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Dipeptidyl peptidase-4 inhibitor improved exercise capacity and mitochondrial biogenesis in mice with heart failure via activation of glucagon-like peptide-1 receptor signalling

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Aims Exercise capacity is reduced in heart failure (HF) patients, due mostly to skeletal muscle abnormalities including impaired energy metabolism, mitochondrial dysfunction, fibre type transition, and atrophy. Glucagon-like peptide-1 (GLP-1) has been shown to improve exercise capacity in HF patients. We investigated the effects of the administration of a dipeptidyl peptidase (DPP)-4 inhibitor on the exercise capacity and skeletal muscle abnormalities in an HF mouse model after myocardial infarction (MI).

Methods and results MI was created in male C57BL/6J mice by ligating the left coronary artery, and a sham operation was performed in other mice. The mice were then divided into two groups according to the treatment with or without a DPP-4 inhibitor, MK-0626 [1 mg/kg body weight (BW)/day] provided in the diet. Four weeks later, the exercise capacity evaluated by treadmill test was revealed to be limited in the MI mice, and it was ameliorated in the MI + MK-0626 group without affecting the infarct size or cardiac function. The citrate synthase activity, mitochondrial oxidative phosphorylation capacity, supercomplex formation, and their quantity were reduced in the skeletal muscle from the MI mice, and these decreases were normalized in the MI + MK-0626 group, in association with the improvement of mitochondrial biogenesis. Immunohistochemical staining also revealed that a shift toward the fast-twitch fibre type in the MI mice was also reversed by MK-0626. Favourable effects of MK-0626 were significantly inhibited by treatment of GLP-1 antagonist, Exendin-(9-39) (150 pmol/kg BW/min, subcutaneous osmotic pumps) in MI + MK-0626 mice. Similarly, exercise capacity and mitochondrial function were significantly improved by treatment of GLP-1 agonist, Exendin-4 (1 nmol/kg/BW/h, subcutaneous osmotic pumps).

Conclusions A DPP-4 inhibitor may be a novel therapeutic agent against the exercise intolerance seen in HF patients by improving the mitochondrial biogenesis in their skeletal muscle.

Keywords Heart failure • Exercise capacity • Skeletal muscle • Mitochondria • DPP-4 inhibitor

1. Introduction

Exercise capacity is limited in patients with heart failure (HF),[1,2] and it is closely related to the prognosis of HF.[3] Aerobic exercise training improves the exercise capacity of HF patients and their prognoses.[4] However, there has been no specific medical therapy that has been demonstrated to effectively improve the exercise capacity of HF patients. The limited exercise capacity in HF largely depends on skeletal muscle

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abnormalities rather than disturbed central hemodynamics.[5] The skeletal muscle abnormalities in HF have been characterized as a reduction of mitochondrial density, abnormal energy metabolism including disturbed oxidative phosphorylation (OXPHOS), the transition of the fibre type from the slow oxidative fibre type I to the fast glycolytic fibre type II, and a reduction in muscular strength and muscle atrophy.[6]

Incretin is the generic name of hormones that include glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 (GLP-1).[7] They are secreted from the intestine when a meal is consumed to promote insulin secretion by acting on pancreatic β cells. Circulating active GLP-1 is degraded within a few minutes by the enzyme dipeptidyl peptidase-4 (DPP-4). DPP-4 inhibitors are the agents that dose dependently inhibit DPP-4 and increase active GLP-1 levels.[8]

GLP-1 receptors have been detected in cardiac myocytes, vascular endothelial cells, and skeletal muscle cells,[9] and GLP-1 may thus have some direct effects on skeletal muscles as well as the cardiovascular system. Treatment with GLP-1 was reported to improve exercise intolerance in HF patients.[10] Moreover, vildagliptin, an inhibitor of DPP-4, enhanced the energy metabolism in the skeletal muscle of diabetes patients.[11] A DPP-4 inhibitor may thus improve skeletal muscle function including energy metabolism and may thereby improve the exercise capacity of HF patients.

However, the effects of a DPP-4 inhibitor on the exercise capacity and skeletal muscle abnormalities of HF patients or models have not been elucidated. In particular, it has not been determined whether a DPP-4 inhibitor affects mitochondrial biogenesis. We created a mouse model of HF after myocardial infarction (MI) with limited exercise capacity and skeletal muscle abnormalities. In this study, we investigated the effects of a DPP-4 inhibitor, MK-0626 (a sitagliptin analogue), on the exercise capacity, skeletal muscle abnormalities, and mitochondrial biogenesis in this model.

2. Methods

All procedures and animal care were approved by our institutional animal research committee and conformed to the animal care guidelines for the Care and Use of Laboratory Animals at the Hokkaido University Graduate School of Medicine. The procedures and care were also in accordance with relevant national and international guidelines and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.1 Experiment I

2.1.1 Experimental animals

Male C57BL/6J mice [10–12 weeks old, body weight (BW): 23–25 g, CLEA Japan, Tokyo] were bred in a pathogen-free environment and housed in an animal room under controlled conditions on a 12-h light/dark cycle maintained at 23–25 °C. Normal diet (ND; CE-2; CLEA Japan, Tokyo) and water were provided. The MI was created by ligating the left coronary artery as described. A sham operation without ligating the coronary artery was also performed. For either operation, the mouse was anesthetized with pentobarbital (50 μg/g BW, ip) throughout the operation, and the adequacy of the anaesthesia was monitored based on the disappearance of the pedal withdrawal reflex.

One day after the sham ($n = 28$) or MI ($n = 70$) surgery, each group of mice was divided into two groups according to treatment with or without the DPP-4 inhibitor MK-0626 (1 mg/kg BW/day provided by Merck Sharp & Dohme, Whitehouse Station, NJ, USA) for 4 weeks. The dose of MK-0626 was chosen on the basis of a pharmacodynamic study. MI mice was divided into two groups in MI + ND ($n = 38$) and MI + MK-0626 ($n = 32$). There was no difference in the mortality rate up to 4 weeks after operation between MI + ND and MI + MK-0626 group (63.2% vs. 53.6%). No mice died after sham operation. This study was performed in the following four groups of mice: (i) sham + ND ($n = 14$), (ii) sham + MK-0626 supplemented in the diet ($n = 14$), (iii) MI + ND ($n = 14$), and (iv) MI + MK-0626 ($n = 14$) in survivors. These assignment procedures were performed using numeric codes to identity the animals.

After 4 weeks, echocardiography, oral glucose test, and treadmill test were performed, and then mice were sacrificed, and skeletal muscle was excised. Biochemical measurement, mitochondrial function and histological analyses, and immunoblotting were performed. An expanded methods section is provided in the Supplementary material online.

2.2 Experiment II

An osmotic minipump (model 2004 ALZET, CA) was implanted into MI mice the next day after MI operation, and these mice were randomly divided to be treated with vehicle (Ve, saline) or Exendin-4 (Ex-4, GLP-1 receptor agonist, 1 nmol/kg BW/h; Abcam, Osaka, Japan) subcutaneously for 4 weeks. Implantation of osmotic minipump was performed under light anaesthesia with avertin (160 mg/kg BW, ip). The dose of Ex-4 and the duration of treatment in this study were chosen based on the previous study of their efficacy.[12] After 4 weeks, exercise capacity and mitochondrial complex I activity in the skeletal muscle were measured in the following two groups: (i) MI + Ve ($n = 5$) and (ii) MI + Ex-4 ($n = 6$) in survivors.

2.3 Experiment III

An osmotic minipump was implanted into MI mice the next day after MI operation, and these mice were randomly divided to be treated with ND or MK-0626, and to be treated with vehicle (0.9% NaCl, 1% bovine serum albumin) or Exendin-(9-39) (Ex-(9-39), GLP-1 receptor antagonist, 150 pmol/kg BW/min; Abcam) subcutaneously for 4 weeks. Implantation of osmotic minipump was performed under light anaesthesia with Avertin (160 mg/kg BW, ip). The dose of Ex-(9-39) and the duration of treatment in this study were chosen based on the previous study of their efficacy.[13] After 4 weeks, exercise capacity and mitochondrial complex I activity in the skeletal muscle were measured in the following three groups: (i) MI + ND + Ve ($n = 7$), (ii) MI + MK-0626 + Ve ($n = 8$), and (iii) MI + MK-0626 + Ex-(9-39) ($n = 11$) in survivors.

2.4 Statistical analysis

Data are expressed as means ± SE. For multiple-group comparisons, two-way analysis of variance followed by the Tukey's test was performed. Survival analysis was performed by the Kaplan–Meier method, and between-group differences in survival were tested using the log-rank test. Student unpaired t tests were performed to compare means between MI + Ve and MI + Ex-4 mice. In oral glucose tolerance test, differences between groups were determined with repeated-measures analysis of variance. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1 Characteristics of the mice

Supplementary material online, *Table S1* shows the characteristics of the mice. The heart weight/BW and lung weight/BW values were significantly increased in the MI mice compared to the sham-operated mice.

The values of fasting blood glucose, plasma insulin levels, total cholesterol, triglyceride, and non-esterified fatty acid were comparable between the sham and MI mice. The echocardiographic and hemodynamic data are shown in Supplementary material online, *Figure S1* and *Table S1*. The left ventricular (LV) diameters and posterior wall thickness were significantly greater, and the LV fractional shortening was significantly lower in the MI mice compared to the sham mice (see Supplementary material online, *Figure S1*).

There was no significant difference in the heart rate or the systolic blood pressure between the sham and MI mice. In addition, our histopathological analysis of non-infarcted LV sections revealed that the myocyte cross-sectional area (CSA) and the collagen volume fraction were significantly increased in the MI mice compared to the sham mice.

The DPP-4 inhibitor MK-0626 did not affect the heart weight/BW, lung weight/BW, or any of the aforementioned echocardiographic, hemodynamic, and structural alterations in the MI mice. MK-0626 also did not affect these parameters in the sham mice. There was no difference in infarct size between the MI + ND and MI + MK-0626 groups.

3.2 Plasma DPP-4 activity, active GLP-1 levels, and oral glucose tolerance test results

The plasma DPP-4 activity was comparable between the sham and MI mice, and MK-0626 significantly decreased the plasma DPP-4 activity in both the sham and MI mice (see Supplementary material online, *Figure S2A*). The plasma active GLP-1 levels were similar between the sham and MI mice, and MK-0626 increased them (see Supplementary material online, *Figure S2B*). However, there were no significant differences in the changes of blood glucose up to 120 min or the area under the curve after the oral glucose load among the four treatment groups (see Supplementary material online, *Figure S2C* and *D*).

