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REVIEW

Energetics and Metabolism

Treatments for skeletal muscle abnormalities in heart failure: sodium-glucose transporter 2 and ketone bodies

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Abstract

Various skeletal muscle abnormalities are known to occur in heart failure (HF) and are closely associated with exercise intolerance. Particularly, abnormal energy metabolism caused by mitochondrial dysfunction in skeletal muscle is a cause of decreased endurance exercise capacity. However, to date, no specific drug treatment has been established for the skeletal muscle abnormalities and exercise intolerance occurring in patients with HF. Sodium-glucose transporter 2 (SGLT2) inhibitors promote glucose excretion by suppressing glucose reabsorption in the renal tubules, which has a hypoglycemic effect independent of insulin secretion. Recently, large clinical trials have demonstrated that treatment with SGLT2 inhibitors suppresses cardiovascular events in patients who have HF with systolic dysfunction. Mechanisms of the therapeutic effects of SGLT2 inhibitors for HF have been suggested to be diuretic, suppression of neurohumoral factor activation, renal protection, and improvement of myocardial metabolism, but this has not been clarified to date. SGLT2 inhibitors are known to increase blood ketone bodies. This suggests that they may improve the abnormal skeletal muscle metabolism in HF, that is, improve fatty acid metabolism, suppress glycolysis, and use ketone bodies in mitochondrial energy production. Ultimately, they may improve aerobic metabolism in skeletal muscle, suppress anaerobic metabolism, and improve aerobic exercise capacity at the level of the anaerobic threshold. The potential actions of such SGLT2 inhibitors explain their effectiveness in HF and may be candidates for new drug treatments aimed at improving exercise intolerance. In this review, we outlined the effects of SGLT2 inhibitors on skeletal muscle metabolism, with a particular focus on ketone metabolism.

cardiac disease; exercise intolerance; mitochondrial dysfunction; muscle atrophy; sodium-glucose transporter 2 inhibitor

INTRODUCTION

Patients with heart failure (HF) demonstrate various skeletal muscle abnormalities, which are the main cause of the decrease in their activities of daily living and quality of life and are closely associated with exercise intolerance and a poor disease prognosis (1–3). Skeletal muscle abnormalities in patients with HF are characterized by impairments in energy metabolism, the transition from oxidative (type I) fibers to glycolytic (type II) fibers, mitochondrial dysfunction, atrophy, weakness, and an increase in muscle cell apoptosis (Fig. 1) (2). These skeletal muscle abnormalities are roughly divided into qualitative and quantitative abnormalities (1, 3). Qualitative abnormalities of skeletal muscle mainly affect aerobic capacity and involve abnormal energy production in mitochondria owing to a metabolic shift from oxidative to glycolytic. On the other hand, quantitative abnormalities mainly affect muscle strength and are caused by an imbalance between protein synthesis and protein degradation. It has been suggested that peripheral hypoperfusion, chronic inflammation, enhanced oxidative stress, neurohormonal activation, and physical inactivity are associated with the development of skeletal muscle abnormalities, although their precise molecular mechanisms remain unclear (2, 4, 5). At present, there are no effective treatment strategies for skeletal muscle abnormalities other than exercise training (2–5). It has recently been reported that the sodium-glucose transporter 2 (SGLT2) inhibitors dapagliflozin and empagliflozin lower the risk of cardiovascular death and the worsening of HF in patients with HF who have a reduced ejection fraction (HFrEF), regardless of the presence or absence of diabetes mellitus (6, 7). The mechanism of action of SGLT2 inhibitors in the treatment of HF is unknown at present, but it has been suggested that they may alter metabolic status, including ketone metabolism (8). In this review, we hence focus on ketone metabolism in the skeletal muscle as

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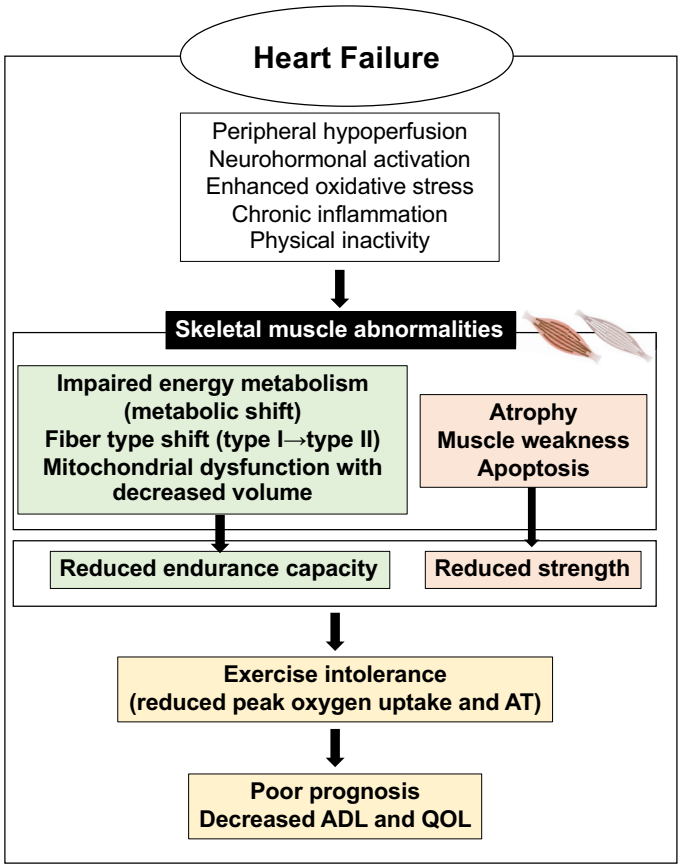

