6. Jang HS, Kim J, Padanilam BJ. Renal sympathetic nerve activation via α2-adrenergic receptors in chronic kidney disease progression. *Kidney Res Clin Pract.* 2019;38:6–14. doi: 10.23876/j.krcp.18.0143
7. Li Q, Deng Y, Liu L, Zhang C, Cai Y, Zhang T, Han M, Xu G. Renal sympathetic denervation ameliorates renal fibrosis via inhibition of cellular senescence. *Front Immunol.* 2022;12:823935. doi: 10.3389/fimmu.2021.823935
8. Fukuta H, Goto T, Wakami K, Kamiya T, Ohte N. Effects of catheter-based renal denervation on heart failure with reduced ejection fraction: a meta-analysis of randomized controlled trials. *Heart Fail Rev.* 2022;27:29–36. doi: 10.1007/s10741-020-09974-4
9. Li J, He Q, Wu W, Li Q, Huang R, Pan X, Lai W. Role of the renal sympathetic nerves in renal sodium/potassium handling and renal damage in spontaneously hypertensive rats. *Exp Ther Med.* 2016;12:2547–2553. doi: 10.3892/etm.2016.3669
10. Majcherczyk S, Mikulski A, Sjölander M, Thorén P. Increase of renal sympathetic nerve activity by metoprolol or propranolol in conscious spontaneously hypertensive rats. *Br J Pharmacol.* 1987;91:711–714. doi: 10.1111/j.1476-5381.1987.tb11267.x
11. Pontes RB, Girardi AC, Nishi EE, Campos RR, Bergamaschi CT. Crosstalk between the renal sympathetic nerve and intrarenal angiotensin II modulates proximal tubular sodium reabsorption. *Exp Physiol.* 2015;100:502–506. doi: 10.1113/EP085075
12. Boer PA, Morelli JM, Figueiredo JF, Gontijo JA. Early altered renal sodium handling determined by lithium clearance in spontaneously hypertensive rats (SHR): role of renal nerves. *Life Sci.* 2005;76:1805–1815. doi: 10.1016/j.lfs.2004.09.029
13. Healy V, Thompson C, Johns EJ. The adrenergic regulation of proximal tubular Na^+/H^+ exchanger 3 in the rat. *Acta Physiol (Oxf).* 2014;210:678–689. doi: 10.1111/apha.12181
14. Katsurada K, Nandi SS, Sharma NM, Patel KP. Enhanced expression and function of renal SGLT2 (sodium-glucose cotransporter 2) in heart failure: role of renal nerves. *Circ Heart Fail.* 2021;14:e008365. doi: 10.1161/CIRCHEARTFAILURE.121.008365
15. Inoue BH, dos Santos L, Pessoa TD, Antonio EL, Pacheco BP, Savignano FA, Carraro-Lacroix LR, Tucci PJ, Malnic G, Girardi AC. Increased NHE3 abundance and transport activity in renal proximal tubule of rats with heart failure. *Am J Physiol Regul Integr Comp Physiol.* 2012;302:R166–R174. doi: 10.1152/ajpregu.00127.2011
16. Borges-Júnior FA, Silva Dos Santos D, Benetti A, Polidoro JZ, Wisnivesky ACT, Crajoinas RO, Antônio EL, Jensen L, Caramelli B, Malnic G, et al. Empagliflozin inhibits proximal tubule NHE3 activity, preserves GFR, and restores euvolemia in nondiabetic rats with induced heart failure. *J Am Soc Nephrol.* 2021;32:1616–1629. doi: 10.1681/ASN.2020071029
17. Onishi A, Fu Y, Patel R, Darshi M, Crespo-Masip M, Huang W, Song P, Freeman B, Kim YC, Soleimani M, et al. A role for tubular Na+/H+ exchanger NHE3 in the natriuretic effect of the SGLT2 inhibitor empagliflozin. *Am J Physiol Renal Physiol.* 2020;319:F712–F728. doi: 10.1152/ajprenal.00264.2020
18. Griffin M, Rao VS, Ivey-Miranda J, Fleming J, Mahoney D, Maulion C, Suda N, Siwakoti K, Ahmad T, Jacoby D, et al. Empagliflozin in heart failure: diuretic and cardiorenal effects. *Circulation.* 2020;142:1028–1039. doi: 10.1161/CIRCULATIONAHA.120.045691
19. Gueguen C, Burke SL, Barzel B, Eikelis N, Watson AMD, Jha JC, Jackson KL, Sata Y, Lim K, Lambert GW, et al. Empagliflozin modulates renal sympathetic and heart rate baroreflexes in a rabbit model of diabetes. *Diabetologia.* 2020;63:1424–1434. doi: 10.1007/s00125-020-05145-0
20. Herat LY, Magno AL, Rudnicka C, Hricova J, Carnagarin R, Ward NC, Arcambal A, Kiuchi MG, Head GA, Schlaich MP, et al. SGLT2 inhibitor-induced sympathoinhibition: a novel mechanism for cardiorenal protection. *JACC Basic Transl Sci.* 2020;5:169–179. doi: 10.1016/j.jacbts.2019.11.007
21. Shimizu W, Kubota Y, Hoshika Y, Mozawa K, Tara S, Tokita Y, Yodogawa K, Iwasaki YK, Yamamoto T, Takano H, et al. Effects of empagliflozin versus placebo on cardiac sympathetic activity in acute myocardial infarction patients with type 2 diabetes mellitus: the EMBODY trial. *Cardiovasc Diabetol.* 2020;19:148. doi: 10.1186/s12933-020-01127-z
22. Boorsma EM, Beusekamp JC, Ter Maaten JM, Figarska SM, Danser AHJ, van Veldhuisen DJ, van der Meer P, Heerspink HJL, Damman K, Voors AA. Effects of empagliflozin on renal sodium and glucose handling in patients with acute heart failure. *Eur J Heart Fail.* 2021;23:68–78. doi: 10.1002/ejhf.2066
23. Mordi NA, Mordi IR, Singh JS, McCrimmon RJ, Struthers AD, Lang CC. Renal and cardiovascular effects of SGLT2 inhibition in combination with loop diuretics in patients with type 2 diabetes and chronic heart failure: the RECEDE-CHF Trial. *Circulation.* 2020;142:1713–1724. doi: 10.1161/CIRCULATIONAHA.120.048739
24. Scholtes RA, Muskiet MHA, van Baar MJB, Hesp AC, Greasley PJ, Hammarstedt A, Karlsson C, Hallow KM, Danser AHJ, Heerspink HJL, et al. The adaptive renal response for volume homeostasis during 2 weeks of dapagliflozin treatment in people with type 2 diabetes and preserved renal function on a sodium-controlled diet. *Kidney Int Rep.* 2022;7:1084–1092. doi: 10.1016/j.ekir.2022.02.023
25. Packer M, Anker SD, Butler J, Filippatos G, Ferreira JP, Pocock SJ, Sattar N, Brueckmann M, Jamal W, Cotton D, et al. Empagliflozin in patients with heart failure, reduced ejection fraction, and volume overload: EMPEROR-Reduced Trial. *J Am Coll Cardiol.* 2021;77:1381–1392. doi: 10.1016/j.jacc.2021.01.033
26. Ferrannini G, Hach T, Crowe S, Sanghvi A, Hall KD, Ferrannini E. Energy balance after sodium-glucose cotransporter 2 inhibition. *Diabetes Care.* 2015;38:1730–1735. doi: 10.2337/dc15-0355
27. Neeland IJ, McGuire DK, Chilton R, Crowe S, Lund SS, Woerle HJ, Broedl UC, Johansen OE. Empagliflozin reduces body weight and indices of adipose distribution in patients with type 2 diabetes mellitus. *Diab Vasc Dis Res.* 2016;13:119–126. doi: 10.1177/1479164115616901
28. Mazer CD, Hare GMT, Connelly PW, Gilbert RE, Shehata N, Quan A, Teoh H, Leiter LA, Zinman B, Jüni P, et al. Effect of empagliflozin on erythropoietin levels, iron stores, and red blood cell morphology in patients with type 2 diabetes mellitus and coronary artery disease. *Circulation.* 2020;141:704–707. doi: 10.1161/CIRCULATIONAHA.119.044235
29. Schmidt WFJ, Wachsmuth N, Jimenez J, Soria R. Hemoglobin mass and blood volume in patients with altitude-related polycythemia. *Front Physiol.* 2022;13:867108. doi: 10.3389/fphys.2022.867108
30. Hallow KM, Helmlinger G, Greasley PJ, McMurray JJV, Boulton DW. Why do SGLT2 inhibitors reduce heart failure hospitalization? A differential volume regulation hypothesis. *Diabetes Obes Metab.* 2018;20:479–487. doi: 10.1111/dom.13126
31. Januzzi JL, Zannad F, Anker SD, Butler J, Filippatos G, Pocock SJ, Ferreira JP, Sattar N, Verma S, Vedin O, Schnee J, et al. Prognostic importance of NT-proBNP and effect of empagliflozin in the EMPEROR-Reduced trial. *J Am Coll Cardiol.* 2021;78:1321–1332. doi: 10.1016/j.jacc.2021.07.046
32. Nassif ME, Qintar M, Windsor SL, Jermyn R, Shavelle DM, Tang F, Lamba S, Bhatt K, Brush J, Civitello A, et al. Empagliflozin effects on pulmonary artery pressure in patients with heart failure: Results From the EMBRACE-HF Trial. *Circulation.* 2021;143:1673–1686. doi: 10.1161/CIRCULATIONAHA.120.052503
33. Omar M, Jensen J, Frederiksen PH, Kistorp C, Videbæk L, Poulsen MK, Möller S, Ali M, Gustafsson F, Køber L, et al. Effect of empagliflozin on hemodynamics in patients with heart failure and reduced ejection fraction. *J Am Coll Cardiol.* 2020;76:2740–2751. doi: 10.1016/j.jacc.2020.10.005