3.3 Exercise capacity and spontaneous physical activity

The work (*Figure 1A*), the run distance (*Figure 1B*), and the run time (*Figure 1C*) were significantly decreased in the mice that had an MI compared to the sham mice, and these values were significantly improved in the MI + MK-0626 mice (respectively, $P < 0.05$). Coincident with the limited exercise capacity, the peak oxygen uptake ($\dot{V}O2$) was significantly lower in the MI mice compared to the sham mice, and this decrease was ameliorated in the MI + MK-0626 mice (*Figure 1D*).

The peak respiratory exchange ratios were >1.0 and did not differ among the four groups of mice, indicating that the mice ran on the treadmill until exhaustion beyond anaerobic threshold in all groups (*Figure 1E*). MK-0626 did not affect these parameters in the sham mice.

The spontaneous physical activity tended to be decreased in the MI mice compared to the sham mice, and MK-0626 did not affect this activity in the sham mice or the MI mice (*Figure 1F*).

3.4 Citrate synthase activity in the whole muscle and in the isolated mitochondria

Citrate synthase (CS, a key enzyme of the tricarboxylic acid cycle) activity was significantly decreased in the skeletal muscle of the MI mice compared to the sham mice, and the decrease was inhibited in MI mice by

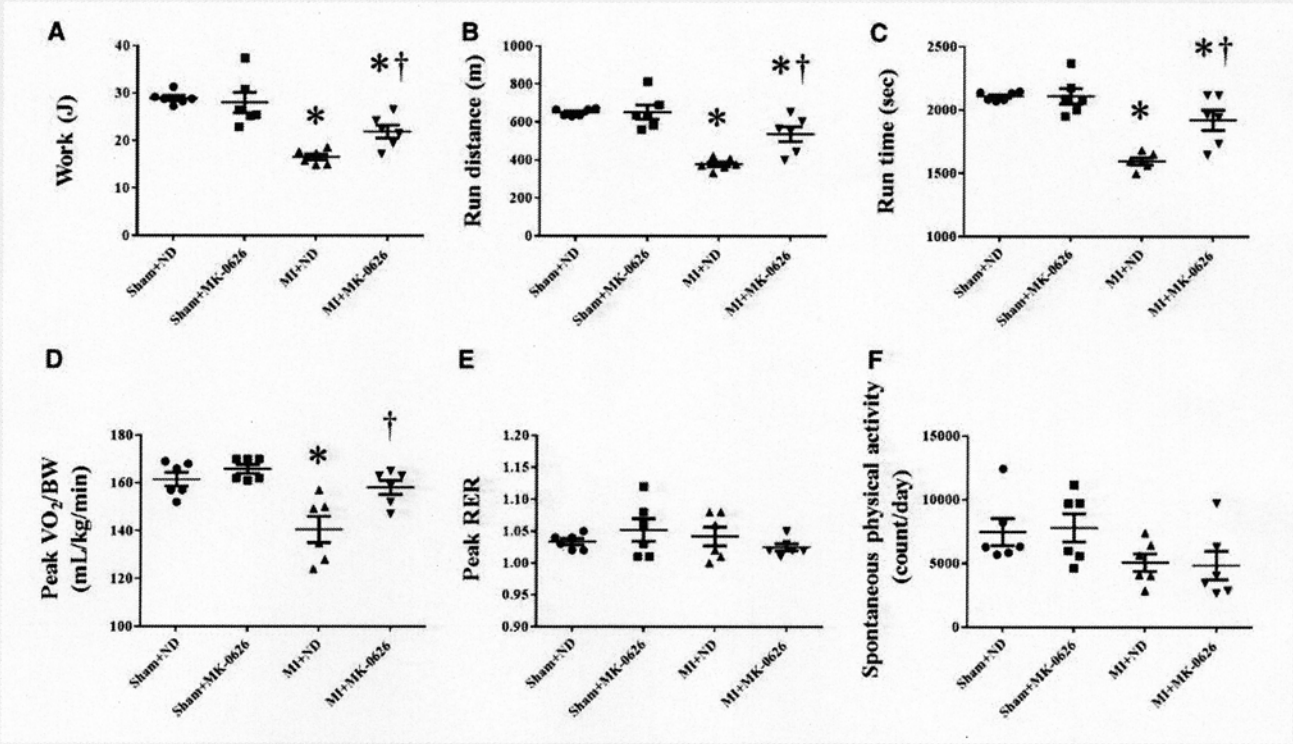

Figure 1 Exercise capacity and spontaneous physical activity. The summarized data of the (*A*) work, (*B*) run distance, (*C*) run time, (*D*) peak oxygen uptake ($\dot{V}O2$)/BW, (*E*) the peak respiratory exchange ratio (RER) to exhaustion, and (*F*) spontaneous physical activity from 4 groups of sham + ND, sham + MK-0626, MI + ND, and MI + MK-0626 mice ($n = 6$ for each group). Data are means ± SE. *$P < 0.05$ vs. sham + ND. †$P < 0.05$ vs. MI + ND.

MK-0626 (*Figure 2A*). This effect of MK-0626 on CS activity was not observed in the sham mice. CS activity in the isolated mitochondria did not differ among the four groups of mice (*Figure 2A*).

3.5 Mitochondrial OXPHOS capacity in permeabilized fibres

We used malate and pyruvate as the non-fatty acid substrate. The non-ADP-stimulated LEAK respiration normalized to muscle weight did not differ significantly among the four groups (*Figure 2B*). The ADP-stimulated OXPHOS respiration was significantly decreased in the MI + ND group, and it was inhibited by MK-0626 (*Figure 2B*). After the addition of glutamate, the same OXPHOS respiration results were observed (*Figure 2B*).

Octanoyl-l-carnitine was added to malate as the fatty acid substrate, and we evaluated the mitochondrial OXPHOS capacity. The non-ADP-stimulated LEAK respiration and ADP-stimulated OXPHOS respiration with only malate did not differ significantly among the four groups (*Figure 2C*). After the addition of octanoyl-l-carnitine, the ADP-stimulated OXPHOS respiration was significantly decreased in the MI + ND mice, and this decrease was inhibited by MK-0626 (*Figure 2C*).

3.6 Mitochondrial complex subunits

Cytochrome c oxidase subunit 1 was significantly decreased in the skeletal muscle from the MI mice compared to the sham mice, and this change was normalized in the MI + MK-0626 group (*Figure 3A*). The protein levels of mitochondrial complex subunits were significantly decreased in the whole skeletal muscle from the MI mice compared to the sham mice, and this change was normalized in the MI + MK-0626 mice (*Figure 3B*). These effects of MK-0626 were not observed in the sham mice. In contrast, the protein levels of mitochondrial complex subunits in the isolated mitochondria did not differ among the four groups of mice (*Figure 3C*). These results suggest that the mitochondrial content is decreased, and the function of each mitochondrion is preserved in the skeletal muscle of HF mice.

The electron microscopic analysis revealed that mitochondrial density was decreased in the MI + ND mice compared to the sham + ND mice and that MK-0626 normalized this decrease (*Figure 3D* and see Supplementary material online, *Figure S3*). Mitochondrial complexes form a supercomplex that contains complexes I/III, III/IV, and I/III/IV.[14] The proper and efficient channelling of electrons[15] and the structural stabilization of the respiratory chain[16,17] are achieved by the formation of this supercomplex. Here, in the MI + ND the mitochondrial supercomplex was decreased, and this decrease was improved by MK-0626 treatment (*Figure 3E*).

3.7 Proteins regulating mitochondrial biogenesis

We also observed that the protein levels of phosphorylated AMP-activated protein kinase α (AMPKα), sirtuin 1 (Sirt-1), peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1), and mitochondrial transcription factor A (Tfam) were significantly decreased in the hindlimb skeletal muscle from the MI mice compared to the sham mice, and these changes were normalized in the MI + MK-0626 group (*Figure 4*). These effects of MK-0626 were not observed in the sham mice.

3.8 Substrate metabolism

Both the level of phosphorylated protein of acetyl-CoA carboxylase-beta and the β-hydroxyacyl CoA dehydrogenase (a key enzyme of fatty acid β-oxidation) activity in the skeletal muscle were significantly decreased in the MI + ND mice compared to the sham + ND mice, and these decreases were inhibited by MK-0626 (*Figure 5A* and *B*).

On the other hand, there were no significant differences in the proteins of hexokinase II (HKII) and pyruvate kinase m2 (key enzymes of glycolysis) in the skeletal muscle among the four groups (*Figure 5C* and *D*). The activity of pyruvate dehydrogenase (a key enzyme of glycolysis) in the skeletal muscle was significantly increased in the MI + ND mice compared to the sham + ND group, but it was not affected by MK-0626 (*Figure 5E*). In contrast, the HKII protein level and the pyruvate dehydrogenase activity in the heart were significantly decreased in the MI + ND mice compared to the sham + ND mice, but these decreases were not affected by MK-0626 (see Supplementary material online,

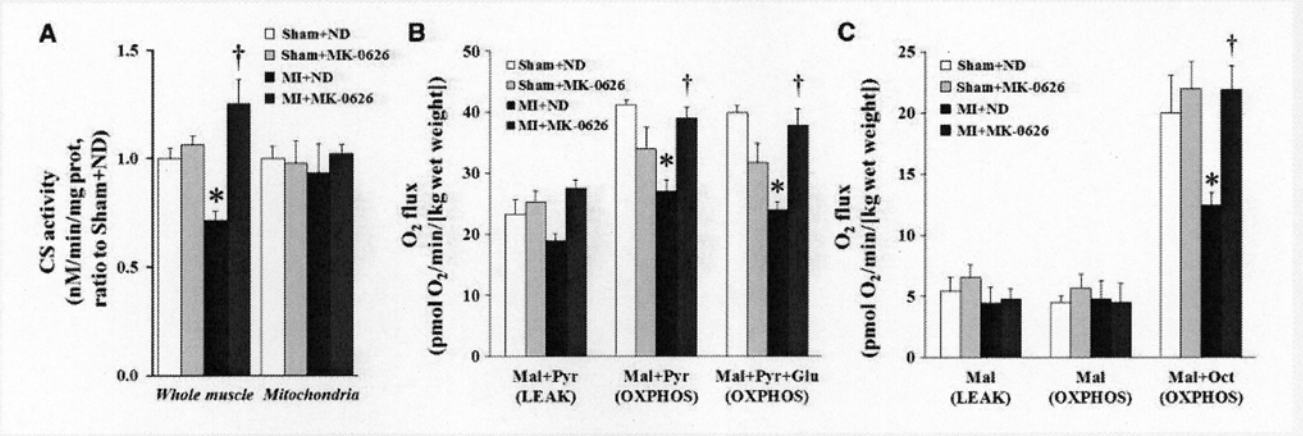

Figure 2 Mitochondrial functions in the skeletal muscle. The summarized data of (*A*) CS activity in the whole muscle ($n = 8$ for each group; left) and in the isolated mitochondria ($n = 9, 9, 7, 5$ for each group; *right*) from the four groups of mice. The mitochondrial OXPHOS capacity with (*B*) a non-fatty-acid substrate ($n = 7, 7, 4, 7$ for each group) and (*C*) a fatty acid substrate in permeabilized fibre in the skeletal muscle ($n = 7, 7, 4, 5$ for each group) from four groups of sham + ND, sham + MK-0626, MI + ND, and MI + MK-0626 mice. Data are means ± SE. *$P < 0.05$ vs. sham + ND. †$P < 0.05$ vs. MI + ND. Mal, malate; Pyr, pyruvate; Glu, glutamate; Oct, octanoyl-l-carnitine.