Figure 1. Skeletal muscle abnormalities in heart failure (HF). In patients with HF, various skeletal muscle abnormalities, including qualitative and quantitative abnormalities, are known to occur, which disrupt muscle endurance capacity and strength, resulting in exercise intolerance. Peripheral hypoperfusion, neurohormonal activation, enhanced oxidative stress, chronic inflammation, and physical inactivity may be associated with these skeletal muscle abnormalities; however, the precise mechanisms involved remain unclear. ADL, activities of daily living; AT, anaerobic threshold; QOL, quality of life

a new treatment target for skeletal muscle abnormalities in HF. We propose that improving skeletal muscle endurance by altering skeletal muscle energy metabolism using SGLT2 inhibitors will improve exercise intolerance in patients with HF and that such effects of SGLT2 inhibitors will improve the cardiovascular outcomes of HF, independently of cardiac dysfunction.

SKELETAL MUSCLE ABNORMALITIES IN PATIENTS WITH HF

Reduced Endurance Capacity

Clinically, peak oxygen uptake and anaerobic threshold (AT) evaluated by the cardiopulmonary exercise test are established indicators of the severity of HF and are independent determinants of disease prognosis (9–11). In fact, it has been reported that the lower the peak oxygen uptake, the higher the mortality rate of patients with HF (9). Oxygen uptake is regulated by oxygen supply (pulmonary function, cardiac function, vascular function, etc.) and oxygen demand (skeletal muscle function), but lowering peak oxygen uptake in patients with HF is associated with skeletal muscle abnormalities, particularly qualitative abnormalities of the skeletal muscle.

The energy used for skeletal muscle contraction is produced from adenosine triphosphate (ATP), but the amount of ATP stored in skeletal muscle cells is very small, and energy via ATP-phosphocreatine system is also depleted by an extremely small amount of exercise (11). Therefore, to perform endurance exercise, ATP needs to be resynthesized, and hence the production of ATP by oxidative phosphorylation in the mitochondrial electron transport chain is important. This aerobic metabolism required to perform mild-to-moderate exercise uses the oxidation of glucose and fatty acids as energy substrates. When exercise intensity becomes high and oxygen supply becomes relatively insufficient, ATP production via anaerobic metabolism by the glycolysis-lactic acid system becomes necessary.

A number of studies showed that skeletal muscle metabolism is impaired during exercise in patients with HF, using phosphorus-31 magnetic resonance spectroscopy (12–17). Okita et al. (16) reported that phosphocreatine is depleted in both patients with HF and healthy subjects at the end of systemic exhaustive exercise; thus, exercise limitation is consistent with metabolic limitation in the skeletal muscle. Furthermore, the phosphocreatine depletion rate was faster, and intramuscular pH was more severely lowered in patients with HF than in healthy subjects. We also demonstrated that the rate of phosphocreatine loss during planter flexion exercise with a constant load is increased in patients with HFrEF compared with healthy subjects (12). In a recent study, patients with HF with a preserved ejection fraction were also shown to have severely impaired skeletal muscle metabolism (17). Such an impairment in skeletal muscle metabolism is caused by the abnormal function of the electron transport chain and decreased mitochondrial volume in the skeletal muscle, leading to decreased muscle endurance and aerobic exercise capacity (18–23). In addition, fatty acid oxidation is impaired and glucose oxidation and glycolysis are enhanced in the skeletal muscle of patients with HF (18, 19, 22, 24), and these effects are closely associated with aerobic exercise capacity, particularly a decrease in the AT (Fig. 2, *A* and *B*). Generally, the oxygen efficiency of glucose oxidation is higher than that of fatty acid oxidation (25). Therefore, these effects are thought to compensate for the poor oxygen supply to skeletal muscle in HF conditions, by energy metabolism that depends on the oxidation of energy-efficient glucose. Indeed, β-oxidation enzymes were reduced, and glycolytic enzymes were maintained or enhanced in the skeletal muscle of patients with HF (13, 26). In addition, in our study and study of other laboratory, intramuscular lipid accumulation was increased in the skeletal muscle of patients with HF (12, 17). This result is also consistent with the impairment in skeletal muscle fatty acid oxidation in patients with HF. Furthermore, consistent with this metabolic shift, the skeletal muscle fiber type demonstrates a decrease in oxidative slow muscles and a relative increase in anaerobic fast muscles.

Skeletal Muscle Atrophy and Weakness

Sarcopenia is defined as skeletal muscle atrophy and muscle weakness or as a decline in physical function with aging