34. Lee MMY, Brooksbank KJM, Wetherall K, Mangion K, Roditi G, Campbell RT, Berry C, Chong V, Coyle L, Docherty KF, et al. Effect of empagliflozin on left ventricular volumes in patients with type 2 diabetes, or prediabetes, and heart failure with reduced ejection fraction (SUGAR-DM-HF). *Circulation.* 2021;143:516–525. doi: 10.1161/CIRCULATIONAHA.120.052186
35. Packer M, Anker SD, Butler J, Filippatos G, Pocock SJ, Carson P, Januzzi J, Verma S, Tsutsui H, Brueckmann M, et al. Cardiovascular and renal outcomes with empagliflozin in heart failure. *N Engl J Med.* 2020;383:1413–1424. doi: 10.1056/NEJMoa2022190
36. Ferrannini E, Baldi S, Frascerra S, Astiarraga B, Heise T, Bizzotto R, Mari A, Pieber TR, Muscelli E. Shift to fatty substrate utilization in response to sodium-glucose cotransporter 2 inhibition in subjects without diabetes and patients with type 2 diabetes. *Diabetes.* 2016;65:1190–1195. doi: 10.2337/db15-1356
37. Zannad F, Ferreira JP, Pocock SJ, Zeller C, Anker SD, Butler J, Filippatos G, Hauske SJ, Brueckmann M, Pfarr E, et al. Cardiac and kidney benefits of empagliflozin in heart failure across the spectrum of kidney function: insights from EMPEROR-Reduced. *Circulation.* 2021;143:310–321. doi: 10.1161/CIRCULATIONAHA.120.051685
38. Shen L, Kristensen SL, Bengtsson O, Böhm M, de Boer RA, Docherty KF, Inzucchi SE, Katova T, Køber L, Kosiborod MN, et al. Dapagliflozin in HFrEF patients treated with mineralocorticoid receptor

antagonists: an analysis of DAPA-HF. *JACC Heart Fail.* 2021;9:254–264. doi: 10.1016/j.jchf.2020.11.009
39. McCallum W, Tighiouart H, Ku E, Salem D, Sarnak MJ. Trends in kidney function outcomes following RAAS inhibition in patients with heart failure with reduced ejection fraction. *Am J Kidney Dis.* 2020;75:21–29. doi: 10.1053/j.ajkd.2019.05.010
40. Vaduganathan M, Ferreira JP, Rossignol P, Neuen B, Claggett BL, Pfeffer MA, McMurray JJV, Pitt B, Zannad F, Solomon SD. Effects of steroidal mineralocorticoid receptor antagonists on acute and chronic estimated glomerular filtration rate slopes in patients with chronic heart failure [published online July 22, 2022]. *Eur J Heart Fail.* doi: 10.1002/ejhf.2635
41. Mc Causland FR, Lefkowitz MP, Claggett B, Packer M, Senni M, Gori M, Jhund PS, McGrath MM, Rouleau JL, Shi V, et al. Angiotensin-neprilysin inhibition and renal outcomes across the spectrum of ejection fraction in heart failure [published online January 5, 2022]. *Eur J Heart Fail.* doi: 10.1002/ejhf.2421
42. van Bommel EJM, Muskiet MHA, van Baar MJB, Tonneijck L, Smits MM, Emanuel AL, Bozovic A, Danser AHJ, Geurts F, Hoorn EJ, et al. The renal hemodynamic effects of the SGLT2 inhibitor dapagliflozin are caused by post-glomerular vasodilatation rather than pre-glomerular vasoconstriction in metformin-treated patients with type 2 diabetes in the randomized, double-blind RED trial. *Kidney Int.* 2020;97:202–212. doi: 10.1016/j.kint.2019.09.013
43. Adamson C, Docherty KF, Heerspink HJL, de Boer RA, Damman K, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, Petrie MC, et al. Initial decline ("dip") in estimated glomerular filtration rate following initiation of dapagliflozin in patients with heart failure and reduced ejection fraction: Insights from DAPA-HF. *Circulation.* 2022;146:438–449. doi: 10.1161/CIRCULATIONAHA.121.058910
44. Vallon V, Rose M, Gerasimova M, Satriano J, Platt KA, Koepsell H, Cunard R, Sharma K, Thomson SC, Rieg T. Knockout of Na-glucose transporter SGLT2 attenuates hyperglycemia and glomerular hyperfiltration but not kidney growth or injury in diabetes mellitus. *Am J Physiol Renal Physiol.* 2013;304:F156–F167. doi: 10.1152/ajprenal.00409.2012
45. Nespoux J, Patel R, Zhang H, Huang W, Freeman B, Sanders PW, Kim YC, Vallon V. Gene knockout of the Na+-glucose cotransporter SGLT2 in a murine model of acute kidney injury induced by ischemia-reperfusion. *Am J Physiol Renal Physiol.* 2020;318:F1100–F1112. doi: 10.1152/ajprenal.00607.2019
46. Herring RA, Shojaee-Moradie F, Stevenage M, Parsons I, Jackson N, Mendis J, Middleton B, Umpleby AM, Fielding BA, Davies M, et al. The SGLT2 inhibitor dapagliflozin increases the oxidation of ingested fatty acids to ketones in type 2 diabetes. *Diabetes Care.* 2022;45:1408–1415. doi: 10.2337/dc21-2043
47. Nielsen R, Møller N, Gormsen LC, Tolbod LP, Hansson NH, Sorensen J, Harms HJ, Frøkiær J, Eiskjaer H, Jespersen NR, et al. Cardiovascular effects of treatment with the ketone body 3-hydroxybutyrate in chronic heart failure patients. *Circulation.* 2019;139:2129–2141. doi: 10.1161/CIRCULATIONAHA.118.036459
48. Li X, Lu Q, Qiu Y, do Carmo JM, Wang Z, da Silva AA, Mouton A, Omoto ACM, Hall ME, Li J, et al. Direct cardiac actions of the sodium glucose co-transporter 2 inhibitor empagliflozin improve myocardial oxidative phosphorylation and attenuate pressure-overload heart failure. *J Am Heart Assoc.* 2021;10:e018298. doi: 10.1161/JAHA.120.018298
49. Lommi J, Kupari M, Koskinen P, Näveri H, Leinonen H, Pulkki K, Härkönen M. Blood ketone bodies in congestive heart failure. *J Am Coll Cardiol.* 1996;28:665–672. doi: 10.1016/0735-1097(96)00214-8
50. Voros G, Ector J, Garweg C, Droogne W, Van Cleemput J, Peersman N, Vermeersch P, Janssens S. Increased cardiac uptake of ketone bodies and free fatty acids in human heart failure and hypertrophic left ventricular remodeling. *Circ Heart Fail.* 2018;11:e004953. doi: 10.1161/CIRCHEARTFAILURE.118.004953
51. Ferrannini E, Baldi S, Frascerra S, Astiarraga B, Barsotti E, Clerico A, Muscelli E. Renal handling of ketones in response to sodium-glucose cotransporter 2 inhibition in patients with type 2 diabetes. *Diabetes Care.* 2017;40:771–776. doi: 10.2337/dc16-2724
52. Verma S, Rawat S, Ho KL, Wagg CS, Zhang L, Teoh H, Dyck JE, Uddin GM, Oudit GY, Mayoux E, et al. Empagliflozin increases cardiac energy production in diabetes: novel translational insights into the heart failure benefits of SGLT2 inhibitors. *JACC Basic Transl Sci.* 2018;3:575–587. doi: 10.1016/j.jacbts.2018.07.006
53. Abdurrachim D, Teo XQ, Woo CC, Chan WX, Lalic J, Lam CSP, Lee PTH. Empagliflozin reduces myocardial ketone utilization while preserving glucose utilization in diabetic hypertensive heart disease: a hyperpolarized 13 C magnetic resonance spectroscopy study. *Diabetes Obes Metab.* 2019;21:357–365. doi: 10.1111/dom.13536
54. Santos-Gallego CG, Requena-Ibanez JA, San Antonio R, Ishikawa K, Watanabe S, Picatoste B, Flores E, Garcia-Ropero A, Sanz J, Hajjar RJ, et al. Empagliflozin ameliorates adverse left ventricular remodeling in nondiabetic heart failure by enhancing myocardial energetics. *J Am Coll Cardiol.* 2019;73:1931–1944. doi: 10.1016/j.jacc.2019.01.056
55. Deng Y, Xie M, Li Q, Xu X, Ou W, Zhang Y, Xiao H, Yu H, Zheng Y, Liang Y, et al. Targeting mitochondria-inflammation circuit by β-hydroxybutyrate mitigates HFpEF. *Circ Res.* 2021;128:232–245. doi: 10.1161/CIRCRESAHA.120.317933
56. Gaborit B, Ancel P, Abdullah AE, Maurice F, Abdesselam I, Calen A, Soghomonian A, Houssays M, Varlet I, Eisinger M, et al. Effect of empagliflozin on ectopic fat stores and myocardial energetics in type 2 diabetes: the EMPACEF study. *Cardiovasc Diabetol.* 2021;20:57. doi: 10.1186/s12933-021-01237-2
57. Tomita I, Kume S, Sugahara S, Osawa N, Yamahara K, Yasuda-Yamahara M, Takeda N, Chin-Kanasaki M, Kaneko T, Mayoux E, et al. SGLT2 inhibition mediates protection from diabetic kidney disease by promoting ketone body-induced mTORC1 inhibition. *Cell Metab.* 2020;32:404–419.e6. doi: 10.1016/j.cmet.2020.06.020
58. Hare GMT, Zhang Y, Chin K, Thai K, Jacobs E, Cazorla-Bak MP, Nghiem L, Wilson DF, Vinogradov SA, Connelly KA, et al. Impact of sodium glucose linked cotransporter-2 inhibition on renal microvascular oxygen tension in a rodent model of diabetes mellitus. *Physiol Rep.* 2021;9:e14890. doi: 10.14814/phy2.14890
