Skeletal Muscle Abnormalities in Heart Failure

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SUMMARY

Exercise capacity is lowered in patients with heart failure, which limits their daily activities and also reduces their quality of life. Furthermore, lowered exercise capacity has been well demonstrated to be closely related to the severity and prognosis of heart failure. Skeletal muscle abnormalities including abnormal energy metabolism, transition of myofibers from type I to type II, mitochondrial dysfunction, reduction in muscular strength, and muscle atrophy have been shown to play a central role in lowered exercise capacity. The skeletal muscle abnormalities can be classified into the following main types: 1) low endurance due to mitochondrial dysfunction; and 2) low muscle mass and muscle strength due to imbalance of protein synthesis and degradation. The molecular mechanisms of these skeletal muscle abnormalities have been studied mainly using animal models. The current review including our recent study will focus upon the skeletal muscle abnormalities in heart failure. (Int Heart J 2015; 56: 475-484)

Key words: Exercise capacity, Mitochondria, Energy metabolism, Fiber type transition, Atrophy, Phosphocreatine, Nox, Renin-angiotensin system, Oxidative stress, Diabetes mellitus

All types of physical activities and exercises need the contraction of muscles. The complex fiber composition of skeletal muscles allows various forms of exercises, such as endurance, sprint, and high-power exercises.¹⁾ The skeletal muscles represent the organ with the largest mass in the body and the blood flow through the skeletal muscle becomes 20 times greater during exercise than that at rest.²⁾ The quality and mass of muscle are thus thought to be very important for maintaining homeostasis of the entire body.

Skeletal Muscle Energy Metabolism

Mitochondrial function plays a key role in the contraction of skeletal muscle.³⁾ ATP is consumed during muscle contraction, however, only very small amounts of ATP are present in the cytoplasm of skeletal muscle cells, and they are rapidly exhausted. When phosphocreatine (PCr), a high-energy phosphorylated metabolite, is broken down to creatine (Cr) and inorganic phosphate, ATP is generated. This ATP is consumed as energy during continuous exercise. On the other hand, PCr also becomes depleted if it is not regenerated or if the intensity of the exercise is too strenuous and the rate of regeneration is insufficient. When PCr is exhausted, the individual is no longer able to continue exercising. In our previous study, we measured the energy metabolism of the quadriceps muscles during peak exercise by a cycle ergometer using magnetic resonance spectroscopy (MRS) in human subjects.^{4,5)} We found that PCr was exhausted in the quadriceps muscles at peak exercise, in-

dicating that the limitation of skeletal muscle energy metabolism coincided with the full-body exercise limitation. During aerobic exercise, PCr levels can be maintained at a certain level allowing continuous contraction by regenerating PCr using ATP produced from mitochondrial oxidative phosphorylation. Therefore, skeletal muscle mitochondria play an important role in energy production for skeletal muscle contraction.

Skeletal Muscle Mass

Muscle mass is important for skeletal muscle to exert force. Skeletal muscle hypertrophy and atrophy are attributed more to increases or decreases in myocyte size than in the number of myofibrils. Generally, muscle strength bears a close relationship with cross-sectional area.⁶⁾ Hypertrophy of skeletal muscle cells occurs when synthesis of proteins such as actin and myosin, the constituents of myofibrils, exceeds their degradation or when degradation is inhibited in the cell. Alternatively, atrophy of skeletal muscle cells occurs when protein degradation exceeds protein synthesis. Therefore, the balance between protein degradation and synthesis plays a crucial role in the maintenance of skeletal muscle strength and muscle mass.