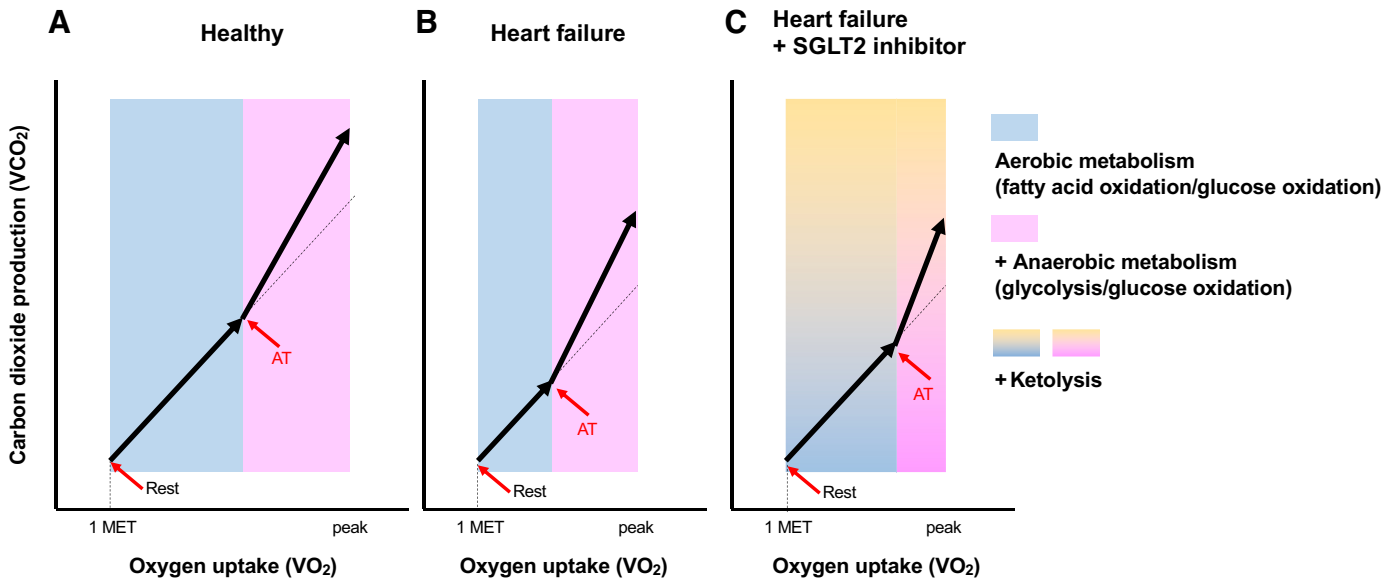

Figure 2. Exercise intolerance in patients with HF and effects of SGLT2 inhibitors (a hypothesis). A schematic diagram of the association between oxygen consumption and carbon dioxide production analyzed by the cardiopulmonary exercise test. This association increases linearly during mild-to-moderate exercise, and the slope of the line becomes steep beyond the AT. In skeletal muscle, aerobic metabolism of fatty acids/glucose oxidation is used below the AT (blue area) and anaerobic metabolism by glycolysis occurs beyond the AT (pink area). Peak oxygen uptake and the AT are decreased in patients with HF compared with healthy subjects (*A* and *B*). In our hypothesis, treatment with SGLT2 inhibitors enhances fatty acid oxidation, limits glycolysis, and promotes ketogenesis (yellow), which improves AT levels, but not peak oxygen uptake (*C*). AT, anaerobic threshold; HF, heart failure; MET, metabolic equivalent; SGLT2, sodium-glucose transporter 2.

(27). Sarcopenia can also occur in patients with HF and is called secondary sarcopenia. The frequency of sarcopenia in patients with HF was reported to be 19.5% in a study of 200 patients with HF (mean age, 69 ± 10 yr, New York Heart Association II–III) (28), and patients with HF and sarcopenia were found to have a higher risk of worsening of their HF than those without sarcopenia (29). In addition, muscle weakness is known to be closely associated with a poor prognosis in patients with HF (30). Furthermore, skeletal muscle atrophy and weakness are strongly associated with reduced exercise capacity and activities of daily living (1, 3, 31). Muscle volume is important for skeletal muscle to produce force. Skeletal muscle atrophy is mainly caused by a decrease in muscle cell size. In general, muscle strength is closely associated with muscle cross-sectional area, and muscle strength per unit area is almost constant among individuals, at 2–3 kg/cm^2 (32).

SKELETAL MUSCLE ABNORMALITIES IN ANIMAL MODELS OF HF

Generally, animal models of HF, created by procedures such as left ventricular (LV) pressure overload by transverse aortic constriction (TAC), ischemic injury by coronary artery ligation or intracoronary microembolization, volume overload by an aortocaval shunt, pacing-induced tachycardia, and drug-induced cardiomyopathy, including by doxorubicin and monocrotaline, have been used to investigate the molecular mechanisms for HF (33–35). In these animal models of HF, skeletal muscle abnormalities, particularly mitochondrial dysfunction, fiber type transition, and metabolic dysfunction in the skeletal muscle have been observed (21, 23, 36–48). However, the detailed molecular mechanism of endurance-associated skeletal muscle abnormalities in HF has not been elucidated. A previous study in postinfarct HF rats reported a close association between peroxisome proliferator-activated receptor- coactivator-1α (PGC-1α) gene expression and mitochondrial dysfunction in the skeletal muscle (48). Furthermore, their mitochondrial dysfunction was ameliorated by the administration of angiotensin-converting enzyme inhibitors (48). We previously demonstrated that mitochondrial dysfunction and fiber type transition occur in the skeletal muscle of postinfarct HF mice, which was owing to enhanced oxidative stress via the activation of xanthine oxidase (31). We also showed that fatty acid oxidation in the skeletal muscle of postinfarct HF mice is impaired (26, 49, 50) and that treatment with recombinant brain-derived neurotrophic factor, a myokine, improves these skeletal muscle abnormalities via the activation of adenosine monophosphate-activated protein kinase-α (AMPKα)-PGC-1α signaling and endurance exercise capacity (49, 51). On the other hand, pharmacological activation of peroxisome proliferator-activated receptor-δ (PPARδ) signaling has been reported to improve oxidative metabolism in the skeletal muscle and endurance exercise capacity in postinfarct HF mice (47). Thus, it is considered that impaired skeletal muscle metabolism, particularly impaired fatty acid oxidation, plays an important role in the exacerbation of skeletal muscle abnormalities and the reduction of endurance exercise capacity in HF.