59. Zanchi A, Burnier M, Muller ME, Ghajarzadeh-Wurzner A, Maillard M, Loncle N, Milani B, Dufour N, Bonny O, Pruijm M. Acute and chronic effects of SGLT2 inhibitor empagliflozin on renal oxygenation and blood pressure control in nondiabetic normotensive subjects: a randomized, placebo-controlled trial. *J Am Heart Assoc.* 2020;9:e016173. doi: 10.1161/JAHA.119.016173
60. Packer M. Role of deranged energy deprivation signaling in the pathogenesis of cardiac and renal disease in states of perceived nutrient overabundance. *Circulation.* 2020;141:2095–2105. doi: 10.1161/CIRCULATIONAHA.119.045561
61. Swedberg K, Young JB, Anand IS, Cheng S, Desai AS, Diaz R, Maggioni AP, McMurray JJ, O'Connor C, Pfeffer MA, et al. Treatment of anemia with darbepoetin alfa in systolic heart failure. *N Engl J Med.* 2013;368:1210–1219. doi: 10.1056/NEJMoa1214865
62. Pfeffer MA, Burdmann EA, Chen CY, Cooper ME, de Zeeuw D, Eckardt KU, Feyzi JM, Ivanovich P, Kewalramani R, Levey AS, et al. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. *N Engl J Med.* 2009;361:2019–2032. doi: 10.1056/NEJMoa0907845
63. Chan LKY, Wang Y, Ng EKW, Leung PS. Na+/H+ exchanger 3 blockade ameliorates type 2 diabetes mellitus via inhibition of sodium-glucose co-transporter 1-mediated glucose absorption in the small intestine. *Diabetes Obes Metab.* 2018;20:709–717. doi: 10.1111/dom.13151
64. Pessoa TD, Campos LC, Carraro-Lacroix L, Girardi AC, Malnic G. Functional role of glucose metabolism, osmotic stress, and sodium-glucose cotransporter isoform-mediated transport on Na+/H+ exchanger isoform 3 activity in the renal proximal tubule. *J Am Soc Nephrol.* 2014;25:2028–2039. doi: 10.1681/ASN.2013060588
65. Nakamura TY, Iwata Y, Arai Y, Komamura K, Wakabayashi S. Activation of Na+/H+ exchanger 1 is sufficient to generate Ca2+ signals that induce cardiac hypertrophy and heart failure. *Circ Res.* 2008;103:891–899. doi: 10.1161/CIRCRESAHA.108.175141
66. Baartscheer A, Schumacher CA, Wüst RC, Fiolet JW, Stienen GJ, Coronel R, Zuurbier CJ. Empagliflozin decreases myocardial cytoplasmic Na+ through inhibition of the cardiac Na+/H+ exchanger in rats and rabbits. *Diabetologia.* 2017;60:568–573. doi: 10.1007/s00125-016-4134-x
67. Uthman L, Baartscheer A, Bleijlevens B, Schumacher CA, Fiolet JWT, Koeman A, Jancev M, Hollmann MW, Weber NC, Coronel R, et al. Class effects of SGLT2 inhibitors in mouse cardiomyocytes and hearts: inhibition of Na+/H+ exchanger, lowering of cytosolic Na+ and vasodilation. *Diabetologia.* 2018;61:722–726. doi: 10.1007/s00125-017-4509-7
68. Chung YJ, Park KC, Tokar S, Eykyn TR, Fuller W, Pavlovic D, Swietach P, Shattock MJ. Off-target effects of sodium-glucose co-transporter 2 blockers: empagliflozin does not inhibit Na+/H+ exchanger-1 or lower [Na+]i in the heart. *Cardiovasc Res.* 2021;117:2794–2806. doi: 10.1093/cvr/cvaa323
69. Chung YJ, Park KC, Tokar S, Eykyn TR, Fuller W, Pavlovic D, Swietach P, Shattock MJ. SGLT2 inhibitors and the cardiac Na+/H+ exchanger-1: the plot thickens. *Cardiovasc Res.* 2021;117:2702–2704. doi: 10.1093/cvr/cvab184

70. Osaka N, Mori Y, Terasaki M, Hiromura M, Saito T, Yashima H, Shiraga Y, Kawakami R, Ohara M, Fukui T, et al. Luseogliflozin inhibits high glucose-induced TGF-β2 expression in mouse cardiomyocytes by suppressing NHE-1 activity. *J Int Med Res.* 2022;50:3000605221097490. doi: 10.1177/03000605221097490
71. Philippaert K, Kalyaanamoorthy S, Fatehi M, Long W, Soni S, Byrne NJ, Barr A, Singh J, Wong J, Palechuk T, et al. Cardiac late sodium channel current is a molecular target for the sodium/glucose cotransporter 2 inhibitor empagliflozin. *Circulation.* 2021;143:2188–2204. doi: 10.1161/CIRCULATIONAHA.121.053350
72. Packer M. Uric acid is a biomarker of oxidative stress in the failing heart: lessons learned from trials with allopurinol and SGLT2 inhibitors. *J Card Fail.* 2020;26:977–984. doi: 10.1016/j.cardfail.2020.08.015
73. Fitchett D, Inzucchi SE, Zinman B, Wanner C, Schumacher M, Schmoor C, Ohneberg K, Ofstad AP, Salsali A, George JT, et al. Mediators of the improvement in heart failure outcomes with empagliflozin in the EMPA-REG OUTCOME trial. *ESC Heart Fail.* 2021;8:4517–4527. doi: 10.1002/ehf2.13615
74. Li J, Neal B, Perkovic V, de Zeeuw D, Neuen BL, Arnott C, Simpson R, Oh R, Mahaffey KW, Heerspink HJL. Mediators of the effects of canagliflozin on kidney protection in patients with type 2 diabetes. *Kidney Int.* 2020;98:769–777. doi: 10.1016/j.kint.2020.04.051
75. Li J, Woodward M, Perkovic V, Figtree GA, Heerspink HJL, Mahaffey KW, de Zeeuw D, Vercruysse F, Shaw W, Matthews DR, et al. Mediators of the effects of canagliflozin on heart failure in patients with type 2 diabetes. *JACC Heart Fail.* 2020;8:57–66. doi: 10.1016/j.jchf.2019.08.004
76. Swe MT, Thongnak L, Jaikumkao K, Pongchaidecha A, Chatsudthipong V, Lungkaphin A. Dapagliflozin not only improves hepatic injury and pancreatic endoplasmic reticulum stress, but also induces hepatic gluconeogenic enzymes expression in obese rats. *Clin Sci (Lond).* 2019;133:2415–2430. doi: 10.1042/CS20190863
77. Umino H, Hasegawa K, Minakuchi H, Muraoka H, Kawaguchi T, Kanda T, Tokuyama H, Wakino S, Itoh H. High basolateral glucose increases sodium-glucose cotransporter 2 and reduces sirtuin-1 in renal tubules through glucose transporter-2 detection. *Sci Rep.* 2018;8:6791. doi: 10.1038/s41598-018-25054-y
78. Herranz N, Gallage S, Mellone M, Wuestefeld T, Klotz S, Hanley CJ, Raguz S, Acosta JC, Innes AJ, Banito A, et al. mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. *Nat Cell Biol.* 2015;17:1205–1217. doi: 10.1038/ncb3225
79. Gangloff YG, Mueller M, Dann SG, Svoboda P, Sticker M, Spetz JF, Um SH, Brown EJ, Cereghini S, Thomas G, et al. Disruption of the mouse mTOR gene leads to early postimplantation lethality and prohibits embryonic stem cell development. *Mol Cell Biol.* 2004;24:9508–9516. doi: 10.1128/MCB.24.21.9508-9516.2004
80. Zhang D, Contu R, Latronico MV, Zhang J, Rizzi R, Catalucci D, Miyamoto S, Huang K, Ceci M, Gu Y, et al. MTORC1 regulates cardiac function and myocyte survival through 4E-BP1 inhibition in mice. *J Clin Invest.* 2010;120:2805–2816. doi: 10.1172/JCI43008
81. Su M, Chen Z, Wang C, Song L, Zou Y, Zhang L, Hui R, Wang J. Cardiac-specific overexpression of miR-222 induces heart failure and inhibits autophagy in mice. *Cell Physiol Biochem.* 2016;39:1503–1511. doi: 10.1159/000447853
82. Gao G, Chen W, Yan M, Liu J, Luo H, Wang C, Yang P. Rapamycin regulates the balance between cardiomyocyte apoptosis and autophagy in chronic heart failure by inhibiting mTOR signaling. *Int J Mol Med.* 2020;45:195–209. doi: 10.3892/ijmm.2019.4407
83. Sang HQ, Jiang ZM, Zhao QP, Xin F. MicroRNA-133a improves the cardiac function and fibrosis through inhibiting Akt in heart failure rats. *Biomed Pharmacother.* 2015;71:185–189. doi: 10.1016/j.biopha.2015.02.030
84. Yamahara K, Kume S, Koya D, Tanaka Y, Morita Y, Chin-Kanasaki M, Araki H, Isshiki K, Araki S, Haneda M, et al. Obesity-mediated autophagy insufficiency exacerbates proteinuria-induced tubulointerstitial lesions. *J Am Soc Nephrol.* 2013;24:1769–1781. doi: 10.1681/ASN.2012111080
85. Inoki K, Mori H, Wang J, Suzuki T, Hong S, Yoshida S, Blattner SM, Ikenoue T, Rüegg MA, Hall MN, et al. mTORC1 activation in podocytes is a critical step in the development of diabetic nephropathy in mice. *J Clin Invest.* 2011;121:2181–2196. doi: 10.1172/JCI44771