Skeletal Muscle, Exercise Capacity and Life Expectancy

Exercise capacity, particularly aerobic exercise capacity, is closely associated to life expectancy. In a prospective study

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where an exercise tolerance test was conducted on 6213 men (3679 diagnosed with cardiovascular disease and 2534 healthy men), lower exercise capacity evaluated by maximum metabolic equivalents indicated a higher risk of mortality.⁷⁾ Also in a study targeting 1263 male diabetic patients, the overall mortality was significantly higher in the low exercise capacity group compared to the high exercise capacity group (adjusted hazard ratio 2.1, 95% confidence interval, 1.5-2.9).⁸⁾ Low exercise capacity evaluated by maximal oxygen consumption was also associated with an increased mortality rate in patients with heart failure (HF).^{7,9)} An important determining factor for exercise capacity, especially aerobic exercise capacity, is endurance of the skeletal muscle; in other words, the function of the skeletal muscle mitochondria.^{3,5)} Determining the mechanisms regulating skeletal muscle mitochondrial function is an extremely important issue for healthy subjects as well as patients with diabetes mellitus or cardiovascular disease.

Muscle strength and mass are also known to be linked to life expectancy. A 12.5-year prospective observational cohort study of 1436 healthy men and 1380 healthy women revealed that the rate of total mortality from any cause increased with small thigh circumference in both men and women.¹⁰⁾ In another study of 2292 healthy subjects, low life expectancy was observed in both men and women who showed low knee extension strength and grip strength.¹¹⁾ Similarly, when a group of HF patients was separated into two groups using a cut-off point of 68 Nm · 100/kg in the strength of the knee flexor muscles, survival rate was significantly lower in the low muscle

strength group.¹²⁾ In recent years, sarcopenia, characterized by the loss of muscle mass and strength and low physical performance, has been attracting attention.¹³⁾ While it often occurs with aging, various causes such as physical inactivity, poor nutrition and endocrine abnormalities are also known to be involved in its pathogenesis.¹⁴⁾ Since sarcopenia directly lowers physical activity and adversely affects prognosis,¹⁵⁾ unraveling the mechanisms regulating muscle mass and strength is an important issue.

Molecular Mechanism of Skeletal Muscle Abnormalities

Skeletal muscle abnormalities can be classified into the following main types: 1) low endurance due to mitochondrial dysfunction; and 2) low muscle mass and muscle strength due to an imbalance of protein synthesis and degradation. The molecular mechanisms of these skeletal muscle abnormalities have been studied mainly using animal models (Figures 1, 2). Conversely, the effects of exercise training on skeletal muscle can also be explained by the same molecular mechanisms. In other words, these are the molecular mechanisms for aerobic exercise training that requires endurance and resistance training that leads to muscle hypertrophy.

Regulation of mitochondrial function (Figure 1): The oxidative capacity of mitochondria is determined primarily by the amount of mitochondrial protein. Peroxisome proliferator-activated receptor γ (PPAR γ) and PPAR γ coactivator-1 (PGC-1) play key roles in mitochondrial biogenesis and functional regu-

Figure 1. Regulation of mitochondrial function. AdipoR indicates adiponectin receptor; eNOS, endothelial nitric oxide synthase; RAS, renin-angiotensin system; AT1R, angiotensin II type 1 receptor; Nox, nicotinamide adenine dinucleotide phosphate-oxidase; CaMKK, calcium-calmodulin-dependent protein kinase kinase; CaMK, calcium-calmodulin-dependent protein kinase; NO, nitric oxide; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; AMPK α , AMP-activated protein kinase α ; SIRT, sirtuin; PGC-1 α , peroxisome proliferator-activated receptor gamma coactivator 1- α ; β -HAD, β -hydroxy-acyl CoA dehydrogenase; TCA, tricarboxylic acid; and NAD, nicotinamide adenine dinucleotide. See text for details.