The molecular mechanism of skeletal muscle atrophy in HF has been reviewed in detail in other articles (1, 5, 31). Skeletal muscle atrophy has been observed in animal models of HF after myocardial infarction (MI) (31, 52, 53). Skeletal muscle atrophy was found to be caused by enhanced protein degradation from activation of the ubiquitin-proteasome

system, which was associated with increased expression of the E3 ubiquitin ligases atrogin-1 and MuRF1. Ubiquitin-proteasome activation on enhanced oxidative stress in the skeletal muscle occurred through nuclear factor (NF)-κB and p38 MAPK activation (52). It has been reported that the insulin-like growth factor-1 (IGF-1)/phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway can prevent the expression of muscle atrophy-induced ubiquitin ligases by inhibiting forkhead box O (FOXO) transcription factors (53). We found that Akt phosphorylation is impaired in the skeletal muscle of postinfarct HF mice (54–57). This finding may suggest that impaired Akt phosphorylation is associated with an increase in ubiquitin ligases by the activation of FOXO transcription factors. On the other hand, the IGF-1-dependent Akt/mammalian target of rapamycin (mTOR) pathway is the main pathway of protein synthesis in the skeletal muscle. We found impairments in Akt phosphorylation and mTOR phosphorylation in the skeletal muscle of postinfarct HF mice, which was associated with enhanced oxidative stress (31). Another important IGF-1-independent protein synthesis pathway is the myostatin-mediated pathway (58). It has been reported that HF-induced skeletal muscle atrophy is inhibited in cardiac-specific myostatin knockout mice, suggesting that cardiac-derived myostatin is involved in the skeletal muscle atrophy that occurs in patients with HF (59).

EFFICACY OF SGLT2 INHIBITORS FOR HF

SGLT2 is a transporter that is expressed in the proximal tubules of the kidneys and reabsorbs ~90% of glomerular-filtered glucose. SGLT2 inhibitors promote urinary glucose excretion, resulting in hypoglycemic and weight-loss effects that are independent of insulin actions (similar to carbohydrate restriction). The effects of the SGLT2 inhibitor empagliflozin on cardiovascular outcomes were investigated in patients with type 2 diabetes with a high cardiovascular risk in the EMPA-REG OUTCOME trial (60). The results of the trial showed that empagliflozin treatment leads to a significantly lower risk of death from cardiovascular causes and hospitalization owing to HF. Similar results were also obtained in trials of other SGLT2 inhibitors (dapagliflozin and canagliflozin), suggesting that the cardiovascular event-suppressing effects observed in patients with diabetes may be a class effect of SGLT2 inhibitors (6, 61). Based on such information, the efficacy of SGLT2 inhibitors was investigated in patients with HFrEF, in the Dapagliflozin and Prevention of Adverse Outcomes in Heart Failure (DAPA-HF) and Empagliflozin Outcome Trial in Patients with Chronic Heart Failure with Reduced Ejection Fraction (EMPEROR-Reduced) trials. SGLT2 inhibitors significantly reduced the frequency of hospitalization owing to HF and cardiovascular death, which were the primary end points, in patients with HFrEF, regardless of the presence or absence of diabetes (6, 7).

Large-scale clinical trials have shown the primary and secondary preventive effects of SGLT2 inhibitors on HF, but the mechanism of their effects remains unclear. A recent excellent review paper by Lopaschuk and Verma (62) discussed the potential mechanisms of the cardioprotective effects of SGLT2 inhibitors. The beneficial effects of SGLT2 inhibitors include hemodynamics effects, such as the lowering of blood pressure and diuretic effects; neurohormonal effects, such as the inhibition of sympathetic nervous activity; inflammation; oxidative stress; systemic and cardiac metabolic changes; and renal protection with increasing erythropoiesis. These effects can ultimately prevent adverse cardiac remodeling.

Because SGLT2 inhibitors have a blood pressure lowering effect (63), probably owing to their diuretic effect, some of the beneficial effects of SGLT2 inhibitors in patients with HF may be associated with a reduction of cardiac afterload. However, in the DAPA-HF trial, the reduction in blood pressure was quite modest (6). Therefore, it is difficult to explain the beneficial effects of SGLT2 inhibitors solely by their blood pressure lowering effect. Treatment of patients with diabetes and HF with empagliflozin for 14 days increased diuretic effects and resulted in decreased body weight and fluid volume (64). However, such effects have also been observed with other diuretics, particularly with the vasopressin V_2 receptor antagonist tolvaptan, although no cardioprotective effects have been demonstrated (65). A subanalysis of the EMPEROR-Reduced trial also demonstrated the beneficial effects of empagliflozin in patients with HFrEF, regardless of volume overload (66), suggesting that actions other than the diuretic effects of SGLT2 inhibitors are the predominant mediators of the reduced hospitalization of patients with HF.

In a large clinical trial, that is, the Dapagliflozin and Prevention of Adverse Outcomes in Chronic Kidney Disease (DAPA-CKD) trial, which analyzed patients with chronic kidney disease, dapagliflozin was found to suppress the deterioration of renal function and death (67). Thus, the protective effects of SGLT2 inhibitors on renal function are being recognized. On the other hand, the renal composite end point, which was a secondary end point of the DAPA-HF study, was not different between the dapagliflozin group and the placebo group, and the actual positive effects on renal function were unclear (6). Therefore, the protective effects of SGLT2 inhibitors on cardiorenal syndrome in patients with HF have not been demonstrated to date. Furthermore, in the DAPA-HF study, SGLT2 inhibitor treatment increased the hematocrit of patients with HF by 2.31 ± 3.90%, which is significantly different from the 0.19 ± 3.81% decrease by placebo treatment (6). This effect is thought to be owing to the increased secretion of erythropoietin in the kidney. It is not clear whether a 2.31% increase in the hematocrit of patients with HF has any clinical efficacy. Although the presence of anemia in patients with HF is associated with an unfavorable prognosis, there is no evidence that the amelioration of anemia improves the prognosis of HF. Moreover, the therapeutic effect of erythropoietin on HF has not been clarified. Therefore, the clinical significance of an increased hematocrit by SGLT2 inhibitors in patients with HF is a topic for future research.