86. Mori H, Inoki K, Masutani K, Wakabayashi Y, Komai K, Nakagawa R, Guan KL, Yoshimura A. The mTOR pathway is highly activated in diabetic nephropathy and rapamycin has a strong therapeutic potential. *Biochem Biophys Res Commun.* 2009;384:471–475. doi: 10.1016/j.bbrc.2009.04.136
87. Kogot-Levin A, Hinden L, Riahi Y, Israeli T, Tirosh B, Cerasi E, Mizrachi EB, Tam J, Mosenzon O, Leibowitz G. Proximal tubule mTORC1 is a central player in the pathophysiology of diabetic nephropathy and its correction by SGLT2 inhibitors. *Cell Rep.* 2020;32:107954. doi: 10.1016/j.celrep.2020.107954
88. Yano T, Shimoshige S, Miki T, Tanno M, Mochizuki A, Fujito T, Yuda S, Muranaka A, Ogasawara M, Hashimoto A, et al. Clinical impact of myocardial mTORC1 activation in nonischemic dilated cardiomyopathy. *J Mol Cell Cardiol.* 2016;91:6–9. doi: 10.1016/j.yjmcc.2015.12.022
89. Haq S, Choukroun G, Lim H, Tymitz KM, del Monte F, Gwathmey J, Grazette L, Michael A, Hajjar R, Force T, et al. Differential activation of signal transduction pathways in human hearts with hypertrophy versus advanced heart failure. *Circulation.* 2001;103:670–677. doi: 10.1161/01.cir.103.5.670
90. Schlingmann KP, Jouret F, Shen K, Nigam A, Arjona FJ, Dafinger C, Houillier P, Jones DP, Kleinerüschkamp F, Oh J, et al. mTOR-activating mutations in RRAGD are causative for kidney tubulopathy and cardiomyopathy. *J Am Soc Nephrol.* 2021;32:2885–2899. doi: 10.1681/ASN.2021030333
91. Mao Z, Tan Y, Tao J, Li L, Wang H, Yu F, Perl A, Zhao M. Renal mTORC1 activation is associated with disease activity and prognosis in lupus nephritis. *Rheumatology (Oxford).* 2022;18:keac037. doi: 10.1093/rheumatology/keac037
92. Sanz MN, Grimbert L, Moulin M, Gressette M, Rucker-Martin C, Lemaire C, Mericskay M, Veksler V, Ventura-Clapier R, Garnier A, et al. Inducible cardiac-specific deletion of Sirt1 in male mice reveals progressive cardiac dysfunction and sensitization of the heart to pressure overload. *Int J Mol Sci.* 2019;20:5005. doi: 10.3390/ijms20205005
93. Guo X, Yan F, Li J, Zhang C, Su H, Bu P. SIRT3 ablation deteriorates obesity-related cardiac remodeling by modulating ROS-NF-κB-MCP-1 signaling pathway. *J Cardiovasc Pharmacol.* 2020;76:296–304. doi: 10.1097/FJC.0000000000000877
94. Huang Y, Zhang J, Xu D, Peng Y, Jin Y, Zhang L. SIRT6-specific inhibitor OSS-128167 exacerbates diabetic cardiomyopathy by aggravating inflammation and oxidative stress. *Mol Med Rep.* 2021;23:367. doi: 10.3892/mmr.2021.12006
95. Yuan YP, Ma ZG, Zhang X, Xu SC, Zeng XF, Yang Z, Deng W, Tang QZ. CTRP3 protected against doxorubicin-induced cardiac dysfunction, inflammation and cell death via activation of Sirt1. *J Mol Cell Cardiol.* 2018;114:38–47. doi: 10.1016/j.yjmcc.2017.10.008
96. Bugyei-Twum A, Ford C, Civitarese R, Seegobin J, Advani SL, Desjardins JF, Kabir G, Zhang Y, Mitchell M, Switzer J, et al. Sirtuin 1 activation attenuates cardiac fibrosis in a rodent pressure overload model by modifying Smad2/3 transactivation. *Cardiovasc Res.* 2018;114:1629–1641. doi: 10.1093/cvr/cvy131
97. Tomczyk MM, Cheung KG, Xiang B, Tamanna N, Fonseca Teixeira AL, Agarwal P, Kereliuk SM, Spicer V, Lin L, Treberg J, et al. Mitochondrial sirtuin-3 (SIRT3) prevents doxorubicin-induced dilated cardiomyopathy by modulating protein acetylation and oxidative stress. *Circ Heart Fail.* 2022;15:e008547. doi: 10.1161/CIRCHEARTFAILURE.121.008547
98. Wu S, Lan J, Li L, Wang X, Tong M, Fu L, Zhang Y, Xu J, Chen X, Chen H, et al. Sirt6 protects cardiomyocytes against doxorubicin-induced cardiotoxicity by inhibiting P53/Fas-dependent cell death and augmenting endogenous antioxidant defense mechanisms [published online October 28, 2021]. *Cell Biol Toxicol.* doi: 10.1007/s10565-021-09649-2
99. Chuang PY, Xu J, Dai Y, Jia F, Mallipattu SK, Yacoub R, Gu L, Premsrirut PK, He JC. In vivo RNA interference models of inducible and reversible Sirt1 knockdown in kidney cells. *Am J Pathol.* 2014;184:1940–1956. doi: 10.1016/j.ajpath.2014.03.016
100. Feng X, Su H, He X, Chen JX, Zeng H. SIRT3 deficiency sensitizes angiotensin-II-induced renal fibrosis. *Cells.* 2020;9:2510. doi: 10.3390/cells9112510
101. Huang W, Liu H, Zhu S, Woodson M, Liu R, Tilton RG, Miller JD, Zhang W. Sirt6 deficiency results in progression of glomerular injury in the kidney. *Aging (Albany NY).* 2017;9:1069–1083. doi: 10.18632/aging.101214
102. Zhang T, Chi Y, Kang Y, Lu H, Niu H, Liu W, Li Y. Resveratrol ameliorates podocyte damage in diabetic mice via SIRT1/PGC-1α mediated attenuation of mitochondrial oxidative stress. *J Cell Physiol.* 2019;234:5033–5043. doi: 10.1002/jcp.27306
103. Locatelli M, Zoja C, Zanchi C, Corna D, Villa S, Bolognini S, Novelli R, Perico L, Remuzzi G, Benigni A, et al. Manipulating Sirtuin 3 pathway ameliorates renal damage in experimental diabetes. *Sci Rep.* 2020;10:8418. doi: 10.1038/s41598-020-65423-0
104. Fan Y, Yang Q, Yang Y, Gao Z, Ma Y, Zhang L, Liang W, Ding G. Sirt6 suppresses high glucose-induced mitochondrial dysfunction and apoptosis in

podocytes through AMPK activation. *Int J Biol Sci.* 2019;15:701–713. doi: 10.7150/ijbs.29323
105. Clark AJ, Parikh SM. Targeting energy pathways in kidney disease: the roles of sirtuins, AMPK, and PGC1α. *Kidney Int.* 2021;99:828–840. doi: 10.1016/j.kint.2020.09.037
106. Kärkkäinen O, Tuomainen T, Mutikainen M, Lehtonen M, Ruas JL, Hanhineva K, Tavi P. Heart specific PGC-1α deletion identifies metabolome of cardiac restricted metabolic heart failure. *Cardiovasc Res.* 2019;115:107–118. doi: 10.1093/cvr/cvy155
107. Akkafa F, Halil Altiparmak I, Erkus ME, Aksoy N, Kaya C, Ozer A, Sezen H, Oztuzcu S, Koyuncu I, Umurhan B. Reduced SIRT1 expression correlates with enhanced oxidative stress in compensated and decompensated heart failure. *Redox Biol.* 2015;6:169–173. doi: 10.1016/j.redox.2015.07.011
108. Sihag S, Cresci S, Li AY, Sucharov CC, Lehman JJ. PGC-1alpha and ERRalpha target gene downregulation is a signature of the failing human heart. *J Mol Cell Cardiol.* 2009;46:201–212. doi: 10.1016/j.yjmcc.2008.10.025
109. Wang S, Fu C, Wang H, Shi Y, Xu X, Chen J, Song X, Sun K, Wang J, Fan X, et al. Polymorphisms of the peroxisome proliferator-activated receptor-gamma coactivator-1alpha gene are associated with hypertrophic cardiomyopathy and not with hypertension hypertrophy. *Clin Chem Lab Med.* 2007;45:962–967. doi: 10.1515/CCLM.2007.189
110. Wang CY, Chen CC, Lin MH, Su HT, Ho MY, Yeh JK, Tsai ML, Hsieh IC, Wen MS. TLR9 binding to Beclin 1 and mitochondrial SIRT3 by a sodium-glucose co-transporter 2 inhibitor protects the heart from doxorubicin toxicity. *Biology (Basel).* 2020;9:369. doi: 10.3390/biology9110369
111. Zhao Y, Wei J, Hou X, Liu H, Guo F, Zhou Y, Zhang Y, Qu Y, Gu J, Zhou Y, et al. SIRT1 rs10823108 and FOXO1 rs17446614 responsible for genetic susceptibility to diabetic nephropathy. *Sci Rep.* 2017;7:10285. doi: 10.1038/s41598-017-10612-7
112. Shao Y, Ren H, Lv C, Ma X, Wu C, Wang Q. Changes of serum Mir-217 and the correlation with the severity in type 2 diabetes patients with different stages of diabetic kidney disease. *Endocrine.* 2017;55:130–138. doi: 10.1007/s12020-016-1069-4
113. Tokarska-Schlattner M, Kay L, Perret P, Isola R, Attia S, Lamarche F, Tellier C, Cottet-Rousselle C, Uneisi A, Hininger-Favier I, et al. Role of cardiac AMP-activated protein kinase in a non-pathological setting: evidence from cardiomyocyte-specific, inducible AMP-activated protein kinase α1α2-knockout mice. *Front Cell Dev Biol.* 2021;9:731015. doi: 10.3389/fcell.2021.731015
114. Sung MM, Zordoky BN, Bujak AL, Lally JS, Fung D, Young ME, Horman S, Miller EJ, Light PE, Kemp BE, et al. AMPK deficiency in cardiac muscle results in dilated cardiomyopathy in the absence of changes in energy metabolism. *Cardiovasc Res.* 2015;107:235–245. doi: 10.1093/cvr/cvv166