Figure S4A and *C*). In addition, there was no significant difference in the protein of pyruvate kinase m2 in the heart among the four groups (see Supplementary material online, *Figure S4B*).

3.9 Muscle fibre type, muscle mass, and autophagy

Figure 6A and *B* provides representative images of staining for myosin ATPase and succinate dehydrogenase. The fibre type staining revealed that the number of type I slow oxidative fibres was decreased in the MI mice compared to the sham mice, and it was increased in the MI + MK-0626 mice. In contrast, the number of type IIb fast glycolytic fibres was increased in the MI mice compared to the sham mice and decreased in the MI + MK-0626 group (*Figure 6D*). The number of type IIa fibres was not altered in four groups. These effects of MK-0626 on skeletal muscle fibre type were not observed in the sham mice.

There were no significant differences in the weights of skeletal muscle including the quadriceps, gastrocnemius, soleus (see Supplementary material online, *Table S1*), or in the muscle cell CSA among the four groups (*Figure 6C*). Similarly, there were no significant differences in the autophagy-related protein levels of phosphorylation of Ulk1, autophagy-related (Atg)12-Atg5, and LC3II and the mRNA expression levels of *becn1*, *Atg7*, *Atg4b*, and microtubule-associated protein 1 light chain 3 beta (*Map1lc3b*) between among the four groups of mice (see Supplementary material online, *Figure S5*).

3.10 Angiogenesis

For the evaluation of angiogenesis, we conducted immunostaining of the skeletal muscle with CD31, a marker of vascular endothelial cells. No significant difference in the CD31 levels was observed among the four groups of mice (see Supplementary material online, *Figure S6*).

3.11 Effects of GLP-1 on exercise capacity and mitochondrial function

The work was significantly increased in MI + Exendin-4 (Ex-4, GLP-1 receptor agonist) mice compared to the MI + vehicle (Ve) mice ($P < 0.05$) (*Figure 7A*). Similarly, mitochondrial complex I activity in the skeletal muscle was significantly increased in MI + EX-4 mice compared to the MI + Ve mice ($P < 0.05$) (*Figure 7B*).

The work was significantly increased in MI + MK-0626 + Ve mice compared to the MI + ND + Ve mice, and this was completely inhibited in the MI + MK-0626 + Ex-(9-39) (GLP-1 receptor antagonist) mice (respectively, $P < 0.05$) (*Figure 7C*). The mitochondrial complex I activity in the skeletal muscle was significantly increased in MI + MK-0626 + Ve mice compared to the MI + ND + Ve mice ($P < 0.05$), and this tended

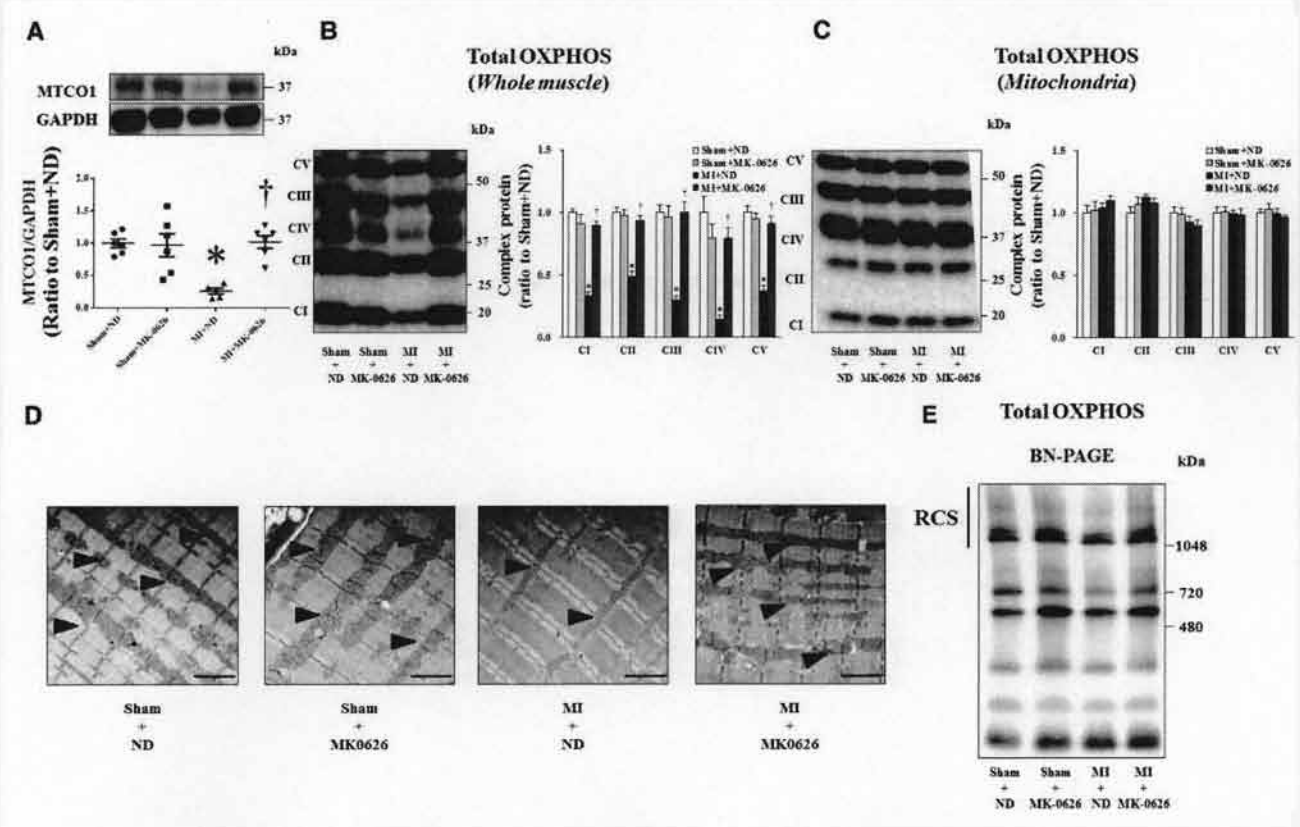

Figure 3 The mitochondrial quantities in the skeletal muscle. The protein levels of (*A*) MTCO1 ($n = 6, 6, 5, 6$ for each group), representative bands (left), and the summarized data (right) of the protein levels of mitochondrial OXPHOS complexes I–V in (*B*) the whole muscles ($n = 8$ for each group) and (*C*) the isolated mitochondria ($n = 9, 9, 7, 5$ for each group) obtained from four groups of sham + ND, sham + MK-0626, MI + ND, and MI + MK-0626 mice. (*D*) Representative transmission electron microscopy image in the skeletal muscle obtained from the four groups. Arrow indicates mitochondrion. Scale bar = 2 μm. (*E*) The representative bands of respiratory chain supercomplex (RCS) in the isolated mitochondria obtained from the four groups of mice. MTCO1, cytochrome c oxidase subunit 1. Data are means ± SE. *$P < 0.05$ vs. sham + ND. †$P < 0.05$ vs. MI + ND.