Figure 2. Skeletal muscle hypertrophy and atrophy. β -AR indicates β -adrenergic receptor; GPCR, G-protein-coupled receptor; IGF, insulin-like growth factor; IGF1R, IGF receptor; ActRIIB, activin receptor IIb; RAS, renin-angiotensin system; AT1R, angiotensin II type 1 receptor; Nox, nicotinamide adenine dinucleotide phosphage-oxidase; TNF α , tumor necrosis factor α ; TNFR, TNF receptor; GR, glucocorticoid receptor; GSK3 β , glycogen synthase kinase-3 β ; PI3K, phosphoinositide-3-kinase; mTORC, mammalian target of rapamycin complex; p70S6K, p70 ribosomal S6 kinase; eIF2B, eukaryotic initiation factor-2B; 4E-BP, eIF4E-binding protein; I κ B, inhibitor of kappa B; IKK, I κ B kinase; NF κ B, nuclear factor kappa B; FoxO, forkhead box O; and MuRF1, muscle RING finger protein 1. See text for details.

lation.^{16,17}) PGC-1 α increases the expression of transcription factors such as nuclear respiratory factor (NRF)-1 and -2.¹⁶ NRFs not only increase the expression of nuclear gene-encoded mitochondrial proteins, but also increase the expression of mitochondrial transcription factor A (Tfam).¹⁶ Tfam binds mitochondrial DNA (mtDNA) in the mitochondria and regulates the protein expression encoded in the mtDNA gene.^{18,19} Overexpression of skeletal muscle-specific PGC-1 α increased mitochondrial biogenesis and an oxidative muscle fiber-type, thereby increasing exercise capacity and creating a phenotypic change that equals aerobic exercise training.^{17,20-22} Conversely, deletion of skeletal muscle-specific PGC-1 α decreased mitochondrial biogenesis and increased the percentage of a glycolytic fiber-type, which caused a decline in exercise capacity.²²

A number of different regulatory mechanisms of PGC-1 α are present in skeletal muscle: 1) AMP-activated protein kinase (AMPK), regulated by intracellular energy deficiency; 2) the sirtuin (SIRT) family, regulated by redox balance; 3) calcium, which increases with cellular contraction and calmodulin-dependent protein kinase (CaMK); and 4) the MAPK family, regulated by stress. In contrast, intracellular energy status, redox balance, and stress response can be altered depending on mitochondrial function. In other words, while PGC-1 α regulates mitochondrial function and numbers, the function of the mitochondrion can itself regulate PGC-1 α .

AMPK is a serine-threonine kinase that regulates intracellular metabolism. AMPK activation is regulated by the deficiency in intracellular energy such as the reduction of ATP and an increase in the AMP/ATP ratio.^{16,23} AMPK is activated by

aerobic exercise training and caloric restriction models and is inhibited in diabetes mellitus and aging models.²⁴⁻²⁶ PGC-1 α is phosphorylated by the activation of AMPK and mitochondrial biogenesis is regulated through this activation.²⁷ In fact, mitochondrial biogenesis is inhibited in skeletal muscle-specific AMPK-deficient mice²⁸ and mitochondrial biogenesis is increased with chronic activation of AMPK by AICAR and gene manipulation.^{26,29}

The SIRT family is a family of class III histone/protein deacetylases responsible for NAD-dependent deacetylation of target proteins.³⁰ NAD⁺ is an electron donor with an important function in energy metabolism and is involved in ATP production within the cell and mitochondria. Activation of SIRT1 and SIRT3 is sensitive to increases in NAD⁺ and the NAD⁺/NADH ratio and activates PGC-1 α through its deacetylation.³¹⁻³³ Like AMPK, SIRTs are also activated through exercise and caloric restriction and are inhibited by diabetes and aging.^{31,32,34,35} It has been reported that activation of SIRT1 by resveratrol increases mitochondria biogenesis and exercise capacity in skeletal muscle.^{29,36-38} Skeletal muscle-specific SIRT1 deficiency, however, showed no obvious phenotypic change in muscle between before and after exercise.³⁹ Research on the role of the SIRT family in skeletal muscle is limited and whether it is an important determining factor for the changes in skeletal muscle phenotype remains unclear.