115. Liu D, Ma Z, Di S, Yang Y, Yang J, Xu L, Reiter RJ, Qiao S, Yuan J. AMPK/PGC1α activation by melatonin attenuates acute doxorubicin cardiotoxicity via alleviating mitochondrial oxidative damage and apoptosis. *Free Radic Biol Med.* 2018;129:59–72. doi: 10.1016/j.freeradbiomed.2018.08.032
116. Wu S, Lu Q, Ding Y, Wu Y, Qiu Y, Wang P, Mao X, Huang K, Xie Z, Zou MH. Hyperglycemia-driven inhibition of AMP-activated protein kinase α2 induces diabetic cardiomyopathy by promoting mitochondria-associated endoplasmic reticulum membranes in vivo. *Circulation.* 2019;139:1913–1936. doi: 10.1161/CIRCULATIONAHA.118.033552
117. Nam DH, Kim E, Benham A, Park HK, Soibam B, Taffet GE, Kaelber JT, Suh JH, Taegtmeyer H, Entman ML, et al. Transient activation of AMPK preceding left ventricular pressure overload reduces adverse remodeling and preserves left ventricular function. *FASEB J.* 2019;33:711–721. doi: 10.1096/fj.201800602R
118. Dugan LL, You YH, Ali SS, Diamond-Stanic M, Miyamoto S, DeCleves AE, Andreyev A, Quach T, Ly S, Shekhtman G, et al. AMPK dysregulation promotes diabetes-related reduction of superoxide and mitochondrial function. *J Clin Invest.* 2013;123:4888–4899. doi: 10.1172/JCI66218
119. Banu K, Lin Q, Basgen JM, Planoutene M, Wei C, Reghuvaran AC, Tian X, Shi H, Garzon F, Garzia A, et al. AMPK mediates regulation of glomerular volume and podocyte survival. *JCI Insight.* 2021;6:e150004. doi: 10.1172/jci.insight.150004
120. Lim JH, Kim HW, Kim MY, Kim TW, Kim EN, Kim Y, Chung S, Kim YS, Choi BS, Kim YS, et al. Cinacalcet-mediated activation of the CaMKKβ-LKB1-AMPK pathway attenuates diabetic nephropathy in db/db mice by modulation of apoptosis and autophagy. *Cell Death Dis.* 2018;9:270. doi: 10.1038/s41419-018-0324-4
121. Tsogbadrakh B, Ryu H, Ju KD, Lee J, Yun S, Yu KS, Kim HJ, Ahn C, Oh KH. AICAR, an AMPK activator, protects against cisplatin-induced acute kidney injury through the JAK/STAT/SOCS pathway. *Biochem Biophys Res Commun.* 2019;509:680–686. doi: 10.1016/j.bbrc.2018.12.159
122. Lopez-Sainz A, Dominguez F, Lopes LR, Ochoa JP, Barriales-Villa R, Climent V, Linschoten M, Tiron C, Chiriatti C, Marques N, et al. Clinical features and natural history of PRKAG2 variant cardiac glycogenosis. *J Am Coll Cardiol.* 2020;76:186–197. doi: 10.1016/j.jacc.2020.05.029
123. Gorski M, Jung B, Li Y, Matias-Garcia PR, Wuttke M, Coassin S, Thio CHL, Kleber ME, Winkler TW, Wanner V, et al. Meta-analysis uncovers genome-wide significant variants for rapid kidney function decline. *Kidney Int.* 2021;99:926–939. doi: 10.1016/j.kint.2020.09.030
124. van Zuydam NR, Ahlqvist E, Sandholm N, Deshmukh H, Rayner NW, Abdalla M, Ladenvall C, Ziemek D, Fauman E, Robertson NR, et al. A genome-wide association study of diabetic kidney disease in subjects with type 2 diabetes. *Diabetes.* 2018;67:1414–1427. doi: 10.2337/db17-0914
125. Meijles DN, Zoumpoulidou G, Markou T, Rostron KA, Patel R, Lay K, Handa BS, Wong B, Sugden PH, Clerk A. The cardiomyocyte "redox rheostat": redox signalling via the AMPK-mTOR axis and regulation of gene and protein expression balancing survival and death. *J Mol Cell Cardiol.* 2019;129:118–129. doi: 10.1016/j.yjmcc.2019.02.006
126. Pillai VB, Sundaresan NR, Gupta MP. Regulation of Akt signaling by sirtuins: its implication in cardiac hypertrophy and aging. *Circ Res.* 2014;114:368–378. doi: 10.1161/CIRCRESAHA.113.300536
127. Ghosh HS, McBurney M, Robbins PD. SIRT1 negatively regulates the mammalian target of rapamycin. *PLoS One.* 2010;5:e9199. doi: 10.1371/journal.pone.0009199
128. Zheng H, Fu Y, Huang Y, Zheng X, Yu W, Wang W. mTOR signaling promotes foam cell formation and inhibits foam cell egress through suppressing the SIRT1 signaling pathway. *Mol Med Rep.* 2017;16:3315–3323. doi: 10.3892/mmr.2017.7032
129. Wang Y, Li X, He Z, Chen W, Lu J. Rapamycin attenuates palmitate-induced lipid aggregation by up-regulating SIRT-1 signaling in AML12 hepatocytes. *Pharmazie.* 2016;71:733–737. doi: 10.1691/ph.2016.6695
130. Cantó C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature.* 2009;458:1056–1060. doi: 10.1038/nature07813
131. Jäger S, Handschin C, St-Pierre J, Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc Natl Acad Sci USA.* 2007;104:12017–12022. doi: 10.1073/pnas.0705070104
132. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE, Shaw RJ. AMPK phosphorylation of Raptor mediates a metabolic checkpoint. *Mol Cell.* 2008;30:214–226. doi: 10.1016/j.molcel.2008.03.003
133. Mizushima N, Levine B. Autophagy in human diseases. *N Engl J Med.* 2020;383:1564–1576. doi: 10.1056/NEJMra2022774
134. Levine B, Kroemer G. Biological functions of autophagy genes: a disease perspective. *Cell.* 2019;176:11–42. doi: 10.1016/j.cell.2018.09.048
135. Galluzzi L, Pietrocola F, Levine B, Kroemer G. Metabolic control of autophagy. *Cell.* 2014;159:1263–1276.
136. Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. *Nature.* 2011;469:323–335. doi: 10.1038/nature09782
137. Xu Y, Wan W. Acetylation in the regulation of autophagy. *Autophagy.* 2022;18:1–9. doi: 10.1080/15548627.2022.2062112
138. Liu Y, Nguyen PT, Wang X, Zhao Y, Meacham CE, Zou Z, Bordieanu B, Johanns M, Vertommen D, Wijshake T, et al. TLR9 and beclin 1 crosstalk regulates muscle AMPK activation in exercise. *Nature.* 2020;578:605–609. doi: 10.1038/s41586-020-1992-7
139. Gatica D, Chiong M, Lavandero S, Klionsky DJ. The role of autophagy in cardiovascular pathology. *Cardiovasc Res.* 2022;118:934–950. doi: 10.1093/cvr/cvab158
140. Chen XC, Li ZH, Yang C, Tang JX, Lan HY, Liu HF. Lysosome depletion-triggered autophagy impairment in progressive kidney injury. *Kidney Dis (Basel).* 2021;7:254–267. doi: 10.1159/000515035
141. Zhu H, Tannous P, Johnstone JL, Kong Y, Shelton JM, Richardson JA, Le V, Levine B, Rothermel BA, Hill JA. Cardiac autophagy is a maladaptive response to hemodynamic stress. *J Clin Invest.* 2007;117:1782–1793. doi: 10.1172/JCI2752
142. Sciarretta S, Maejima Y, Zablocki D, Sadoshima J. The role of autophagy in the heart. *Annu Rev Physiol.* 2018;80:1–26. doi: 10.1146/annurev-physiol-021317-121427
143. Nah J, Shirakabe A, Mukai R, Zhai P, Sung EA, Ivessa A, Mizushima W, Nakada Y, Saito T, Hu C, et al. Ulk1-dependent alternative mitophagy plays a protective role during pressure overload in the heart. *Cardiovasc Res.* 2022;9:cvac003. doi: 10.1093/cvr/cvac003

144. Ghosh R, Gillaspie JJ, Campbell KS, Symons JD, Boudina S, Pattison JS. Chaperone mediated autophagy protects cardiomyocytes against hypoxic-cell death [published online May 18, 2022]. *Am J Physiol Cell Physiol.* doi: 10.1152/ajpcell.00369.2021
145. Han YC, Tang SQ, Liu YT, Li AM, Zhan M, Yang M, Song N, Zhang W, Wu XQ, Peng CH, et al. AMPK agonist alleviate renal tubulointerstitial fibrosis via activating mitophagy in high fat and streptozotocin induced diabetic mice. *Cell Death Dis.* 2021;12:925. doi: 10.1038/s41419-021-04184-8
146. Yuan L, Yuan Y, Liu F, Li L, Liu J, Chen Y, Cheng J, Lu Y. PGC-1α alleviates mitochondrial dysfunction via TFEB-mediated autophagy in cisplatin-induced acute kidney injury. *Aging (Albany NY).* 2021;13:8421–8439. doi: 10.18632/aging.202653
147. Kanamori H, Yoshida A, Naruse G, Endo S, Minatoguchi S, Watanabe T, Kawaguchi T, Tanaka T, Yamada Y, Takasugi N, et al. Impact of autophagy on prognosis of patients with dilated cardiomyopathy. *J Am Coll Cardiol.* 2022;79:789–801. doi: 10.1016/j.jacc.2021.11.059
148. Nandi SS, Duryee MJ, Shahshahan HR, Thiele GM, Anderson DR, Mishra PK. Induction of autophagy markers is associated with attenuation of miR-133a in diabetic heart failure patients undergoing mechanical unloading. *Am J Transl Res.* 2015;7:683–696.