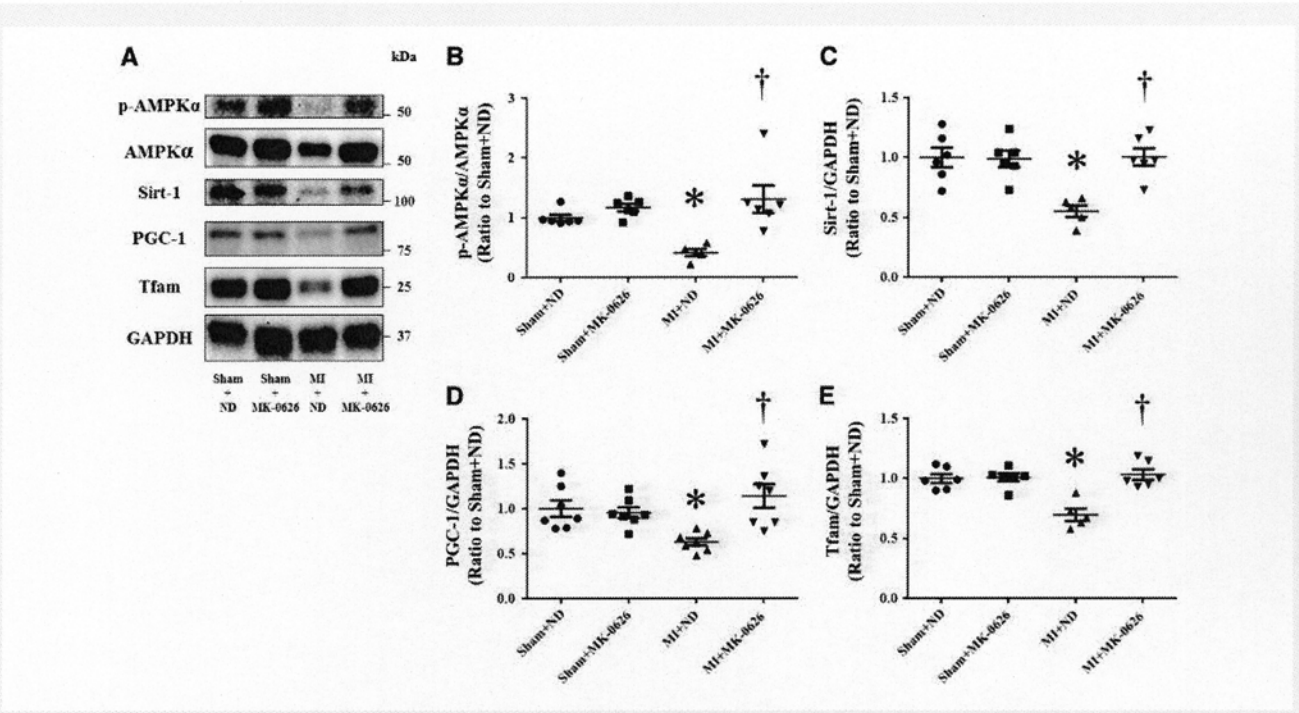

Figure 4 AMPK-Sirt1-PGC1 signalling in the skeletal muscle. The protein levels of (*A*) ^{T172}p-AMPKα/AMPKα, (*B*) sirt-1, (*C*) PGC-1, and (*D*) Tfam in the skeletal muscle from four groups of sham + ND, sham + MK-0626, MI + ND, and MI + MK-0626 mice ($n = 6, 6, 5, 6$ for each group). AMPK, AMP-activated protein kinase; sirt-1, sirtuin-1; PGC-1, peroxisome proliferator-activated receptor gamma coactivator 1; Tfam, mitochondrial transcription factor A. Data are means ± SE. $^{*}P < 0.05$ vs. sham + ND. $^{\dagger}P < 0.05$ vs. MI + ND.

to be inhibited in the MI + MK-0626 + Ex-(9-39) mice ($P = 0.07$) (*Figure 7D*).

4. Discussion

We studied a mouse model with HF-induced skeletal muscle abnormalities, and the results of our analyses demonstrated that the DPP-4 inhibitor MK-0626 significantly ameliorated the limited exercise capacity of mice with an MI, without affecting their LV function and remodelling. MK-0626 in the diet also shifted the fibre type from type IIb glycolytic fibre to type I oxidative fibre, and it normalized the mitochondrial oxidative capacity, CS activity, mitochondrial supercomplex formation, and fatty acid oxidation in the skeletal muscle of the MI mice. These characteristics were accompanied by the normalization of decreased protein levels of p-AMPKα, Sirt-1, PGC-1, and Tfam, which regulate mitochondrial biogenesis. These beneficial effects of MK-0626 were independent of glucose lowering and glucose metabolism.

4.1 Limited exercise capacity and skeletal muscle abnormalities in HF after MI

In HF patients, limited exercise capacity is due to the abnormalities of the energy metabolism in the skeletal muscle,[1,18–20] which depends on mitochondrial function. However, at this time, it is not clear how mitochondrial function in the skeletal muscle is impaired in HF. We created an HF-induced model with skeletal muscle abnormalities and limited exercise capacity to investigate this question. In our MI mouse model, we observed significant reductions of the CS activity, the number of mitochondrial OXPHOS complex subunits in the whole muscle, and the mitochondrial respiration in permeabilized fibres (*Figures 2* and *3B*). Similarly, the mitochondrial supercomplexes and the mitochondrial density in the skeletal muscle were decreased in the MI mice compared to the sham + ND mice (*Figure 3A*, *D*, and *E* and see Supplementary material online, *Figure S3*).

These findings can be explained by the results of mitochondrial biogenesis-related signalling protein. The protein levels of phosphorylated AMPK and Sirt-1, PGC-1, and Tfam were significantly decreased in the skeletal muscle from the MI mice compared to the sham mice (*Figure 4*). These signalling pathways are crucial in the metabolic flexibility of the skeletal muscle, and they regulate the substrate metabolism balance between fatty acid and glucose oxidation.[21] The pathways are also associated with the fibre type composition in the skeletal muscle. Our results suggest that in the HF mice, the main substrate of energy production in the skeletal muscle was shifted from fatty acid to glucose oxidation, and the fibre type shifted from slow twitch to fast twitch (*Figures 2C*, *5*, and *6*), which is one of the determinants of aerobic capacity.[6,22]

Previous articles showed that main determinants of exercise capacity in patients with HF was skeletal muscle abnormalities but not central circulatory disturbances including cardiac dysfunction.[6] However, cardiac function can affect a part of exercise capacity. On the other hand, main determinants of exercise capacity in mice model of HF have never been known. Although mitochondrial function and density in the skeletal muscle was completely improved by MK-0626 treatment in MI mice (*Figures 2*, *3*, and see Supplementary material online, *Figure S3*), work was significantly and partially improved in this study (*Figure 1*). These results suggest that some of the impaired exercise capacity is affected by other factors including cardiac dysfunction other than skeletal muscle abnormalities.

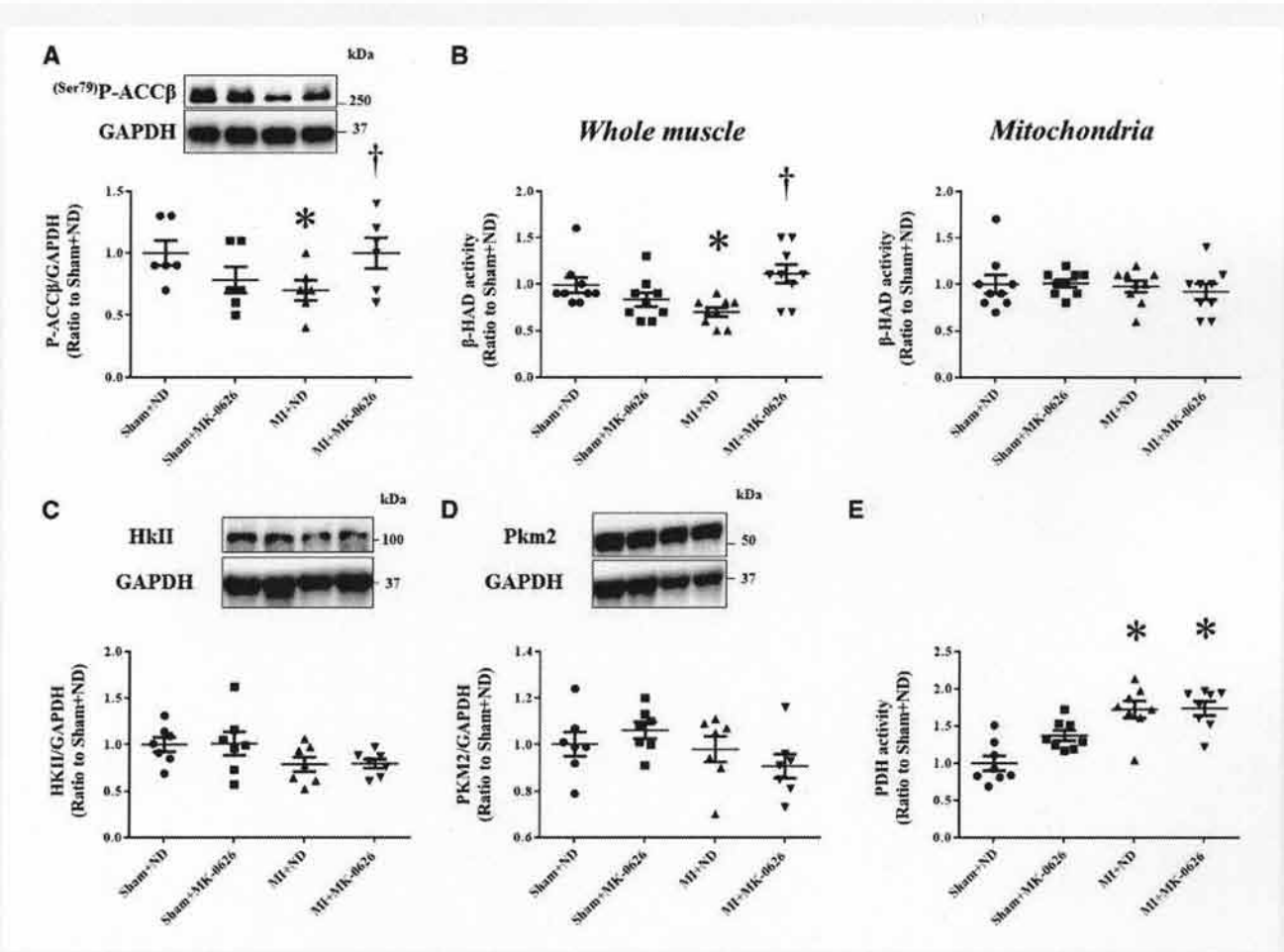

Figure 5 Fatty acid and glucose metabolism in the skeletal muscle. The summarized data of the protein expression of (*A*) ^{Ser79}p-acetyl-CoA carboxylase β ($n =$, 6, 5, 6 for each group), (*B*) β-hydroxyacyl-CoA dehydrogenase activity in the whole muscles ($n =$, 10, 9, 9 for each group) and the isolated mitochondria ($n =$ for each group), protein expression of (*C*) HKII, (*D*) pyruvate kinase (PK)M2 ($n =$ for each group), and (*E*) pyruvate dehydrogenase (PDH) activity ($n =$ for each group) in skeletal muscle from 4 groups of sham + ND, sham + MK-0626, MI + ND, and MI + MK-0626 mice. Data are means ± SE. *$P < 0.05$ vs. sham + ND. †$P < 0.05$ vs. MI + ND.