A large amount research has been performed to clarify whether SGLT2 inhibitors exert their cardioprotective effects by suppressing cardiac remodeling. To date, an animal study has shown that SGLT2 inhibitors suppress the progression of cardiac remodeling, and their effects are mediated by a reduction of chronic inflammation and oxidative stress (68). SGLT2 inhibitors have also been shown to suppress

sympathetic nervous activity, mainly in studies using diabetes model animals (69, 70). These neurohormonal suppressive effects may be involved in suppressing the onset of HF events and improving cardiac remodeling. On the other hand, in the DAPH-HF trial, most of the subjects were treated with β-blockers and renin-angiotensin system inhibitors (6). It is unclear whether SGLT2 inhibitors have a further inhibitory effect on neurohormonal factors, including sympathetic nervous activity, under the administration of sufficient β-blockers and renin-angiotensin system inhibitors.

ASSOCIATION BETWEEN METABOLIC CHANGES IN THE FAILING HEART AND SGLT2 INHIBITORS

SGLT2 inhibitors can affect cardiac metabolism and may be associated with the suppression of cardiac remodeling. With the progression of HF, there is a change in the ability to use metabolic substrates in the failing heart. The phenomenon in which the origin of myocardial ATP production in the failing heart shifts from mitochondrial oxidative phosphorylation owing to the oxidation of glucose and fatty acids to glycolysis is called a metabolic shift (71–74). Energy production of the failing heart as a whole is reduced, resulting in a fuel-starved heart (75, 76). In addition, the uncoupling between glucose oxidation and glycolysis causes an increase in intracellular protons in cardiomyocytes, leading to cardiomyocyte injury. Recent reports have shown that in the failing heart, ketone metabolism is more than doubled and antagonizes glucose metabolism (77, 78). From the viewpoint of the (phosphate to oxygen) P:O ratio, ketone metabolism is not considered to be an efficient energy substrate for the heart compared with glucose (79) but as a thrifty substrate, which is an additional energy substrate for the fuel-starved heart (80, 81). Ketone metabolism may be involved in the cardiac protection that alleviates myocardial injury owing to the uncoupling of glucose metabolism (82).

SGLT2 inhibitors are known to promote glucose excretion, in individuals with or without diabetes, resulting in an increase in free fatty acids owing to lipolysis and an increase in ketone bodies. The heart, which originally has high metabolic flexibility, is thought to be able to select an advantageous metabolic substrate against such changes in the metabolic environment caused by SGLT2 inhibitors. SGLT2 inhibitors compensate for the myocardial energy deficit by increasing ketone body metabolism, maintaining some level of energy efficiency, and minimizing the effects of uncoupling on glucose metabolism (83). Metabolic regulation in the failing heart is considered to be an important factor in the cardioprotective action of SGLT2 inhibitors. In fact, in a diabetic cardiomyopathy model, increased myocardial ketone oxidation by empagliflozin provided an additional energy substrate in the heart and improved cardiac performance (83). It has also been reported that SGLT2 inhibitors modify the metabolic changes occurring in the failing heart after MI in pigs (84); that is, fatty acid metabolism is suppressed, and glucose metabolism is enhanced as a compensatory measure in the failing heart, but SGLT2 inhibitors suppress glucose metabolism and partially restore fatty acid metabolism. In addition, SGLT2 inhibitors maintain myocardial metabolism by increasing hepatic ketone production and enhancing ketone metabolism in the failing heart. It has been suggested that the modification of myocardial metabolism by SGLT2 inhibitors plays an important role in suppressing the progression of cardiac remodeling. However, recently reported results of the therapeutic effects of SGLT2 inhibitors in patients with HF did not show an improvement in left ventricular ejection fraction (LVEF) (85). Therefore, it remains unclear at this time whether SGLT2 inhibitors have a direct effect on the myocardium.

EFFECTS OF SGLT2 INHIBITORS ON SKELETAL MUSCLE METABOLISM

We investigated the effects of the SGLT2 inhibitor empagliflozin on the skeletal muscle of a mouse HF model with post-MI (86). Two weeks after MI surgery, the MI mice were divided into two groups according to treatment with or without empagliflozin (300 mg/kg body wt/day orally) for 4 wk. In this protocol, the administration of empagliflozin did not improve LV diameter or LV contractility. In the HF mouse model that was used, urinary glucose excretion, abdominal fat, blood free fatty acid, and β-hydroxybutyrate (β-OHB) levels were not different compared with sham mice. The level of circulating ketone bodies has been reported to be increased in animal models of decompensated HF with pressure overload via TAC, combined with a small apical MI or pacing-induced HF (77, 87). In contrast, it has been reported that other animal models of compensated HF with pressure overload via TAC do not show any differences compared with controls (77, 88, 89). The discrepancies among these reports are believed to be owing to differences in the models and the severity of HF (90). On the other hand, empagliflozin markedly increased urinary glucose excretion, decreased abdominal fat, and increased blood free fatty acid and β-OHB levels in MI mice. We measured mitochondrial respiration in permeabilized skeletal muscle fibers using a high-resolution respirometer (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria). It has been shown that mitochondrial respiration, particularly mitochondrial respiration with fatty acid substrate, is improved in the skeletal muscle extracted from MI mice treated with empaglifozin (Fig. 3*A*). Skeletal muscle endurance increased in association with increased mitochondrial respiration with fatty acid substrate (Fig. 3*B*). On the other hand, mitochondrial respiration with pyruvic acid substrate and without fatty acid substrate did not increase, and exercise capacity to exhaustion evaluated by the treadmill test did not increase (Fig. 3, *A* and *B*). Substrate-induced differences in mitochondrial respiratory capacity caused by empagliflozin may be explained by changes in pyruvate dehydrogenase (PDH) activity. In the skeletal muscle of mice with HF, there is a shift from fatty acid-dependent metabolism to glucose- and glycolytic-dependent metabolism, but SGLT2 inhibitors increase fatty acid oxidation by limiting the use of glucose (91). On the other hand, SGLT2 inhibitors did not alter the expression of proteins associated with fatty acid uptake and fatty acid oxidation in the skeletal muscle, and hence the mechanism by which fatty acid oxidation is increased remains unclear. Metabolic changes in the skeletal muscle of HF mice are also considered to be a compensatory mecha-