Although AMPK and SIRT have been believed to regulate PGC-1 α independently, it has been also suggested that they may regulate one another and regulate PGC-1 α together.^{16,31} A recent study has shown that adiponectin and its recep-

tor, adiponectin receptor 1, regulate AMPK/SIRT and PGC-1 α , and CaMK, which is discussed later, is involved in this regulatory mechanism.⁴⁰⁾ Endothelial nitric oxide synthase (eNOS)-derived NO levels are known to increase with exercise, and it has been involved in the phosphorylation and activation of AMPK.⁴¹⁾ It was also shown that eNOS-derived NO plays an important role in PGC-1 α expression and mitochondrial biogenesis.^{42,43)}

Calcium is necessary for muscle contraction. Its temporary elevation affects the cross-bridge interaction between myosin and actin, leading to the contraction of muscle cells. On the other hand, this calcium elevation regulates CaMK activation. CaMK is a serine/threonine kinase that targets transcription factors involved in the gene expression of various mitochondrial regulatory proteins.⁴⁰⁾ Exercise increases the phosphorylation of CaMK II in an exercise intensity-dependent manner.⁴⁴⁾ Skeletal muscle-specific overexpression of CaMK IV in mice resulted in increased PGC-1 gene and protein expression and elevated mitochondrial biogenesis.⁴⁵⁾

Mitogen-activated protein kinases (MAPKs) are stress-response kinases that have an important role in mitochondrial biogenesis. MAPKs are activated by the mechanical stress of contraction in the skeletal muscle and stimulation of cytokines and oxidative stress.⁴⁶⁾ This is related to MAPK activation during exercise. An MAPK family member, p38 MAPK, stimulates the upstream transcription factor of the PGC-1 α gene and increases mitochondrial biogenesis in skeletal muscle by regulating the expression of PGC-1 α .^{24,47)} On the other hand, in the absence of p38 γ isoforms, an increase in mitochondrial biogenesis induced by exercise is inhibited.⁴⁸⁾

Muscle hypertrophy and muscle atrophy (Figure 2):

Protein synthesis system Skeletal muscle hypertrophy is attained through increased protein synthesis.⁴⁹⁾ The most important mechanism that leads to protein synthesis is a signal mediated by insulin-like growth factor (IGF-1). IGF-1 expression was increased in the skeletal muscle from rats with compensatory hypertrophy.⁵⁰⁾ In addition, overexpression of skeletal muscle-specific IGF-1 increased muscle size in mice.^{51,52)} When IGF-1 binds to its receptor, IGF-1 tyrosine kinase receptor, it triggers the phosphorylation of the receptor and continues to phosphorylate the insulin receptor substrate 1 (IRS1). In addition, this binding activates a downstream signal, phosphatidylinositol-3-kinase (PI3K), and activates a serine/threonine kinase known as Akt. The activation of Akt is an extremely important molecular mechanism for protein synthesis. In fact, skeletal muscle hypertrophy was induced in mice overexpressed with skeletal muscle-specific Akt.⁵³⁾ On the other hand, in mice lacking both Akt1 and Akt2, significant skeletal muscle atrophy was observed.⁵⁴⁾ The mammalian target of rapamycin (mTOR) is one of the few known target molecules of muscle hypertrophy by Akt activation, and is made up of two complexes, TORC1 and TORC2.⁵⁵⁾ TORC1 contains rapamycin-sensitive raptor and stimulates protein synthesis through phosphorylation and activation of p70S6K and inhibition of 4E-BP1.⁵⁶⁻⁵⁸⁾ Resistance training model rats by electrical stimulation of skeletal muscles showed a strong correlation between the degree of muscle hypertrophy and the degree of p70S6K1 phosphorylation.⁵⁹⁾ On the other hand, TORC2 contains rapamycin-insensitive rictor, which phosphorylates Akt and makes a feedback loop while being involved in the inhibition of forkhead box O (FoxO).^{57,60,61)} IGF-1 induces protein syn-

thesis not only through a protein synthesis pathway mediated by PI3K/Akt/mTOR, but also in an mTOR-independent manner.^{55,62-66)} Akt phosphorylates and inactivates glycogen synthase kinase 3 β (GSK3 β), leading to the activation of eIF2B, which is involved in protein synthesis. The PI3K/Akt/GSK3 β -mediated pathway may also be involved in muscle hypertrophy.^{55,62-66)}