4.2 Effects of MK-0626 on the skeletal muscle abnormalities

The most important findings in this study were that MK-0626 improved both the skeletal muscle abnormalities and the limited exercise capacity in the MI mice. This included mitochondrial biogenesis and a substrate and fibre-type shift (*Figures 2*, *3*, *5*, *6*, and see Supplementary material online, *Figure S3*). These favourable effects of MK-0626 were due to the increased phosphorylation of AMPK (*Figure 4*). A single 1 mg/kg oral dose of MK-0626 has been shown to inhibit DPP-4 activity by more than 90% and increase the active GLP-1 levels by up to 2–3-fold in mice.[23] In this study, MK-0626 at the dose of 1 mg/kg BW/day significantly inhibited the plasma DPP-4 activity by 42% and increased the plasma active GLP-1 levels by 1.6-fold, reaching 1 pmol/L (see Supplementary material online, *Figure S2A* and *B*). It has been reported that AMPK was phosphorylated through a GLP-1-dependent mechanism by the administration of a DPP-4 inhibitor.[24] The effects of MK-0626 may thus be due to the increased active GLP-1 levels. To investigate the effects of GLP-1 on exercise capacity and skeletal muscle mitochondrial function, we treated MI mice with GLP-1 receptor agonist (Ex-4). Exercise capacity and mitochondrial function in the skeletal muscle were significantly improved by GLP-1 receptor agonist compared to vehicle in MI mice (*Figure 7*). To further investigate the role of GLP-1 in the favourable effect of MK-0626, we treated MI mice with MK-0626 and GLP-1 receptor antagonist (Ex-(9-39)) or vehicle. The treatment with Ex-(9-39) cancelled the favourable effects of MK-0626 (*Figure 7*).

In addition, MK-0626 did not affect the exercise capacity, mitochondrial biogenesis, metabolism, or muscle fibre type in the sham-operated mice. These results suggested that MK-0626 directly affected the causes of impaired metabolism and the muscle fibre type in MI mice, possible through an increase in GLP-1.

4.3 Other possible mechanisms for the favourable effects of MK-0626

DPP-4 has many substrates, including glucose-dependent insulinotropic polypeptide, stromal cell-derived factor (SDF)-1α, substance P, and neuropeptide Y.[25,26] SDF-1α was reported to promote angiogenesis,[27,28] which may contribute to the beneficial effect of MK-0626. We measured the plasma SDF-1α levels and found that they were similar between the

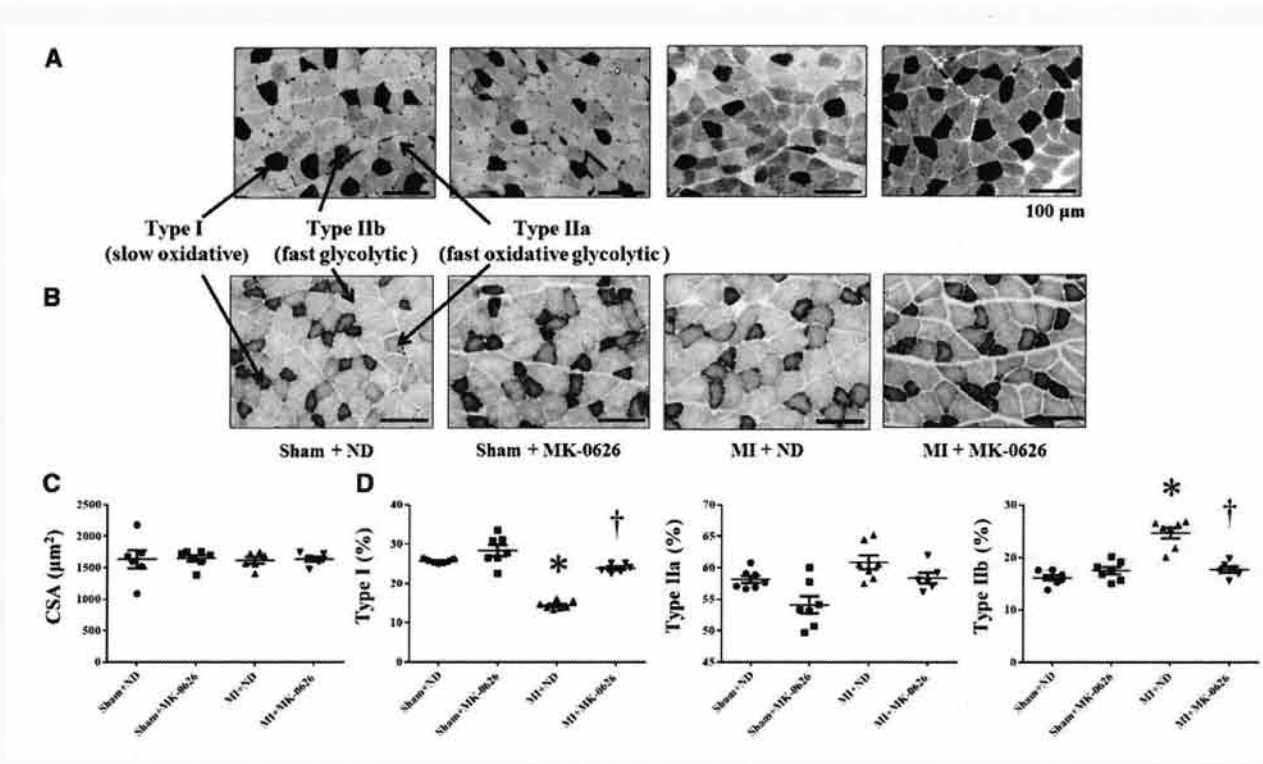

Figure 6 Skeletal muscle fibre types. Representative high-power photomicrographs of skeletal muscle tissue sections stained with (*A*) myosin ATPase and (*B*) succinate dehydrogenase (SDH) from the four groups of mice. Scale bar, 100 μm. The summarized data of (*C*) myocyte CSA ($n = 6, 7, 6, 6$ for each group) and (*D*) the quantitative analysis of SDH staining in the skeletal muscle from 4 groups of sham + ND, sham + MK-0626, MI + ND, and MI + MK-0626 mice ($n = 7, 7, 7, 6$ for each group). Data are means ± SE. $^*P < 0.05$ vs. sham + ND. †$P < 0.05$ vs. MI + ND.

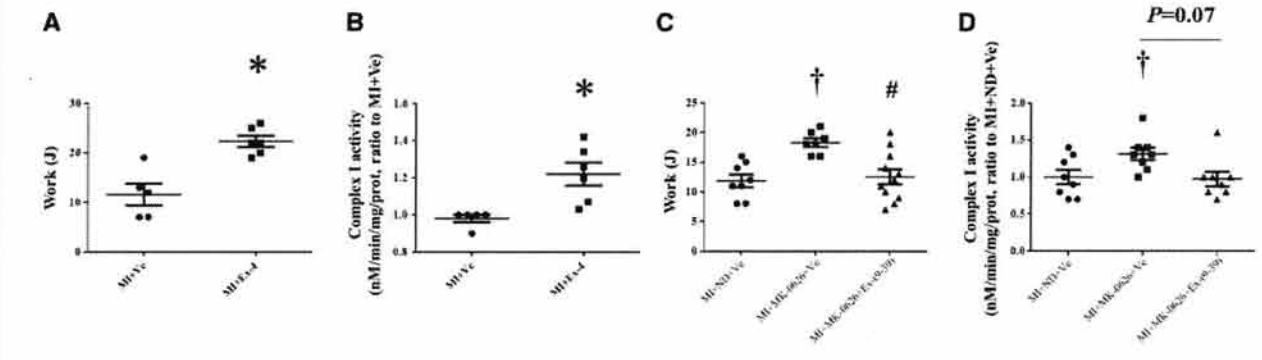

Figure 7 GLP-1 receptor agonist and antagonist in exercise capacity and mitochondrial function in the skeletal muscle. The summarized data of (*A*) work and (*B*) mitochondrial complex I activity in the skeletal muscle from the MI + Ve ($n = 5$) and MI + Ex-4 (GLP-1 agonist, $n = 6$) group. The summarized data of (*C*) work and (*D*) mitochondrial complex I activity in the skeletal muscle from the MI + ND + Ve ($n = 8$), MI + MK-0626 + Ve ($n = 7$), and MI + MK-026 + Ex-(9-39) (GLP-1 agonist, $n = 11$) group. Ve, vehicle; Ex, exendin. Data are expressed as means ± SE. $^*P < 0.05$, vs. MI + Ve; †$P < 0.05$, vs. MI + ND + Ve; #$P < 0.05$, vs. MI + Ve.

sham and MI mice (0.56 ± 0.03 vs. 0.57 ± 0.05, P = NS), and MK-0626 did not affect the SDF-1α levels in the MI mice (0.54 ± 0.09 ng/mL). CD31 staining revealed that there was no significant difference in the angiogenesis of skeletal muscle among the four groups of mice (see Supplementary material online, *Figure S6*). We therefore concluded that angiogenesis does not have a major role in the favourable effects of MK-0626.

MK-0626 did not affect the glucose metabolism or insulin (see Supplementary material online, *Figure S2C and D* and *5C–E* and *Table S1*). Therefore, the effects of MK-0626 on exercise capacity and skeletal muscle were not due to a glucose-lowering effect. In a previous study, the DPP-4 inhibitor vildagliptin at a dose of 10 mg/kg BW/day in mice with transverse aortic constriction increased the plasma active GLP-1 levels by 3.2-fold to 3 pmol/L, and it improved glucose metabolism and

cardiac function.[29] Inthachai et al.[30] reported that the treatment with vildagliptin (3 mg/kg BW/day) for 8 weeks partially improved cardiac remodelling after MI in rats compared to the treatment with vehicle. In contrast, we observed that the treatment with MK-0626 (sitagliptin analogue, 1 mg/kg BW/day) for 4 weeks did not altered cardiac remodelling after MI in mice compared to the treatment with vehicle (see Supplementary material online, *Table S1* and *Figure S1*). Therefore, these discrepancies might be due to the differences in kind (vildagliptin vs. sitagliptin analogue) and dose (3 mg/kg BW/day vs. 1 mg/kg BW/day) of DPP-4 inhibitor, animal species used in the experiments (rats vs. mice), period of drug administration (8 weeks vs. 4 weeks), and degree of infarct size (about 35% vs. 55%). Importantly, the effects of MK-0626 might not be due to its favourable metabolic or hemodynamic effects in our model.

Earlier studies reported that skeletal muscle atrophy often occurs in HF patients.[6,22] GLP-1 and its analogues have been reported to confer benefit by activating autophagy in β cells and hepatocytes.[31,32] However, MK-0626 did not affect the autophagy-related protein and mRNA levels in the skeletal muscle in this study (see Supplementary material online, *Figure S5*). Jannig et al.[33] reported that autophagy activates in the atrophic skeletal muscle from MI mice. However, we observed no significant differences in BW, skeletal muscle weight, CSA, or autophagy between the sham and MI mice, and these parameters were not affected by MK-0626 treatment (see Supplementary material online, *Table S1* and *Figure S5*). Therefore, since the MK0626 treatment did not affect autophagy, skeletal muscle atrophy did not occur in our HF model mice.