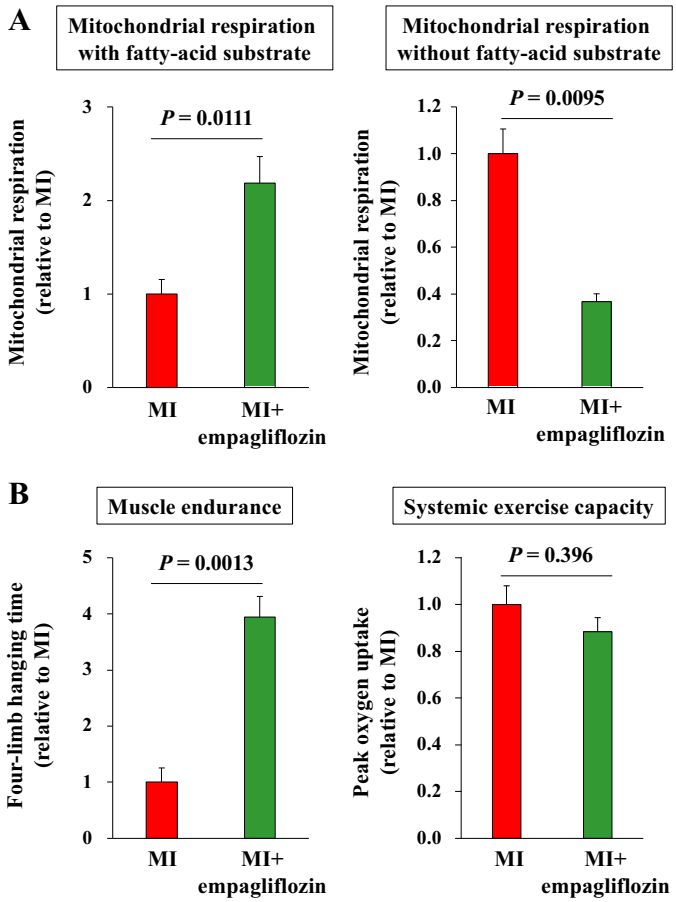

Figure 3. Effects of empagliflozin on endurance capacity and mitochondrial respiration in the skeletal muscle. *A*: mitochondrial respiration in the skeletal muscle with fatty-acid substrate (*left*), and without fatty-acid substrate (*right*) evaluated by a high-resolution respirometer (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria) in MI mice treated with and without empagliflozin. *B*: muscle endurance evaluated by four-limb hanging time (*left*) and systemic exercise capacity evaluated by peak oxygen uptake during treadmill exercise (*right*) in MI mice treated with and without empagliflozin. All values were means ± SD and expressed as relative to MI. Statistical analyses were performed using Student's *t* test for comparisons between MI and MI + empagliflozin, and a P value < 0.05 was considered significant. MI, myocardial infarction.

nism in terms of more energy-efficient substrate utilization, and the restriction of glucose utilization by SGLT2 inhibitors may limit energy efficiency and energy production capacity in the skeletal muscle. To compensate for this, increased ketone bodies in the blood may be used for skeletal muscle energy metabolism. We did not analyze ketone body utilization or ketone oxidation in the skeletal muscle of HF model mice (86). On the other hand, Janardhan et al. (92) reported that the use of ketone bodies in the skeletal muscle was impaired in patients with HF. They also considered that this finding was a result of skeletal muscle abnormalities and is a factor in the worsening of skeletal muscle abnormalities. This report supports our hypothesis that increased levels of blood ketone bodies by SGLT2 inhibitors maintain skeletal muscle energy metabolism by increasing skeletal muscle ketone body utilization. Further detailed analyses to clarify these mechanisms are required in the future. There are still some important unresolved points regarding metabolism in HF. Both cardiac dysfunction and skeletal muscle abnormalities are associated with exercise intolerance in patients with HF, and both cardiac dysfunction and skeletal muscle abnormalities may affect each other. This is a fundamental issue in the pathogenesis of HF. The effects of SGLT2 inhibitors can also affect the metabolism of both the heart and skeletal muscle, and it is difficult to clearly distinguish between the two in the results of previous studies.