Myostatin-mediated signaling is also important as one of the mechanisms of IGF-1-independent muscle hypertrophy. Myostatin, a member of the transforming growth factor (TGF)- β family, is produced by skeletal muscle and negatively regulates skeletal muscle mass.⁶⁷⁾ Mature myostatin binds to the activin receptor in skeletal muscle and both Smad2 and Smad3 are subsequently phosphorylated and activated.^{68,69)} These phosphorylations inhibit Akt phosphorylation. In fact, significant skeletal muscle hypertrophy is known to occur with treatments such as myostatin gene mutation and myostatin inhibitors.^{67,70)} On the other hand, IGF-1 is known to have the ability to compete with the negative regulation of myostatin on skeletal muscle.⁷¹⁾ Smad2 phosphorylation is inhibited by the IGF-1/mTORC1 signal.⁶⁸⁾ Therefore, IGF-1 and myostatin compete and interact with one another to regulate skeletal muscle mass.

Another important signal for regulating skeletal muscle mass is the G protein-coupled receptor (GPCR)-mediated signal. The β 2 adrenaline receptor stimulants, such as clenbuterol, are known to induce skeletal muscle hypertrophy.⁷²⁻⁷⁴⁾ The effect is mediated by GTP binding to a subunit, G α_s , followed by Akt/mTOR signal cascade. Also, the activation of another GPCR, frizzled (Fzd)/Wnt7a, is mediated through the G α_s pathway.⁷⁵⁾ Skeletal muscle atrophy has been observed in G α_s -deficient mice.⁷⁶⁾ Conversely, in a recent study, skeletal muscle hypertrophy was observed through a PKC signal mediated by G α_{i2} activation.⁷⁶⁾ G α_{i2} activation phosphorylated the downstream target of mTOR, particularly p70S6K and GSK3 β , but not Akt.

Protein degradation system The ubiquitin-proteasome (UP) system plays a critical role in protein breakdown in muscle atrophy. In various models of muscle atrophy, UP system activation is thought to be an important mechanism in protein degradation.^{77,78)} Three enzymes trigger UP system activation: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin-ligase (E3). E3 is a post-translational modification enzyme that specifically identifies the target protein and binds ubiquitin. While many E3s are now known, Muscle Ring Finger 1 (MuRF1) and Muscle Atrophy F-box (MAFbx)/Atrogin-1 are E3 ubiquitin ligases showing increased expression in previously reported models of muscle atrophy and are considered important markers for muscle atrophy.^{77,78)} These gene-deficient mice were resistant to muscle atrophy.⁷⁷⁾

Inflammatory cytokines are known causative factors in muscle atrophy. Tumor necrosis factor (TNF)- α , in particular, is involved.⁷⁹⁻⁸¹⁾ In fact, many atrophy models show an increase in this inflammatory cytokine. TNF- α binds to its receptor and activates nuclear factor (NF)- κ B, followed by increases in the expression of MuRF1.⁸²⁾ NF- κ B thus plays a central role in muscle atrophy induced by inflammatory cytokines.⁸³⁻⁸⁵⁾ While NF- κ B is regulated by the inhibitor of κ B (I κ B) kinase (IKK) complex, the role of activation and inhibition of NF- κ B in skeletal muscle atrophy was identified through the overexpres-

sion of skeletal muscle-specific IKK β and the dominant inhibitory form of I κ B α .⁸³ NF- κ B activation in the skeletal muscle is now known to increase MuRF1 expression, while expression of MAFbx is not changed. On the other hand, p38MAPK is involved in the increased expression of MAFbx. The inhibitor of p38MAPK inhibited the increased expression of MAFbx induced by TNF- α stimulation.⁸⁶