149. Yan H, Du D, Wang C, Tian M. Downregulation of autophagy-related circular RNA (ACR) is correlated with poor survival of patients with chronic heart failure. *Bioengineered.* 2022;13:13141–13149. doi: 10.1080/21655979.2022.2059862
150. Saito T, Asai K, Sato S, Hayashi M, Adachi A, Sasaki Y, Takano H, Mizuno K, Shimizu W. Autophagic vacuoles in cardiomyocytes of dilated cardiomyopathy with initially decompensated heart failure predict improved prognosis. *Autophagy.* 2016;12:579–587. doi: 10.1080/15548627.2016.1145326
151. Liu WJ, Shen TT, Chen RH, Wu HL, Wang YJ, Deng JK, Chen QH, Pan Q, Huang Fu CM, Tao JL, et al. Autophagy-lysosome pathway in renal tubular epithelial cells is disrupted by advanced glycation end products in diabetic nephropathy. *J Biol Chem.* 2015;290:20499–20510. doi: 10.1074/jbc.M115.666354
152. Gui Z, Suo C, Wang Z, Zheng M, Fei S, Chen H, Sun L, Han Z, Tao J, Ju X, et al. Impaired ATG16L-dependent autophagy promotes renal interstitial fibrosis in chronic renal graft dysfunction through inducing EndMT by NF-κB signal pathway. *Front Immunol.* 2021;12:650424. doi: 10.3389/fimmu.2021.650424
153. Zeng C, Fan Y, Wu J, Shi S, Chen Z, Zhong Y, Zhang C, Zen K, Liu Z. Podocyte autophagic activity plays a protective role in renal injury and delays the progression of podocytopathies. *J Pathol.* 2014;234:203–213. doi: 10.1002/path.4382
154. da Silva CA, Monteiro MLGdR, Araújo LS, Urzedo MG, Rocha LB, Dos Reis MA, Machado JR. In situ evaluation of podocytes in patients with focal segmental glomerulosclerosis and minimal change disease. *PLoS One.* 2020;15:e0241745. doi: 10.1371/journal.pone.0241745
155. Xu C, Wang W, Zhong J, Lei F, Xu N, Zhang Y, Xie W. Canagliflozin exerts anti-inflammatory effects by inhibiting intracellular glucose metabolism and promoting autophagy in immune cells. *Biochem Pharmacol.* 2018;152:45–59. doi: 10.1016/j.bcp.2018.03.013
156. Mizuno M, Kuno A, Yano T, Miki T, Oshima H, Sato T, Nakata K, Kimura Y, Tanno M, Miura T. Empagliflozin normalizes the size and number of mitochondria and prevents reduction in mitochondrial size after myocardial infarction in diabetic hearts. *Physiol Rep.* 2018;6:e13741. doi: 10.14814/phy2.13741
157. Aragón-Herrera A, Feijóo-Bandín S, Otero Santiago M, Barral L, Campos-Toimil M, Gil-Longo J, Costa Pereira TM, García-Caballero T, Rodríguez-Segade S, Rodríguez J, et al. Empagliflozin reduces the levels of CD36 and cardiotoxic lipids while improving autophagy in the hearts of Zucker diabetic fatty rats. *Biochem Pharmacol.* 2019;170:113677. doi: 10.1016/j.bcp.2019.113677
158. Fukushima K, Kitamura S, Tsuji K, Sang Y, Wada J. Sodium glucose co-transporter 2 inhibitor ameliorates autophagic flux impairment on renal proximal tubular cells in obesity mice. *Int J Mol Sci.* 2020;21:4054. doi: 10.3390/ijms21114054
159. Korbut AI, Taskaeva IS, Bgatova NP, Muraleva NA, Orlov NB, Dashkin MV, Khotskina AS, Zavyalov EL, Konenkov VI, Klein T, et al. SGLT2 inhibitor empagliflozin and dpp4 inhibitor linagliptin reactivate glomerular autophagy in db/db mice, a model of type 2 diabetes. *Int J Mol Sci.* 2020;21:2987. doi: 10.3390/ijms21082987
160. Arab HH, Al-Shorbagy MY, Saad MA. Activation of autophagy and suppression of apoptosis by dapagliflozin attenuates experimental inflammatory bowel disease in rats: targeting AMPK/mTOR, HMGB1/RAGE and Nrf2/HO-1 pathways. *Chem Biol Interact.* 2021;335:109368. doi: 10.1016/j.cbi.2021.109368
161. Jaikumkao K, Promsan S, Thongnak L, Swe MT, Tapanya M, Htun KT, Kothan S, Intachai N, Lungkaphin A. Dapagliflozin ameliorates pancreatic injury and activates kidney autophagy by modulating the AMPK/mTOR signaling pathway in obese rats. *J Cell Physiol.* 2021;236:6424–6440. doi: 10.1002/jcp.30316
162. Li L, Li Q, Huang W, Han Y, Tan H, An M, Xiang Q, Zhou R, Yang L, Cheng Y. Dapagliflozin alleviates hepatic steatosis by restoring autophagy via the AMPK-mTOR pathway. *Front Pharmacol.* 2021;12:589273. doi: 10.3389/fphar.2021.589273
163. Meng Z, Liu X, Li T, Fang T, Cheng Y, Han L, Sun B, Chen L. The SGLT2 inhibitor empagliflozin negatively regulates IL-17/IL-23 axis-mediated inflammatory responses in T2DM with NAFLD via the AMPK/mTOR/autophagy pathway. *Int Immunopharmacol.* 2021;94:107492. doi: 10.1016/j.intimp.2021.107492
164. Nasiri-Ansari N, Nikolopoulou C, Papoutsi K, Kyrou I, Mantzoros CS, Kyriakopoulos G, Chatzigeorgiou A, Kalotychou V, Randeva MS, Chatha K, et al. Empagliflozin attenuates non-alcoholic fatty liver disease (NAFLD) in high fat diet fed ApoE(-/-) mice by activating autophagy and reducing ER stress and apoptosis. *Int J Mol Sci.* 2021;22:818. doi: 10.3390/ijms22020818
165. Ren C, Sun K, Zhang Y, Hu Y, Hu B, Zhao J, He Z, Ding R, Wang W, Liang C. Sodium-glucose cotransporter-2 inhibitor empagliflozin ameliorates sunitinib-induced cardiac dysfunction via regulation of AMPK-mTOR signaling pathway-mediated autophagy. *Front Pharmacol.* 2021;12:664181. doi: 10.3389/fphar.2021.664181
166. Xu J, Kitada M, Ogura Y, Liu H, Koya D. Dapagliflozin restores impaired autophagy and suppresses inflammation in high glucose-treated HK-2 cells. *Cells.* 2021;10:1457. doi: 10.3390/cells10061457
167. Ala M, Khoshdel MRF, Dehpour AR. Empagliflozin enhances autophagy, mitochondrial biogenesis, and antioxidant defense and ameliorates renal ischemia/reperfusion in nondiabetic rats. *Oxid Med Cell Longev.* 2022;2022:1197061. doi: 10.1155/2022/1197061
168. Cai C, Guo Z, Chang X, Li Z, Wu F, He J, Cao T, Wang K, Shi N, Zhou H, et al. Empagliflozin attenuates cardiac microvascular ischemia/reperfusion through activating the AMPKα1/ULK1/FUNDC1/mitophagy pathway. *Redox Biol.* 2022;52:102288. doi: 10.1016/j.redox.2022.102288
169. Li X, Flynn ER, do Carmo JM, Wang Z, da Silva AA, Mouton AJ, Omoto ACM, Hall ME, Hall JE. Direct cardiac actions of sodium-glucose cotransporter 2 inhibition improve mitochondrial function and attenuate oxidative stress in pressure overload-induced heart failure. *Front Cardiovasc Med.* 2022;9:859253. doi: 10.3389/fcvm.2022.859253
170. Park CH, Lee B, Han M, Rhee WJ, Kwak MS, Yoo TH, Shin JS. Canagliflozin protects against cisplatin-induced acute kidney injury by AMPK-mediated autophagy in renal proximal tubular cells. *Cell Death Discov.* 2022;8:12. doi: 10.1038/s41420-021-00801-9
171. Yang L, Liang B, Li J, Zhang X, Chen H, Sun J, Zhang Z. Dapagliflozin alleviates advanced glycation end product induced podocyte injury through AMPK/mTOR mediated autophagy pathway. *Cell Signal.* 2022;90:110206. doi: 10.1016/j.cellsig.2021.110206
172. Xu Z, Hu W, Wang B, Xu T, Wang J, Wei D. Canagliflozin ameliorates nonalcoholic fatty liver disease by regulating lipid metabolism and inhibiting inflammation through induction of autophagy. *Yonsei Med J.* 2022;63:619–631. doi: 10.3349/ymj.2022.63.7.619
173. Yu YW, Que JQ, Liu S, Huang KY, Qian L, Weng YB, Rong FN, Wang L, Zhou YY, Xue YJ, et al. Sodium-glucose co-transporter-2 inhibitor of dapagliflozin attenuates myocardial ischemia/reperfusion injury by limiting NLRP3 inflammasome activation and modulating autophagy. *Front Cardiovasc Med.* 2022;8:768214. doi: 10.3389/fcvm.2021.768214
174. Oshima H, Miki T, Kuno A, Mizuno M, Sato T, Tanno M, Yano T, Nakata K, Kimura Y, Abe K, et al. Empagliflozin, an SGLT2 inhibitor, reduced the mortality rate after acute myocardial infarction with modification of cardiac metabolomes and antioxidants in diabetic rats. *J Pharmacol Exp Ther.* 2019;368:524–534. doi: 10.1124/jpet.118.253666
175. Shao Q, Meng L, Lee S, Tse G, Gong M, Zhang Z, Zhao J, Zhao Y, Li G, Liu T. Empagliflozin, a sodium glucose co-transporter-2 inhibitor, alleviates atrial remodeling and improves mitochondrial function in high-fat diet/streptozotocin-induced diabetic rats. *Cardiovasc Diabetol.* 2019;18:165. doi: 10.1186/s12933-019-0964-4
176. Ren FF, Xie ZY, Jiang YN, Guan X, Chen QY, Lai TF, Li L. Dapagliflozin attenuates pressure overload-induced myocardial remodeling in mice via activating SIRT1 and inhibiting endoplasmic reticulum stress

[published online December 1, 2021]. *Acta Pharmacol Sin.* doi: 10.1038/s41401-021-00805-2.
177. Quagliariello V, De Laurentiis M, Rea D, Barbieri A, Monti MG, Carbone A, Paccone A, Altucci L, Conte M, Canale ML, et al. The SGLT-2 inhibitor empagliflozin improves myocardial strain, reduces cardiac fibrosis and pro-inflammatory cytokines in non-diabetic mice treated with doxorubicin. *Cardiovasc Diabetol.* 2021;20:150. doi: 10.1186/s12933-021-01346-y
178. Ke Q, Shi C, Lv Y, Wang L, Luo J, Jiang L, Yang J, Zhou Y. SGLT2 inhibitor counteracts NLRP3 inflammasome via tubular metabolite itaconate in fibrosis kidney. *FASEB J.* 2022;36:e22078. doi: 10.1096/fj.202100909RR
179. Tian G, Yu Y, Deng H, Yang L, Shi X, Yu B. Empagliflozin alleviates ethanol-induced cardiomyocyte injury through inhibition of mitochondrial apoptosis via a SIRT1/PTEN/Akt pathway. *Clin Exp Pharmacol Physiol.* 2021;48:837–845. doi: 10.1111/1440-1681.13470