4.4 Study limitations

This study has several limitations. First, MK-0626 did not affect the exercise capacity, mitochondrial biogenesis, metabolism, or muscle fibre type in the sham mice as it did in the MI mice. These results suggested that MK-0626 directly affected the causes of the impairment of mitochondrial biogenesis in the MI mice. However, the detailed molecular mechanisms responsible for the improvement of mitochondrial biogenesis by MK-0626 have not been explored. Further studies are needed to elucidate the effects by DPP-4 inhibitor treatment. Second, we used only MK-0626 and did not examine the effects of other DPP-4 inhibitors. We thus cannot speculate whether our findings are specific for MK-0626 or whether other DPP-4 inhibitors could provide similar beneficial effects.

4.5 Clinical perspective

Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus—Thrombolysis in Myocardial Infarction (SAVOR-TIMI 53) trial reported that saxagliptin treatment was associated with an increased risk for hospitalization for HF in patients with diabetes mellitus.[34] In contrast, the Examination of Cardiovascular Outcomes with Alogliptin vs. Standard of Care (EXAMINE) trial and the Trial Evaluating Cardiovascular Outcomes with Sitagliptin showed that alogliptin and sitagliptin did not increase the overall risk of hospitalization for HF.[35,36] Recent meta-analysis of randomized clinical trials and multicentre observational study reported that DPP-4 inhibitors were not associated with an increased risk of hospitalization for HF.[37,38] On the other hand, it has never been known whether DPP-4 inhibitors worsen the outcomes in patients with HF. In a secondary exploratory analysis of the EXAMINE trial, alogliptin did not increase the risk of hospitalization for heart HF among patients with a history of HF.[39] Therefore, there is no evidence that DPP-4 inhibitors worsen the outcomes in patients with HF. Moreover, it has never been investigated whether DPP-4 inhibitor affect functional capacity in patients with HF. We clearly showed that MK-0626, sitagliptin analogue, improved exercise capacity in the model of HF, and our results suggested to verify whether the effect of DPP-4 inhibitors on exercise capacity in patients with HF was very significant.

5. Conclusions

After MI in the mice, MK-0626 improved the exercise capacity and normalized the mitochondrial function and fatty acid oxidation, based on the improvement of mitochondrial biogenesis as well as the skeletal muscle fibre type switch. Importantly, these beneficial effects of MK-0626 on exercise capacity were independent of glucose metabolism and cardiac function, and capillary density. DPP-4 inhibitors may be a novel therapeutic agent against skeletal muscle dysfunction in HF.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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Conflict of interest: none declared.

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Serum myostatin levels are independently associated with skeletal muscle wasting in patients with heart failure

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ABSTRACT

Background: It has been reported that skeletal muscle mass and strength are decreased in patients with heart failure (HF), and HF is associated with both reduced exercise capacity and adverse clinical outcomes. Myostatin has been known as a negative regulator of muscle growth, follistatin as the myostatin antagonist, maintaining tissue homeostasis. We thus determined serum myostatin levels in HF patients and whether they are associated with skeletal muscle wasting.

Methods and results: Forty one consecutive HF patients (58 ± 15 years old, New York Heart Association class I–III) and 30 age-matched healthy subjects as controls (53 ± 8 years old) were studied. Serum myostatin levels were significantly lower in HF patients than controls (18.7 ± 7.4 vs. 23.6 ± 5.2 ng/mL, $P < 0.001$). Circumference of the thickest part of the right thigh was significantly small (468 ± 72 vs. 559 ± 37 mm, $P = 0.001$) and lower extremity muscular strength was lower in patients with HF (129 ± 55 vs. 219 ± 52 N × m, $P < 0.001$). Fourteen HF patients (34%) had muscle wasting. By univariate analysis, higher age, higher serum follistatin, and lower serum myostatin were significantly associated with the presence of muscle wasting. By multivariate analysis, serum myostatin levels were independently associated with muscle wasting (OR = 0.77, 95% CI [0.58, 0.93], $P = 0.02$).

Conclusion: Serum myostatin levels were significantly decreased in HF patients and associated with lower extremity muscle wasting, suggesting that myostatin may be an important factor for maintaining skeletal muscle mass and strength in HF.

1. Introduction

Skeletal muscle abnormalities including impaired muscle energy metabolism, transition of fiber type, and muscle atrophy are frequently observed in patients with heart failure (HF), which is not always related to resting cardiac function, and may contribute to symptoms such as fatigue and dyspnea [1]. These muscle abnormalities are associated with both reduced aerobic exercise capacity and adverse clinical outcomes [2]. It has been also reported that muscle strength and muscle mass are an independent predictor of adverse cardiac event in patients with HF [3,4]. Muscle strength is closely associated with muscle mass. Therefore, to clarify the regulation of muscle mass in HF is an important issue.

Myostatin, a member of the transforming growth factor-β superfamily maintaining tissue homeostasis, has been known as a negative regulator of muscle growth in mammals and an increase in its expression is reported to be involved in a decrease in muscle mass [5]. Indeed, a child whose myostatin gene naturally occurred a loss-of-function mutation had greater quadriceps muscle in the cross-sectional area assessed by echography than age- and sex-matched controls [6]. Myostatin also plays a crucial role in regulating adult muscle growth and size. Mice with conditional postnatal inactivation of the myostatin gene showed that muscular hypertrophy in skeletal muscle was induced by increasing the size of muscle fibers rather than their number [7].

It was reported that plasma myostatin levels were shown to be increased in HF patients compared to in healthy controls [8]. However, this study has not reported the association between myostatin level and muscle wasting. On the other hand, in cardiac cachexia, characterized by a severe loss of skeletal muscle, weakness, and exercise intolerance, serum myostatin levels were decreased [9]. Therefore, it is highly

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controversial whether myostatin levels were increased or decreased in patients with HF. In the present study, we thus determined whether serum myostatin levels were altered in HF patients and were associated with skeletal muscle wasting.

2. Methods

2.1. Patient subjects

Forty one consecutive patients suffering from HF (31 men, 58 ± 15 years, left ventricular ejection fraction (LVEF) 32.9 ± 10.8%) and 30 age-matched healthy individuals as controls (26 men, 52 ± 8 years, LVEF 61.9 ± 5.9%) were studied in the present study. HF was diagnosed on the basis of the Framingham criteria by 2 or more cardiologists [10]. Informed consent was obtained from all participating subjects and the protocol, conformed to the ethical guidelines of the Declaration of Helsinki, was approved by the medical ethics committee of Hokkaido University Hospital.

2.2. Demographic, clinical characteristics, and body composition

Causes of HF were determined based on medical information. Body weight and height were measured, and body mass index (BMI) (body weight/[height]^2, kg/m^2) was calculated. Air displacement plethysmograph, termed BOD POD (Life Measurement Instruments, Concord, CA, USA), was used to evaluate body composition. The BOD POD measures total lean body weight and total fat weight, which is highly reliable method in Japanese population and is considered to be accurate as much as Dual Energy X-ray Absorptiometry (DEXA) [11,12]. The appendicular lean body mass (aLBM) was estimated from height and total fat weight as follows [13]:

$$\text{aLBM (kg) for men} = -22.48 + 24.14 \times \text{height (m)} + 0.21 \times \text{total fat mass (kg)}$$

$$\text{aLBM (kg) for women} = -13.19 + 14.75 \times \text{height (m)} + 0.23 \times \text{total fat mass (kg)}$$

2.3. Assessment of muscle strength

The knee extension strength was assessed using an isokinetic dynamometer (Multitrace 2, Lectromed, Jersey, Channel Islands). The maximal strength was measured in both legs in a sitting position with the patient's legs hanging freely, the ankle fixed by a pressure transducer. The best of three measurements was used. Arm strength was analyzed using the handgrip dynamometer (Saehan Corporation Korea Hydraulic Hand Dynamometer, model SH5001). Likewise, the best of three measurements was used.

2.4. Definition of muscle wasting

Muscle wasting was defined according to previously published criteria suggested to diagnose sarcopenia. According to previous study, we defined muscle wasting as both an aLBM and the knee extension strength 2 SD below the mean of a healthy young reference group aged 18–40 years [14].

2.5. Serum myostatin and follistatin, cytokines, and biochemistry

Peripheral venous blood samples were collected in serum tubes from all subjects between 6:00 and 9:00 am. All samples were allowed to clot before centrifuged at 1000 *g* for 15 min and were stored at −80 °C until analysis.

Serum myostatin levels were determined by a commercially available enzyme immunoassay kit (R&D System, Inc., Minneapolis, USA) according to the manufacturer's protocol as previously described [15] and its detection limit was 20 pg/mL. Serum follistatin levels were determined by a commercially available enzyme immunoassay kit (R&D System, Inc., Minneapolis, USA) according to the manufacturer's protocol as previously described [15] and its detection limit was 20 pg/mL.

Serum levels of interleukin (IL)-1β, IL-6, and the tumor necrosis factor-α (TNF-α) were analyzed using magnetic cytokine assays purchased from Bio-Rad Laboratories GmbH (Munich, Germany), the lower limits of detection being 0.1, 0.1, and 0.4 pg/mL, respectively.

Hemoglobin, serum albumin, fasting blood glucose, and B-type natriuretic peptide (BNP) were also measured. The estimated glomerular filtration rate (eGFR) was calculated from serum creatinine value and age using the Japanese equation as follows [16]:

$$\text{eGFR} = 194 \times (\text{serum creatinine in mg/dL})^{-1.094} \times (\text{age in years})^{-0.287} \times (0.739 \text{ if female}).$$

The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated from the fasting blood glucose (FBG) and fasting serum insulin (FIRI) concentrations by the formula: HOMA-IR = FBG (mg/dL) × FIRI (μU/mL) / 405.

All analyses were performed by investigators blinded to clinical information.

2.6. Echocardiography

Left ventricular (LV) end-diastolic dimension (EDD) and LV end-systolic dimension (ESD) were measured in the parasternal long axis view by transthoracic echocardiography. LVEF was measured with biplane Simpson's method via the apical 4- and 2-chamber views [17].