Other crucial factors involved in atrophy are glucocorticoids. Glucocorticoids promote protein degradation, leading to muscle atrophy.⁸⁷⁻⁸⁹ In this case, the expressions of MuRF1 and MAFbx increase, but these increases are not mediated by NF- κ B or p38 MAPK. Expressions of these E3 ligase were inhibited by PI3/Akt system-mediated IGF-1. In a genetically modified model with constitutive activation of Akt, the increased expression of MuRF1 and MAFbx, both associated with muscle atrophy, is inhibited.⁸⁹ The inhibitory mechanism of E3 ligase expressions by Akt is thought to be associated with the inhibition of activation of FoxO phosphorylation.⁸⁹

Skeletal Muscle Abnormalities in HF

Exercise capacity in HF patients declines according to its severity. The skeletal muscle plays an important role in the reduced exercise capacity.^{5,90,91} Many skeletal muscle abnormalities have been reported in association with HF. They include

impaired skeletal muscle energy metabolism, mitochondrial dysfunction, fiber-type transition, and atrophy.^{5,90-97} Skeletal muscle atrophy shows a decrease in muscle fiber size, and closely associates with the limited exercise capacity. Fiber-type transitions, the decreased ratio of type 1 (slow) fibers, and the relatively increased ratio of type 2 (fast) fibers were observed in skeletal muscle biopsy samples from patients with HF,^{92,93,95-98} which was coincident with a shift from myosin heavy chain (MHC) 1 to fast fatigable MHC 2.^{94,98} These fiber-type transitions are also known to be closely associated with exercise capacity.^{94,98} We measured skeletal muscle energy metabolism during local exercise by lower limb using MRS in patients with HF.⁵ They demonstrated a large PCr loss compared to healthy subjects (Figure 3A). A larger PCr loss indicated an impaired aerobic ATP production in skeletal muscle mitochondria. This PCr loss during localized exercise and the whole-body exercise tolerance (peak oxygen uptake; peak VO₂, and anaerobic threshold; AT) was closely linked (Figure 3B, C). Furthermore, we measured intramyocellular lipid (IMCL) levels by MRS (Figure 3D). IMCL was increased in HF patients compared to healthy subjects (Figure 3E) and its content was closely associated with skeletal muscle energy metabolism and whole-body exercise tolerance (Figure 3F, G).⁹¹ This suggests that fatty acid oxidation is impaired in the mitochondria, and these results are coincident with a previous

Figure 3. Skeletal muscle energy metabolism in patients with heart failure. Representative ³¹P MR spectra recorded at rest and during exercise in triceps surae muscle from a healthy subject (upper) and a patient with HF (under) in the quantification of PCr content (A). Scatter plots between PCr loss and peak VO₂ (B) or AT (C) in healthy subjects (○; n = 12) and patients with HF (●; n = 18). Each plot represents the individual data of exercise capacity and ³¹P-MRS obtained from the same subject. Representative ¹H MR spectra recorded at the resting tibialis anterior muscle (D) from a healthy subject (upper) and a patient with HF (under) in the quantification of IMCL content (E). Scatter plots between IMCL content and peak VO₂ (F) or AT (G) in healthy subjects (○; n = 12) and patients with HF (●; n = 18). Each plot represents the individual data of exercise capacity and ¹H-MRS obtained from the same subject. HF indicates heart failure; PCr, phosphocreatine; ATP, adenosine triphosphate; VO₂, oxygen uptake; AT, anaerobic threshold; IMCL, intramyocellular lipid; EMCL, extramyocellular lipid; and TCr, total creatine. Reproduced with permission from Hirabayashi, *et al.* Int J Cardiol 2014; 176: 1110-2.⁹¹

report that a key enzyme of β -oxidation, 3-hydroxyacyl-CoA dehydrogenase (β -HAD), a fatty acid metabolic enzyme in skeletal muscle, is decreased in patients with HF.⁹⁷ As shown above, many clinical observations for patients with HF have shown skeletal muscle abnormalities. However, many uncertainties remain in terms of the mechanisms underlying the occurrence of these skeletal muscle abnormalities.