KETONE BODIES AND SKELETAL MUSCLE ENERGY METABOLISM

Ketone bodies are generally produced in the liver and enter the circulation to be mainly used by extrahepatic tissues as a source of acetyl-coenzyme A (CoA) and thus have the potential to maintain ATP production by mitochondrial respiration (Fig. 4) (93). Lipid-derived ketone bodies [acetoacetate (AcAc), β-OHB, and acetone] are increased by ketogenesis during fasting, starvation, intake of a ketogenic diet (i.e., carbohydrate-restricted diet), and by SGLT2 inhibitors (31, 94, 95). β-OHB and AcAc have been recognized as energy substrates for skeletal muscle, as are fatty acids and glucose (96). The P:O ratio for ATP production is 2.50 for ketone bodies compared with 2.33 for palmitate and 2.58 for glucose, and thus the efficiency of ATP production per oxygen atom in cardiac muscle for ketone bodies is higher than that for fatty acids but lower than that for glucose (25). Interestingly, ketone-derived mitochondrial energy production is minimal when other substrates, such as glucose and fatty acids, are readily available in skeletal muscle cells in vitro (97). Therefore, ketone bodies are thought to be important energy substrates in mitochondria in situations in which other energy substrates are not available.

An increase in ketone bodies can alter fuel selection for mitochondrial energy production in the skeletal muscle during exercise. Ketones suppress fatty acid synthesis, preferentially use intramuscular triglyceride, and enhance fatty acid oxidation in the skeletal muscle of humans (94, 96, 98, 99). Administration of a β-OHB mineral salt for 2 wk increased endurance performance in mice (100), and a single administration of a nutritional β-OHB ketone salt was shown to increase fat oxidation during steady-state exercise of mild-to-moderate intensity (30%–60% ventilator threshold) in healthy men (99). On the other hand, the administration of ketones was shown to suppress glycolysis (94, 96–98, 100) and to impair the ability to perform high-intensity exercise in healthy men (99). β-OHB can be oxidized to AcAc, which increases the amounts of acetyl-CoA and citrate and inhibits the glycolysis pathway (pyruvate oxidation) by inhibiting PDH, phosphofructokinase, and lactate by increasing the $NADH:NAD^+$ ratio, the acetyl-CoA:CoA ratio, and citrate level (94, 96–98, 100, 101). Ketones as an alternative fuel for glucose may not have any advantages for performing high-intensity exercise, which relies almost solely on anaerobic glycolysis, or an extremely high glycolytic flux for ATP production (98).

Most studies on the effects of ketone bodies on skeletal muscle metabolism have been conducted in healthy subjects and athletes, and it remains unclear whether skeletal muscle has similar effects in patients with chronic diseases, such as