2.7. Cardiopulmonary exercise testing

Cardiopulmonary exercise testing was performed using an upright electromechanical bicycle ergometer (Aerobike 75XLII, Combi Wellness, Tokyo, Japan) with ramp protocol as described previously [18]. Briefly, after 3 min of unloaded cycling, the exercise load was increased in 10–15 W/min increments in HF patients and 25 W/min increments in control subjects to symptom-limited maximal work. Patients stopped exercise when they had severe leg fatigue and/or dyspnea. Oxygen uptake (VO_2) was measured at rest and throughout the exercise period using a 280E Aero-monitor (Aeromonitor AE-300S, Minato Medical Science, Osaka, Japan). Anaerobic threshold (AT) was determined by the V-slope method, as described previously [19]. Peak VO_2 was defined as the maximal VO_2 attained during exercise.

2.8. Statistical analysis

The effect size was calculated to be 1.355 based on the comparison of the serum myostatin levels between normal subjects and patients with chronic obstructive pulmonary disease by Ju et al. [20]. To detect the effect compared with the threshold change of 0 under the conditions of $\alpha = 0.05$, $\beta = 0.1$ and allocation ratio = 1.5 (HF/control), sample sizes of the study patients needed were calculated to be 24 for HF and 16 for control. Data are expressed as means ± SD for continuous variables and as numbers and percentages for categorical variables. Myostatin data was normally distributed as proven by the Shapiro–Wilk test. Student's *t* test was used to compare continuous variables. When data were not distributed normally, the Mann–Whitney *U* test was used. Chi-square test was used to compare categorical variables. Univariate linear regression model was used to determine the correlation between variables and serum myostatin levels. Multivariate linear regression analysis including variables with a P-value < 0.05 in the univariate model or clinical parameters was performed to identify the independent variables associated with serum myostatin. All analyses were performed using JMP 9.0.2 (SAS Institute Inc., Cary, NC, USA). The differences were considered statistically significant when P-values were less than 0.05.

3. Results

3.1. Baseline characteristics in controls and in patients with HF

The baseline characteristics of the study subjects are summarized in Table 1. Two groups were matched for age, male to female ratio, and BMI. There were 2 patients with NYHA functional class I, 28 patients with class II, and 11 patients with class III. The etiology of HF was ischemic cardiomyopathy in 11 patients, non-ischemic cardiomyopathy

Table 1
Clinical, echocardiographic, and cardiopulmonary exercise parameters in control subjects and in patients with HF.

	Controls (n = 30)	HF (n = 41)	P-value
Baseline characteristics			
Age, years (mean ± SD)	52 ± 8	58 ± 15	0.069
Male, n (%)	26 (87)	31 (76)	0.247
BMI, kg/m^2	23.8 ± 3.2	23.1 ± 4.1	0.462
NYHA (I/II/III)	-	2/28/11	
Cause of HF, n (%)			
Ischemic	-	11 (27)	
Non-ischemic	-	30 (73)	
Medical history, n (%)			
Hypertension	4 (13)	12 (29)	0.112
Diabetes mellitus	-	14 (33)	
Medication use, n (%)			
ACEI	-	24 (60)	
ARB	4 (13)	12 (29)	0.112
β-blocker	-	38 (93)	
Diuretics	-	32 (78)	
Echocardiographic parameters			
LV EDD, mm	46.6 ± 3.2	63.9 ± 11.0	<0.001
LV ESD, mm	30.2 ± 3.8	54.8 ± 12.6	<0.001
LVEF, %	61.9 ± 5.9	32.9 ± 10.8	<0.001
Cardiopulmonary exercise variables			
Peak VO_2, mL/kg/min	29.5 ± 6.7	13.6 ± 3.2	<0.001
AT, mL/kg/min	15.5 ± 4.1	8.8 ± 2.1	<0.001

Values are means ± SD; HF indicates heart failure; BMI, body mass index; NYHA, New York Association; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; LV, left ventricle; EDD, end-diastolic diameter; ESD, end-systolic diameter; EF, ejection fraction; VO_2, oxygen uptake; AT, anaerobic threshold.

in 30 patients, including idiopathic dilated cardiomyopathy, valvular heart disease and hypertensive heart disease. At the time of the study, patients were treated with angiotensin-converting enzyme inhibitors/angiotensin II type I receptor antagonists (ARB) (89%), β-blockers (93%), and diuretics (78%). Only four control subjects had hypertension and received ARBs (13%). Echocardiographic examination revealed that patients with HF had significantly larger LVEDD and ESD with a significantly reduced LVEF of 32.9 ± 10.8%. Peak VO_2 and anaerobic threshold were also lower in patients with HF.

3.2. Serum levels of neurohormones, muscle mass and strength

Serum levels of myostatin and follistatin have been shown in Fig. 1. Serum myostatin levels were significantly decreased in HF patients compared to control subjects (18.7 ± 7.4 vs. 23.6 ± 5.2 ng/mL, P = 0.0027) (Fig. 1A). On the contrary, serum follistatin levels were increased (2.7 ± 1.2 vs. 1.9 ± 0.6 ng/mL, P = 0.0015) (Fig. 1B).

The body composition, muscle strength, and blood biomarkers of the study subjects are summarized in Table 2. There were no significant differences in body weight and body fat weight. In contrast, lean body weight was lower in HF patients than in control subjects. Circumference of the thickest part of right thigh was significantly small (468 ± 72 vs. 559 ± 37 mm, P = 0.001) and grip strength (45 ± 9 vs. 33 ± 11 kg, P < 0.001) and lower extremity muscular strength (129 ± 54 vs. 219 ± 51 N × m, P < 0.001) were significantly decreased in patients with HF compared to control.

Hemoglobin, serum albumin, and eGFR were decreased in HF patients compared to control subjects. There were no significant differences in serum IL-6, TNF-α levels and renin activity between groups. In contrast, IL-1β was decreased in HF patients compared to control subjects. Plasma Ang II and BNP were increased in HF patients compared to control subjects.

3.3. Univariate and multivariate linear model of muscle wasting in patients with HF

The relationships between serum myostatin and parameters of muscle strength in lower extremity muscle were investigated. There was a significant positive correlation between serum myostatin levels and lower extremity muscular strength (r = 0.580, P = 0.0003) (Fig. 2A), and circumference of thigh (r = 0.481, P = 0.0022) among all study subjects (Fig. 2B).

By univariate analysis, higher age (OR = 1.06, 95% CI [1.01, 1.14], P = 0.04), higher serum follistatin (OR = 2.28, 95% CI [1.20, 5.80], P = 0.04), and lower serum myostatin (OR = 0.76, 95% CI [0.62, 0.88], P = 0.001) were significantly associated with the presence of muscle wasting.

Table 2
Body composition, muscle strength, and blood biomarkers in control subject and in patients with HF.

	Controls (n = 30)	HF (n = 41)	P-value
Body weight, kg	68 ± 9	63 ± 15	0.087
Body fat weight, kg	16 ± 6	16 ± 9	0.989
Lean body weight, kg	52 ± 6	46 ± 10	0.006
Circumference of thigh, cm	56 ± 4	47 ± 7	<0.001
Grip strength, kg	45 ± 9	33 ± 11	<0.001
Lower extremity muscular strength, N × m	219 ± 51	129 ± 54	<0.001
Hemoglobin, g/dL	14.8 ± 0.9	13.2 ± 1.8	<0.001
Serum albumin, g/dL	4.6 ± 0.3	4.1 ± 0.4	<0.001
eGFR, mL × min^{-1} × 1.73 m^{-2}	75.7 ± 13.2	60.2 ± 23.9	0.001
HOMA-IR	1.85 ± 1.91	2.31 ± 2.30	0.375
Interleukin-6, ng/mL	1.1 ± 3.0	1.0 ± 1.8	0.827
Tumor necrosis factor-α, pg/mL	0.095 ± 0.23	0.064 ± 0.17	0.516
Interleukin-1β, pg/mL	2.6 ± 2.4	0.50 ± 1.4	<0.001
Renin activity, ng/mL/h	2.5 ± 6.0	10.9 ± 11.3	0.220
Ang II, pg/mL	10.2 ± 13.0	22.5 ± 28.7	0.039
BNP, pg/mL	13.8 ± 9.04	403.8 ± 473.1	<0.001

Values are means ± SD; HF indicates heart failure; eGFR, estimated glomerular filtration rate; HOMA-IR, homeostasis model assessment – insulin resistance; Ang, angiotensin; BNP, B-type natriuretic peptide.

Multivariate analysis showed that serum myostatin levels were independently associated with muscle wasting (OR = 0.77, 95% CI [0.58, 0.93], P = 0.024) (Table 3).

4. Discussion

The major finding of the present study was that serum myostatin levels were significantly decreased and serum follistatin levels were increased in HF patients, and serum myostatin levels were independently associated with lower extremity muscle wasting.

Previous papers reported that myostatin expression was upregulated in the sheep's hearts after myocardial infarction and the rat HF model of chronic pressure overload [21,22]. In patients with HF, some reports have also shown an increase in serum myostatin levels [8,23]. However, in the present study, serum myostatin levels were significantly lower in HF patients than control subjects. Serum follistatin, known as the myostatin antagonist, levels were increased. Our results have been supported by previous reports that serum myostatin levels were decreased in HF patients with compensatory status, cachexia, or with treatment of exercise training [9,24,25]. Previous animal study reported that myostatin levels were increased in muscle atrophy due to denervation (a model of disuse), and stretching and electrical stimulation to muscle, which mimic exercise training, decreased myostatin levels [26]. Therefore, serum myostatin levels depend on the various conditions including severity of HF and treatment including exercise therapy. In the present study, all patients with HF were already compensated and about 70% of HF patients performed exercise training when they tested.

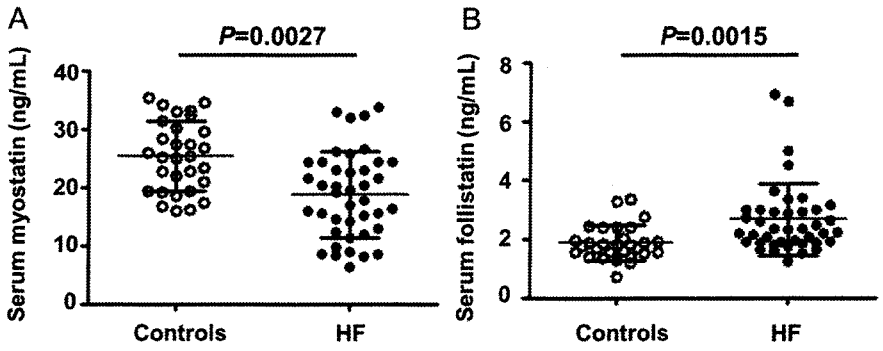

Fig. 1. Serum myostatin levels in control subjects (open circles, n = 30) and in patients with heart failure (closed circled, n = 41) (A), and serum follistatin levels in control subjects (open circles, n = 30) and in patients with heart failure (closed circles, n = 41) (B). HF indicates heart failure.