Skeletal muscle atrophy was observed in HF animal models 12 weeks after the induction of myocardial infarction (MI).^{99,100} The atrophy was due to the enhanced protein degradation from activation of the UP system rather than a defect in protein synthesis, and the expressions of MAFbx and MuRF1 were also increased.^{99,100} These were inhibited in mice with overexpression of skeletal muscle-specific IGF-1.¹⁰⁰ Furthermore, the activation of the UP system in skeletal muscle from post-infarct HF increased in mice lacking β 2 adrenoreceptors, which was associated with a decline in Akt phosphorylation.¹⁰¹ On the other hand, UP system activation occurred through NF- κ B and p38 MAPK activation by NAD(P)H oxidase-derived oxidative stress in skeletal muscle from the same animal model.⁹⁹ We found that impairment of Akt phosphorylation in skeletal muscle from post-infarct HF mice occurred 4 weeks after surgery,^{102,103} which was associated with NAD(P)H oxidase-derived oxidative stress and increased local angiotensin II in skeletal muscles.^{102,103} Angiotensin II is thus considered to be important in skeletal muscle atrophy in HF. In this model, protein synthesis decreased due to impairment of Akt phosphorylation and protein degradation enhanced through protein ubiquitination.¹⁰⁴ A recent study reported that HF-induced skeletal muscle atrophy was inhibited in cardiac-specific myostatin-knockout mice.¹⁰⁵ This suggests that a cardiac-derived

myostatin is involved in skeletal muscle atrophy in HF, thereby indicating a new mechanism of skeletal muscle abnormalities in HF.

In the previous studies, skeletal muscle mitochondrial dysfunction and fiber-type transitions were observed in various HF models including post-infarct, aortic constriction, rapid pacing, and monocrotaline-induced pulmonary hypertension models.¹⁰⁶⁻¹¹⁵ However, none of these reports demonstrated the mechanism of skeletal muscle mitochondrial defects due to HF. A previous study in post-infarct HF rats reported a close association between decreased PGC-1 α gene expression and mitochondrial dysfunction in skeletal muscle.¹¹⁵ Furthermore, they were ameliorated by the administration of angiotensin-converting enzyme (ACE) inhibitor.¹¹⁵

We have shown that angiotensin II is possibly involved in skeletal muscle abnormalities in HF. Firstly, we administered a subpressor dose (50 ng/kg/minute) of angiotensin II into mice, and skeletal muscle mitochondrial dysfunction occurred and exercise capacity decreased without affecting skeletal muscle mass.¹¹⁶ We also found that reactive oxygen species from NAD(P)H oxidase are involved in this model.¹¹⁶ Furthermore, we investigated the effects of a pressor dose (1000 ng/kg/minute) of angiotensin II on the skeletal muscle abnormalities.¹¹⁷ Angiotensin II directly induced mitochondrial dysfunction (Figure 4A) and fiber type transition to glycolytic (type IIb) from oxidative (type I) fibers (Figure 4B), followed by skeletal muscle atrophy (Figure 4C). Consequently, it reduced the exercise capacity (Figure 4D). Therefore, angiotensin II can be a key molecule that causes a series of skeletal muscle abnormalities observed in HF.