180. Ye Y, Jia X, Bajaj M, Birnbaum Y. Dapagliflozin attenuates Na+/H+ exchanger-1 in cardiofibroblasts via AMPK activation. *Cardiovasc Drugs Ther.* 2018;32:553–558. doi: 10.1007/s10557-018-6837-3
181. Koyani CN, Plastira I, Sourij H, Hallström S, Schmidt A, Rainer PP, Bugger H, Frank S, Malle E, von Lewinski D. Empagliflozin protects heart from inflammation and energy depletion via AMPK activation. *Pharmacol Res.* 2020;158:104870. doi: 10.1016/j.phrs.2020.104870
182. Lu Q, Liu J, Li X, Sun X, Zhang J, Ren D, Tong N, Li J. Empagliflozin attenuates ischemia and reperfusion injury through LKB1/AMPK signaling pathway. *Mol Cell Endocrinol.* 2020;501:110642. doi: 10.1016/j.mce.2019.110642
183. Radlinger B, Hornsteiner F, Folie S, Salvenmoser W, Haubner BJ, Schuetz T, Haas S, Ress C, Adolph TE, Salzmann K, et al. Cardioprotective effects of short-term empagliflozin treatment in db/db mice. *Sci Rep.* 2020;10:19686. doi: 10.1038/s41598-020-76698-8
184. Kondo H, Akoumianakis I, Badi I, Akawi N, Kotanidis CP, Polkinghorne M, Stadiotti I, Sommariva E, Antonopoulos AS, Carena MC, et al. Effects of canagliflozin on human myocardial redox signalling: clinical implications. *Eur Heart J.* 2021;42:4947–4960. doi: 10.1093/eurheartj/ehab420
185. Lee CC, Chen WT, Chen SY, Lee TM. Dapagliflozin attenuates arrhythmic vulnerabilities by regulating connexin43 expression via the AMPK pathway in post-infarcted rat hearts. *Biochem Pharmacol.* 2021;192:114674. doi: 10.1016/j.bcp.2021.114674
186. Liu Y, Wu M, Xu J, Xu B, Kang L. Empagliflozin prevents from early cardiac injury post myocardial infarction in non-diabetic mice. *Eur J Pharm Sci.* 2021;161:105788. doi: 10.1016/j.ejps.2021.105788
187. Sun P, Wang Y, Ding Y, Luo J, Zhong J, Xu N, Zhang Y, Xie W. Canagliflozin attenuates lipotoxicity in cardiomyocytes and protects diabetic mouse hearts by inhibiting the mTOR/HIF-1α pathway. *iScience.* 2021;24:102521. doi: 10.1016/j.isci.2021.102521
188. Tsai KL, Hsieh PL, Chou WC, Cheng HC, Huang YT, Chan SH. Dapagliflozin attenuates hypoxia/reoxygenation-caused cardiac dysfunction and oxidative damage through modulation of AMPK. *Cell Biosci.* 2021;11:44. doi: 10.1186/s13578-021-00547-y
189. He L, Ma S, Zuo Q, Zhang G, Wang Z, Zhang T, Zhai J, Guo Y. An effective sodium-dependent glucose transporter 2 inhibition, canagliflozin, prevents development of hypertensive heart failure in dahl salt-sensitive rats. *Front Pharmacol.* 2022;13:856386. doi: 10.3389/fphar.2022.856386
190. Moellmann J, Mann PA, Kappel BA, Kahles F, Klinkhammer BM, Boor P, Kramann R, Ghesquiere B, Lebherz C, Marx N, et al. The sodium-glucose co-transporter-2 inhibitor ertugliflozin modifies the signature of cardiac substrate metabolism and reduces cardiac mTOR signalling, endoplasmic reticulum stress and apoptosis [published online July 8, 2022]. *Diabetes Obes Metab.* doi: 10.1111/dom.14814
191. Mancini SJ, Boyd D, Katwan OJ, Strembitska A, Almabrouk TA, Kennedy S, Palmer TM, Salt IP. Canagliflozin inhibits interleukin-1β-stimulated cytokine and chemokine secretion in vascular endothelial cells by AMP-activated protein kinase-dependent and -independent mechanisms. *Sci Rep.* 2018;8:5276. doi: 10.1038/s41598-018-23420-4
192. Zhou H, Wang S, Zhu P, Hu S, Chen Y, Ren J. Empagliflozin rescues diabetic myocardial microvascular injury via AMPK-mediated inhibition of mitochondrial fission. *Redox Biol.* 2018;15:335–346. doi: 10.1016/j.redox.2017.12.019
193. D'Onofrio N, Sardu C, Trotta MC, Scisciola L, Turriziani F, Ferraraccio F, Panarese I, Petrella L, Fanelli M, Modugno P, et al. Sodium-glucose co-transporter2 expression and inflammatory activity in diabetic atherosclerotic plaques: effects of sodium-glucose co-transporter2 inhibitor treatment. *Mol Metab.* 2021;54:101337. doi: 10.1016/j.molmet.2021.101337
194. Tian J, Zhang M, Suo M, Liu D, Wang X, Liu M, Pan J, Jin T, An F. Dapagliflozin alleviates cardiac fibrosis through suppressing EndMT and fibroblast activation via AMPKα/TGF-β/Smad signalling in type 2 diabetic rats. *J Cell Mol Med.* 2021;25:7642–7659. doi: 10.1111/jcmm.1660
195. Uthman L, Kuschma M, Römer G, Boomsma M, Kessler J, Hermanides J, Hollmann MW, Preckel B, Zuurbier CJ, Weber NC. Novel anti-inflammatory effects of canagliflozin involving hexokinase II in lipopolysaccharide-stimulated human coronary artery endothelial cells. *Cardiovasc Drugs Ther.* 2021;35:1083–1094. doi: 10.1007/s10557-020-07083-w
196. Faridvand Y, Kazemzadeh H, Vahedian V, Mirzajanzadeh P, Nejabati HR, Safaie N, Maroufi NF, Pezeshkian M, Nouri M, Jodati A. Dapagliflozin attenuates high glucose-induced endothelial cell apoptosis and inflammation through AMPK/SIRT1 activation. *Clin Exp Pharmacol Physiol.* 2022;49:643–651. doi: 10.1111/1440-1681.13638
197. He L, Li Y, Zhang D, Song H, Xu D, Song Z. Dapagliflozin improves endothelial cell dysfunction by regulating mitochondrial production via the SIRT1/PGC-1α pathway in obese mice. *Biochem Biophys Res Commun.* 2022;615:123–130. doi: 10.1016/j.bbrc.2022.05.022
198. Chang YK, Choi H, Jeong JY, Na KR, Lee KW, Lim BJ, Choi DE. Dapagliflozin, SGLT2 inhibitor, attenuates renal ischemia-reperfusion injury. *PLoS One.* 2016;11:e0158810. doi: 10.1371/journal.pone.0158810
199. Birnbaum Y, Bajaj M, Yang HC, Ye Y. Combined SGLT2 and DPP4 inhibition reduces the activation of the NLRP3/ASC inflammasome and attenuates the development of diabetic nephropathy in mice with type 2 diabetes. *Cardiovasc Drugs Ther.* 2018;32:135–145. doi: 10.1007/s10557-018-6778-x
200. Inoue MK, Matsunaga Y, Nakatsu Y, Yamamotoya T, Ueda K, Kushiyama A, Sakoda H, Fujishiro M, Ono H, Iwashita M, et al. Possible involvement of normalized Pin1 expression level and AMPK activation in the molecular mechanisms underlying renal protective effects of SGLT2 inhibitors in mice. *Diabetol Metab Syndr.* 2019;11:57. doi: 10.1186/s13098-019-0454-6
201. Kim JH, Ko HY, Wang HJ, Lee H, Yun M, Kang ES. Effect of dapagliflozin, a sodium-glucose co-transporter-2 inhibitor, on gluconeogenesis in proximal renal tubules. *Diabetes Obes Metab.* 2020;22:373–382. doi: 10.1111/dom.13905
202. Li J, Liu H, Takagi S, Nitta K, Kitada M, Srivastava SP, Takagaki Y, Kanasaki K, Koya D. Renal protective effects of empagliflozin via inhibition of EMT and aberrant glycolysis in proximal tubules. *JCI Insight.* 2020;5:e129034. doi: 10.1172/jci.insight.129034
203. Liu X, Xu C, Xu L, Li X, Sun H, Xue M, Li T, Yu X, Sun B, Chen L. Empagliflozin improves diabetic renal tubular injury by alleviating mitochondrial fission via AMPK/SP1/PGAM5 pathway. *Metabolism.* 2020;111:154334. doi: 10.1016/j.metabol.2020.154334
204. Mohamed HE, Asker ME, Keshawy MM, Hasan RA, Mahmoud YK. Inhibition of tumor necrosis factor-α enhanced the antifibrotic effect of empagliflozin in an animal model with renal insulin resistance. *Mol Cell Biochem.* 2020;466:45–54. doi: 10.1007/s11010-020-03686-x
205. Zhang Z, Ni L, Zhang L, Zha D, Hu C, Zhang L, Feng H, Wei X, Wu X. Empagliflozin regulates the AdipoR1/p-AMPK/p-ACC pathway to alleviate lipid deposition in diabetic nephropathy. *Diabetes Metab Syndr Obes.* 2021;14:227–240. doi: 10.2147/DMSO.S289712
206. Chi PJ, Lee CJ, Hsieh YJ, Lu CW, Hsu BG. Dapagliflozin ameliorates lipopolysaccharide related acute kidney injury in mice with streptozotocin-induced diabetes mellitus. *Int J Med Sci.* 2022;19:729–739. doi: 10.7150/ijms.69031
207. Zhai R, Liu Y, Tong J, Yu Y, Yang L, Gu Y, Niu J. Empagliflozin ameliorates preeclampsia and reduces postpartum susceptibility to adriamycin in a mouse model induced by angiotensin receptor agonistic autoantibodies. *Front Pharmacol.* 2022;13:826792. doi: 10.3389/fphar.2022.826792
208. Hawley SA, Ford RJ, Smith BK, Gowans GJ, Mancini SJ, Pitt RD, Day EA, Salt IP, Steinberg GR, Hardie DG. The Na+/glucose cotransporter inhibitor canagliflozin activates AMPK by inhibiting mitochondrial function and increasing cellular AMP levels. *Diabetes.* 2016;65:2784–2794. doi: 10.2337/db16-0058
209. Xu L, Nagata N, Nagashimada M, Zhuge F, Ni Y, Chen G, Mayoux E, Kaneko S, Ota T. SGLT2 inhibition by empagliflozin promotes fat utilization and browning and attenuates inflammation and insulin resistance by polarizing M2 macrophages in diet-induced obese mice. *EBioMedicine.* 2017;20:137–149. doi: 10.1016/j.ebiom.2017.05.028
210. Osataphan S, Macchi C, Singhal G, Chimene-Weiss J, Sales V, Kozuka C, Dreyfuss JM, Pan H, Tangcharoenpaisan Y, Morningstar J, et al. SGLT2 inhibition reprograms systemic metabolism via FGF21-dependent and -independent mechanisms. *JCI Insight.* 2019;4:e123130. doi: 10.1172/jci.insight.123130
211. Chiang H, Lee JC, Huang HC, Huang H, Liu HK, Huang C. Delayed intervention with a novel SGLT2 inhibitor NGI001 suppresses diet-induced

metabolic dysfunction and non-alcoholic fatty liver disease in mice. *Br J Pharmacol.* 2020;177:239–253. doi: 10.1111/bph.14859