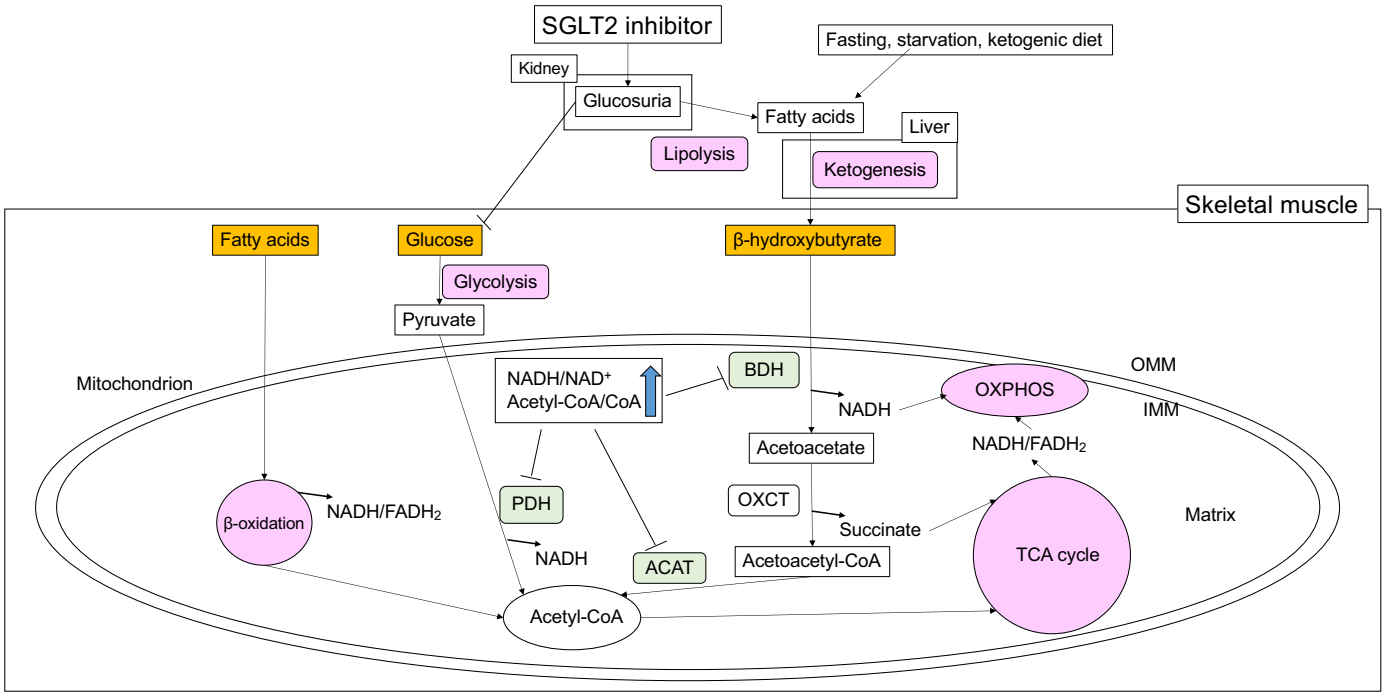

Figure 4. Skeletal muscle energy metabolism and effects of SGLT2 inhibitors. A schematic diagram of skeletal muscle metabolism is shown. The major energy substrates for skeletal muscle metabolism are fatty acids and glucose. When these substrates can be sufficiently oxidized, the nicotinamide adenine dinucleotide hydorate (NADH)/nicotinamide adenine dinucleotide (NAD)$^+$ ratio and acetyl-CoA:CoA ratio in mitochondria are high, and the β-hydroxybutyrate-metabolizing enzymes β-hydroxybutyrate dehydrogenase (BDH) and acetyl-CoA acetyltransferase (ACAT) are suppressed. SGLT2 inhibitors cause glucosuria and lipolysis, and as a result, they limit glucose utilization and enhance fatty acid oxidation in the skeletal muscle. On the other hand, fatty acid-derived ketone bodies are synthesized in the liver, and these ketone bodies can be used as an energy substrate for the skeletal muscle. When β-hydroxybutyrate metabolism is increased, pyruvate dehydrogenase (PDH), which is a pyruvate metabolizing enzyme, is suppressed as the NADH:NAD$^+$ ratio and acetyl-CoA:CoA ratio are increased. IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; OXCT, succinyl-CoA:3-ketoacid CoA transferase; OXPHOS, oxidative phosphorylation; SGLT2, sodium-glucose transporter 2; TCA, tricarboxylic acid cycle.

HF. However, characteristics of the effects of ketone bodies on skeletal muscle metabolism suggest that ketone bodies may be effective for improving skeletal muscle metabolism in patients with HF. In the skeletal muscle of patients with HF, energy metabolism by fatty acid oxidation is impaired, and compensatory energy metabolism by glycolysis is increased. The increased levels of circulating ketones may improve the ability to perform moderate-intensity endurance exercise in patients with HF by increasing fatty acid oxidation of the skeletal muscle, suppressing glycolysis, and using ketone bodies themselves as energy substrates. On the other hand, performing maximum levels of high-intensity exercise may not be effective. The effects of SGLT2 inhibitors that were observed in HF model mice are fully consistent with the effects observed for ketone bodies in these mice. If the clinical efficacy of SGLT2 inhibitors in HF is associated with the increased levels of ketone bodies in the blood, this will limit maximal exercise capacity beyond the AT and increase exercise capacity to the AT level in patients with HF. Therefore, SGLT2 inhibitors are considered to be ideal drugs for patients with HF (Fig. 2*C*).

KETONE BODIES AND SKELETAL MUSCLE ATROPHY

The intravenous infusion of sodium β-OHB into healthy young men was shown to increase muscle protein synthesis by decreasing leucine oxidation (102). Analogous anabolic effects of ketones have also been reported in C2C12 myotubes and in rodent skeletal muscle injury models (94). The intake of a ketone ester drink during recovery from a bout of intense glycogen-depleting exercise promoted protein synthesis via enhancing mTOR complex 1 signaling in the skeletal muscle of healthy humans (103). Moreover, ketone bodies increased both leucine-mediated mTOR complex 1 activation and protein synthesis in cultured muscle cells (103). On the other hand, ketone bodies have been reported to exert anticatabolic effects in skeletal muscle (104). Thomsen et al. (105) reported that β-OHB exerts robust anticatabolic effects in the skeletal muscle of men via a reduction of protein degradation, in conditions of lipopolysaccharide-induced acute inflammation. Moreover, a ketogenic formula was shown to prevent muscle atrophy caused by cancer cachexia in Colon 26 tumor-bearing mice (106). Similarly, ketone diesters were found to attenuate muscle wasting via inhibition of the ubiquitin-proteasome system in mouse atrophy models of both cancer anorexia cachexia syndrome and lipopolysaccharide-induced sepsis (107). These anticatabolic effects of ketone bodies in the skeletal muscle may be mediated by the suppression of oxidative stress associated with histone deacetylase inhibition or the attenuation of NLR family pyrin domain-containing 3 inflammasome-mediated inflammation (108, 109). Therefore, muscle atrophy and weakness induced by an imbalance between protein synthesis and

degradation may be a therapeutic target for HF, via an increase in ketone bodies, such as by the administration of an SGLT2 inhibitor. It has been reported that treatment with an SGLT2 inhibitor increases grip strength in patients with type 2 diabetes (110). On the other hand, an SGLT2 inhibitor has been reported to reduce skeletal muscle mass in high-fat diet-fed mice (111, 112). This is thought to be owing to the increased catabolism of skeletal muscle as a compensatory mechanism for the increase in glucose excretion caused by an SGLT2 inhibitor when ingesting a high-fat diet with a low carbohydrate content. Clinically, administration of an SGLT2 inhibitor to patients with diabetes has been shown to reduce lean mass (113, 114). Therefore, the administration of SGLT2 inhibitors with the main purpose of improving muscle strength and mass requires caution in clinical practice.

CONCLUSIONS AND FUTURE DIRECTIONS

SGLT2 inhibitors have been established to suppress cardiovascular death and the readmission of patients with HFrEF, and their use is recommended in the Heart Failure Guidelines of Japan, the United States, and Europe. On the other hand, the mechanism of their effectiveness remains unclear. Based on our previous research, we focused on skeletal muscle metabolism, particularly ketone body metabolism, as a possible mechanism of action of SGLT2 inhibitors. If improvement in skeletal muscle metabolism by SGLT2 inhibitors contributes to the improvement in exercise tolerance of patients with HF, particularly at the AT level, these SGLT2 inhibitors are expected to become a treatment for HF based on a new paradigm.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.T. and S.K. conceived and designed research; S.T. performed experiments; S.T. analyzed data; S.T. and S.K. interpreted results of experiments; S.T. prepared figures; S.T. and S.K. drafted manuscript; S.T., H.S., and S.K. edited and revised manuscript; S.T., H.S., and S.K. approved final version of manuscript.

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