Figure 4. Skeletal muscle abnormalities in mice treated with angiotensin II. Citrate synthase activity (A, left), mitochondrial complex I (A, middle) and III (A, right) activities in the skeletal muscle tissue from vehicle and Ang II mice. Representative high-power photomicrographs of skeletal muscle tissue sections stained with nicotinamide adenine dinucleotide (NADH, B) and hematoxylin-eosin (HE) from vehicle and Ang II mice (C). Work during peak exercise in vehicle and Ang II mice (D). Scale bar, 100 μ m. Ang II indicates angiotensin II; and CS, citrate synthase. Data are expressed the mean $\pm$ SE. * P < 0.05 versus vehicle. Reproduced with permission from Kadoguchi, *et al.* Exp Physiol 2015; 100: 312-22.¹¹⁷

HF and Diabetes Mellitus

Diabetes mellitus increases the risk of the development of HF, and also aggravates the severity of HF.¹¹⁸⁻¹²⁰ The occurrence of HF itself is also known to trigger insulin resistance and diabetes.^{102,103,121-123} Therefore, a strong link exists between HF and diabetes, and this modifies the clinical condition. The presence of diabetes exacerbates the cardiac interstitial fibrosis, triggers cardiac myocyte hypertrophy, and exacerbates myocardial remodeling, which are thought to largely contribute in modifying the clinical condition of HF.¹²⁰ On the other hand, diabetes is strongly associated with skeletal muscle abnormalities and declines in exercise capacity. In fact, skeletal muscle abnormalities of energy metabolism and IMCL deposition are known to occur in diabetic and insulin-resistant patients,^{124,125} and these are linked to skeletal muscle mitochondrial dysfunction. Our study demonstrated that the exercise tolerance in patients with metabolic syndrome and insulin-resistance were significantly lower compared to healthy individuals.^{124,125} Skeletal muscle energy metabolism measured by MRS during exercise was impaired along with an increase in IMCL deposition, indicating that these skeletal muscle abnormalities were closely linked to exercise intolerance.¹²⁴ In addition, blood oxidative stress markers and impaired skeletal muscle energy metabolism were found to be closely linked.¹²⁵ Therefore, we believe that a decline in exercise tolerance was potentially due to the elevated oxidative stress from insulin-resistance triggering skeletal muscle mitochondrial dysfunction.

We showed that exercise capacity was significantly decreased in a high fat diet (HFD)-induced diabetic mice model.¹²⁶⁻¹²⁹ Mitochondrial respiratory capacity, complex activities and citrate synthase activity in skeletal muscle were also impaired along with declines in numbers of mitochondria and type I muscle fibers in this model. In agreement with these results, both mitochondrial DNA and PGC-1 α gene expression were decreased. In addition, a rise in plasma angiotensin II levels increased and NAD(P)H oxidase activation and oxidative stress in skeletal muscle were increased. When this diabetic mice model was treated with apocynin (an NAD(P)H oxidase inhibitor), olmesartan (an angiotensin II receptor blocker) and pioglitazone (an insulin sensitizing drug), exercise capacity and skeletal muscle mitochondrial dysfunction were all improved. Therefore, skeletal muscle abnormalities in diabetes mellitus are thought to be associated with mitochondrial dysfunction through an activated renin-angiotensin system and activated NAD(P) oxidase or the decreased mitochondrial biogenesis via decreased PGC-1 α expression. It has been also reported that adiponectin and SIRT-1 play an important role in the regulation of mitochondrial function in the same mouse model.^{40,130-135} Therefore, the common mechanisms for skeletal muscle mitochondrial abnormalities could be involved in diabetes and HF.

Conclusions: The number of patients with HF is on the rise and the development of new treatment strategies for HF is a critical issue. Exercise intolerance adversely affects not only prognosis, but also quality of life in patients with HF, and can be a target of treatment for HF. Skeletal muscle abnormalities are known to be important in determining factors of exercise intolerance in HF. However, the mechanisms underlying skeletal muscle abnormalities in HF have not been demonstrated and hence specific treatments for skeletal muscle abnormalities

and methods to improve exercise intolerance other than exercise therapy are lacking. Further research in this field is crucial.

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