212. Suga T, Sato K, Ohyama T, Matsui S, Kobayashi T, Tojima H, Horiguchi N, Yamazaki Y, Kakizaki S, Nishikido A, et al. Ipragliflozin-induced improvement of liver steatosis in obese mice may involve sirtuin signaling. *World J Hepatol.* 2020;12:350–362. doi: 10.4254/wjh.v12.i7.350
213. Wei D, Liao L, Wang H, Zhang W, Wang T, Xu Z. Canagliflozin ameliorates obesity by improving mitochondrial function and fatty acid oxidation via PPARα in vivo and in vitro. *Life Sci.* 2020;247:117414. doi: 10.1016/j.lfs.2020.117414
214. Yang X, Liu Q, Li Y, Tang Q, Wu T, Chen L, Pu S, Zhao Y, Zhang G, Huang C, et al. The diabetes medication canagliflozin promotes mitochondrial remodelling of adipocyte via the AMPK-Sirt1-Pgc-1α signalling pathway. *Adipocyte.* 2020;9:484–494. doi: 10.1080/21623945.2020.1807850
215. Chen H, Birnbaum Y, Ye R, Yang HC, Bajaj M, Ye Y. SGLT2 inhibition by dapagliflozin attenuates diabetic ketoacidosis in mice with type-1 diabetes [published online August 27, 2021]. *Cardiovasc Drugs Ther.* doi: 10.1007/s10557-021-07243-6
216. Lee JY, Lee M, Lee JY, Bae J, Shin E, Lee YH, Lee BW, Kang ES, Cha BS. Ipragliflozin, an SGLT2 inhibitor, ameliorates high-fat diet-induced metabolic changes by upregulating energy expenditure through activation of the AMPK/ SIRT1 pathway. *Diabetes Metab J.* 2021;45:921–932. doi: 10.4093/dmj.2020.0187
217. Luo J, Sun P, Wang Y, Chen Y, Niu Y, Ding Y, Xu N, Zhang Y, Xie W. Dapagliflozin attenuates steatosis in livers of high-fat diet-induced mice and oleic acid-treated L02 cells via regulating AMPK/mTOR pathway. *Eur J Pharmacol.* 2021;907:174304. doi: 10.1016/j.ejphar.2021.174304
218. Xu L, Xu C, Liu X, Li X, Li T, Yu X, Xue M, Yang J, Kosmas CE, Moris D, et al. Empagliflozin induces white adipocyte browning and modulates mitochondrial dynamics in KK Cg-Ay/J mice and mouse adipocytes. *Front Physiol.* 2021;12:745058. doi: 10.3389/fphys.2021.745058
219. Yang X, Liu Q, Li Y, Ding Y, Zhao Y, Tang Q, Wu T, Chen L, Pu S, Cheng S, et al. Inhibition of the sodium-glucose co-transporter SGLT2 by canagliflozin ameliorates diet-induced obesity by increasing intra-adipose sympathetic innervation. *Br J Pharmacol.* 2021;178:1756–1771. doi: 10.1111/bph.15381
220. Thirunavukarasu S, Jex N, Chowdhary A, Hassan IU, Straw S, Craven TP, Gorecka M, Broadbent D, Swoboda P, Witte KK, et al. Empagliflozin treatment is associated with improvements in cardiac energetics and function and reductions in myocardial cellular volume in patients with type 2 diabetes. *Diabetes.* 2021;70:2810–2822. doi: 10.2337/db21-0270
221. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature.* 2005;434:113–118. doi: 10.1038/nature03354
222. Wolf P, Fellinger P, Pfleger L, Beiglböck H, Krumpolec P, Barbieri C, Gastaldelli A, Harreiter J, Metz M, Scherer T, et al. Gluconeogenesis, but not glycogenolysis, contributes to the increase in endogenous glucose production by SGLT-2 inhibition. *Diabetes Care.* 2021;44:541–548. doi: 10.2337/dc20-1983
223. Hirschey MD, Shimazu T, Capra JA, Pollard KS, Verdin E. SIRT1 and SIRT3 deacetylate homologous substrates: AceCS1,2 and HMGCS1,2. *Aging (Albany NY).* 2011;3:635–642. doi: 10.18632/aging.100339
224. Chen R, Xu M, Hogg RT, Li J, Little B, Gerard RD, Garcia JA. The acetylase/deacetylase couple CREB-binding protein/Sirtuin 1 controls hypoxia-inducible factor 2 signaling. *J Biol Chem.* 2012;287:30800–30811. doi: 10.1074/jbc.M111.244780
225. Wang J, Zhu XX, Liu L, Xue Y, Yang X, Zou HJ. SIRT1 prevents hyperuricemia via the PGC-1α/PPARγ-ABCG2 pathway. *Endocrine.* 2016;53:443–452. doi: 10.1007/s12020-016-0896-7
226. Huang DY, Gao H, Boini KM, Osswald H, Nürnberg B, Lang F. In vivo stimulation of AMP-activated protein kinase enhanced tubuloglomerular feedback but reduced tubular sodium transport during high dietary NaCl intake. *Pflugers Arch.* 2010;460:187–196. doi: 10.1007/s00424-010-0803-7
227. Hasegawa K. Novel tubular-glomerular interplay in diabetic kidney disease mediated by sirtuin 1, nicotinamide mononucleotide, and nicotinamide adenine dinucleotide: Oshima Award Address 2017. *Clin Exp Nephrol.* 2019;23:987–994. doi: 10.1007/s10157-019-01719-4
228. Zannad F, Ferreira JP, Butler J, Filippatos G, Januzzi JL, Sumin M, Zwick M, Saadati M, Pocock SJ, Sattar N, et al. Effect of empagliflozin on circulating proteomics in heart failure: mechanistic insights from the EMPEROR program [published online August 26, 2022]. *Eur Heart J.* doi: 10.1093/eurheartj/ehac495
229. Gan L, Han Y, Bastianetto S, Dumont Y, Unterman TG, Quirion R. FoxO-dependent and -independent mechanisms mediate SirT1 effects on IGFBP-1 gene expression. *Biochem Biophys Res Commun.* 2005;337:1092–1096. doi: 10.1016/j.bbrc.2005.09.169
230. Crane FL, Navas P, Low H, Sun IL, de Cabo R. Sirtuin activation: a role for plasma membrane in the cell growth puzzle. *J Gerontol A Biol Sci Med Sci.* 2013;68:368–370. doi: 10.1093/gerona/gls184
231. Josephrajan A, Hertzel AV, Bohm EK, McBurney MW, Imai SI, Mashek DG, Kim DH, Bernlohr DA. Unconventional secretion of adipocyte fatty acid binding protein 4 is mediated by autophagic proteins in a sirtuin-1-dependent manner. *Diabetes.* 2019;68:1767–1777. doi: 10.2337/db18-1367
232. Ferrannini E, Murthy AC, Lee YH, Muscelli E, Weiss S, Ostroff RM, Sattar N, Williams SA, Ganz P. Mechanisms of sodium-glucose cotransporter 2 inhibition: insights from large-scale proteomics. *Diabetes Care.* 2020;43:2183–2189. doi: 10.2337/dc20-0456
233. Thiele K, Rau M, Hartmann NK, Möllmann J, Jankowski J, Böhm M, Keszei AP, Marx N, Lehrke M. Effects of empagliflozin on erythropoiesis in patients with type 2 diabetes: data from a randomized, placebo-controlled study. *Diabetes Obes Metab.* 2021;23:2814–2818. doi: 10.1111/dom.14517
234. Bae HR, Kim DH, Park MH, Lee B, Kim MJ, Lee EK, Chung KW, Kim SM, Im DS, Chung HY. β-Hydroxybutyrate suppresses inflammasome formation by ameliorating endoplasmic reticulum stress via AMPK activation. *Oncotarget.* 2016;7:66444–66454. doi: 10.18632/oncotarget.12119
235. Jung J, Park WY, Kim YJ, Kim M, Choe M, Jin K, Seo JH, Ha E. 3-hydroxybutyrate ameliorates the progression of diabetic nephropathy. *Antioxidants (Basel).* 2022;11:381. doi: 10.3390/antiox11020381
236. Yu Y, Yu Y, Zhang Y, Zhang Z, An W, Zhao X. Treatment with D-β-hydroxybutyrate protects heart from ischemia/reperfusion injury in mice. *Eur J Pharmacol.* 2018;829:121–128. doi: 10.1016/j.ejphar.2018.04.019
237. Kim DH, Park MH, Ha S, Bang EJ, Lee Y, Lee AK, Lee J, Yu BP, Chung HY. Anti-inflammatory action of β-hydroxybutyrate via modulation of PGC-1α and FoxO1, mimicking calorie restriction. *Aging (Albany NY).* 2019;11:1283–1304. doi: 10.18632/aging.101838
238. Nakamura M, Odanovic N, Nakada Y, Dohi S, Zhai P, Ivessa A, Yang Z, Abdellatif M, Sadoshima J. Dietary carbohydrates restriction inhibits the development of cardiac hypertrophy and heart failure. *Cardiovasc Res.* 2021;117:2365–2376. doi: 10.1093/cvr/cvaa298
239. Mikami D, Kobayashi M, Uwada J, Yazawa T, Kamiyama K, Nishimori K, Nishikawa Y, Morikawa Y, Yokoi S, Takahashi N, et al. β-Hydroxybutyrate, a ketone body, reduces the cytotoxic effect of cisplatin via activation of HDAC5 in human renal cortical epithelial cells. *Life Sci.* 2019;222:125–132. doi: 10.1016/j.lfs.2019.03.008
240. Byrne NJ, Soni S, Takahara S, Ferdaoussi M, Al Batran R, Darwesh AM, Levasseur JL, Beker D, Vos DY, Schmidt MA, et al. Chronically elevating circulating ketones can reduce cardiac inflammation and blunt the development of heart failure. *Circ Heart Fail.* 2020;13:e006573. doi: 10.1161/CIRCHEARTFAILURE.119.006573
241. Sokolov V, Yakovleva T, Chu L, Tang W, Greasley PJ, Johansson S, Peskov K, Helmlinger G, Boulton DW, Penland RC. Differentiating the sodium-glucose cotransporter 1 inhibition capacity of canagliflozin vs. dapagliflozin and empagliflozin using quantitative systems pharmacology modeling. *CPT Pharmacometrics Syst Pharmacol.* 2020;9:222–229. doi: 10.1002/psp4.12498
242. Liang Y, Arakawa K, Ueta K, Matsushita Y, Kuriyama C, Martin T, Du F, Liu Y, Xu J, Conway B, et al. Effect of canagliflozin on renal threshold for glucose, glycemia, and body weight in normal and diabetic animal models. *PLoS One.* 2012;7:e30555. doi: 10.1371/journal.pone.0030555
243. Mustroph J, Wagemann O, Lücht CM, Trum M, Hammer KP, Sag CM, Lebek S, Tarnowski D, Reinders J, Perbellini F, et al. Empagliflozin reduces Ca/calmodulin-dependent kinase II activity in isolated ventricular cardiomyocytes. *ESC Heart Fail.* 2018;5:642–648. doi: 10.1002/ehf2.12336
244. Hu Y-X, Han X-S, Jing Q. Ca(2+) ion and autophagy. *Adv Exp Med Biol.* 2019;1206:151–166. doi: 10.1007/978-981-15-0602-4_7
245. Ying Y, Jiang C, Zhang M, Jin J, Ge S, Wang X. Phloretin protects against cardiac damage and remodeling via restoring SIRT1 and anti-inflammatory effects in the streptozotocin-induced diabetic mouse model. *Aging (Albany NY).* 2019;11:2822–2835. doi: 10.18632/aging.101954