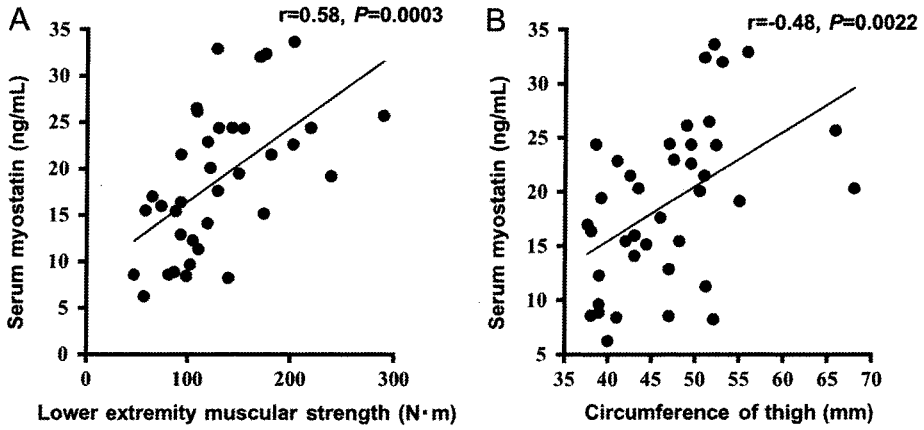

Fig. 2. Correlation between serum myostatin levels and lower extremity muscular strength (A), and correlation between serum myostatin levels and circumference of thigh (B) in patients with heart failure (n = 41).

Lower extremity muscular strength and circumference of thigh were significantly lower in HF patients than control subjects, and thus patients with HF in our cohort showed skeletal muscle wasting. By univariate and multivariate analysis, serum myostatin levels were significantly and independently associated with muscle wasting. Myostatin has been known as a negative regulator of muscle mass, and mainly expressed in and secreted from the skeletal muscle. In general, its increase leads to muscle atrophy, and its decrease leads to muscle hypertrophy. Therefore, muscle wasting in patients with HF might be caused by the increase in myostatin. However, our results suggest that lower serum myostatin and higher serum follistatin could be compensatory mechanisms of skeletal muscle for muscle wasting induced by HF.

The mechanisms for muscle wasting in patients with HF has never been know. Some animal studies have reported its molecular signal [27]. We previously reported that infusion of high-dose of angiotensin II into mice caused skeletal muscle atrophy via the activation of ubiquitin–proteasome pathway [28]. Other reports showed that inflammatory cytokines were increased in patients with HF [29]. Therefore, we investigated the association between serum inflammatory cytokines, renin activity, or angiotensin II levels and muscle wasting. However, we could not find significant association. This may suggest that several factors but not single factor are associated with muscle wasting. Other possible explanation for discrepancy between our results and animal studies is that we did not measure these in local level of the skeletal muscle. Further studies are needed to clarify the mechanisms for skeletal muscle wasting in patients with HF.

Table 3
Independent predictors of muscle wasting in patients with HF.

Variable	Univariate analysis		Multivariate analysis		
	OR	P-value	OR	95% CI	P-value
Age, years	1.06	0.038	1.03	0.95–1.16	0.411
Gender	0.41	0.224			
BMI, kg/m^2	0.61	0.005	0.66	0.37–0.95	0.080
LVEF, %	1.03	0.240	1.15	1.02–1.36	0.011
Peak VO_2, mL/kg/min	0.87	0.238			
Serum myostatin, ng/mL	0.76	0.001	0.77	0.58–0.93	0.024
Serum follistatin, ng/mL	2.28	0.036	1.89	0.59–8.44	0.374
Interleukin-6, ng/mL	0.68	0.291			
Tumor necrosis factor-α, pg/mL	0.29	0.571			
Interleukin-1β, pg/mL	0.49	0.314			
Plasma renin activity, ng/mL/h	0.95	0.156			
Plasma Ang II, pg/mL	0.98	0.195			
Plasma BNP, pg/mL	1.00	0.256			

Male and female were assigned values of 0 and 1, respectively. HF indicates heart failure; OR, odds ratio; CI, confidence interval; BMI, body mass index; LVEF, left ventricular ejection fraction; VO_2, oxygen uptake; Ang, angiotensin; BNP, B-type natriuretic peptide.

4.1. Study limitations

There are several limitations in our observational study, including its cross-sectional design, the relatively small sample size, and the lack of measurements of muscle mass by computed tomography, which is thought to be standard method of measurement of lower extremity muscle mass, to fully explain the complex underlying pathophysiology. However, our observations are unique, because the relationship between myostatin and skeletal muscle wasting in patient with HF has not been previously reported. Future studies are necessary to better understand the exact pathophysiology underlying the mechanism of how myostatin work in the body and whether the main source of myostatin is skeletal muscle.

4.2. Clinical implication

During the acute phase, bed in rest causes the progressive decline in skeletal muscle in patients with HF, especially elderly, which leads to the impairment in activities of daily living. This is one of clinical problem to be resolved. The increase in myostatin level could be associated with the development in this process. At the chronic phase, minimum stimulation to the muscle (i.e. standing, walking, and exercise training) inhibits an excess myostatin expression in or secretion from muscle, which maintains muscle mass. In stable patients with HF, serum myostatin is secreted from skeletal muscle and their levels reflect muscle mass, which could be a novel marker for muscle mass.

5. Conclusions

Serum myostatin levels were significantly decreased in HF patients and associated with lower extremity muscle wasting, suggesting that myostatin may be important factor for maintaining skeletal muscle mass and strength in this disease state.

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Disclosures

The authors have no conflicts of interest to disclose.

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Cardiovascular pharmacology

Direct renin inhibitor ameliorates insulin resistance by improving insulin signaling and oxidative stress in the skeletal muscle from post-infarct heart failure in mice

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ABSTRACT

Insulin resistance can occur as a consequence of heart failure (HF). Activation of the renin-angiotensin system (RAS) may play a crucial role in this phenomenon. We thus investigated the effect of a direct renin inhibitor, aliskiren, on insulin resistance in HF after myocardial infarction (MI). MI and sham operation were performed in male C57BL/6 J mice. The mice were divided into 4 groups and treated with sham-operation (Sham, n=10), sham-operation and aliskiren (Sham+Aliskiren; 10 mg/kg/day, n=10), MI (n=11), or MI and aliskiren (MI+Aliskiren, n=11). After 4 weeks, MI mice showed left ventricular dilation and dysfunction, which were not affected by aliskiren. The percent decrease of blood glucose after insulin load was significantly smaller in MI than in Sham (14 ± 5% vs. 36 ± 2%), and was ameliorated in MI+Aliskiren (34 ± 5%) mice. Insulin-stimulated serine-phosphorylation of Akt and glucose transporter 4 translocation were decreased in the skeletal muscle of MI compared to Sham by 57% and 69%, and both changes were ameliorated in the MI+Aliskiren group (91% and 94%). Aliskiren administration in MI mice significantly inhibited plasma renin activity and angiotensin II (Ang II) levels. Moreover, (pro)renin receptor expression and local Ang II production were upregulated in skeletal muscle from MI and were attenuated in MI+Aliskiren mice, in tandem with a decrease in superoxide production and NAD(P)H oxidase activities. In conclusion, aliskiren ameliorated insulin resistance in HF by improving insulin signaling in the skeletal muscle, at least partly by inhibiting systemic and (pro)renin receptor-mediated local RAS activation, and subsequent NAD(P)H oxidase-induced oxidative stress.

1. Introduction

Insulin resistance is highly prevalent and an established risk factor for heart failure (HF), and it has been associated with reduced functional capacity and poor prognosis (Doehner et al., 2005; Ingelsson et al., 2005; Lopaschuk et al., 2010). Conversely, HF itself is known to trigger the occurrence of insulin resistance, accounting for a vicious cycle of functional exacerbation of these two conditions (AlZadjali et al., 2009; Witteles et al., 2004). Indeed, the peripheral effects of insulin resistance are likely to represent a major metabolic feature of the pathophysiology of HF, contributing to key clinical symptoms such as breathlessness and early muscle fatigue (Kinugawa et al., 2015; Okita et al., 2013; Wilson et al., 1993). Multiple mechanisms of insulin resistance have already been identified, including increased oxidative stress and hyperactivation of the renin-angiotensin system (RAS) (Of®-cers et al., 2002; Wei et al., 2006). We previously reported that insulin resistance was induced in experimental HF in mice (Ohta et al., 2011), and a later study showed that this induction was accompanied by increased local angiotensin II (Ang II) in the skeletal muscle and subsequent NAD(P)H oxidase-derived oxidative stress (Fukushima et al., 2014). In addition, the recent discovery of a (pro)renin receptor for renin and its precursor, prorenin, raises the possibility that these components of RAS may play significant pathophysiological roles in the insulin resistance of rats with high fructose diet-induced diabetes or in post-infarct HF mice (Fukushima et al., 2014; Nagai et al., 2009).

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Aliskiren is a potent direct renin inhibitor that blocks the first rate-limiting step in RAS, preventing the compensatory rise in plasma renin activity and other downstream components of this system from occurring during angiotensin converting enzyme (ACE) inhibitor or Ang II receptor blocker treatment (Gradman and Traub, 2007). Aliskiren has been shown to protect against the development of insulin resistance in an animal model of diabetes by improving skeletal muscle glucose transport as well as to improve insulin sensitivity in hypertensive patients with metabolic syndrome (Fogari et al., 2010; Iwai et al., 2010; Marchionne et al., 2012). In addition, it has been reported that aliskiren can inhibit the free form of mature renin and the (pro)renin receptor-bound forms of renin and prorenin, suggesting that these dual inhibitory effects play roles in both systemic RAS and (pro)renin receptor-mediated tissue RAS (Biswas et al., 2010). To date, clinical trials to investigate the effect of aliskiren on myocardial infarction (MI) and HF have failed to improve outcomes by administration of aliskiren in combination with an ACE inhibitor or Ang II receptor blocker (Gheorghiade et al., 2013; Solomon et al., 2011). However, it remains to be determined whether low-dose single treatment with aliskiren could ameliorate the insulin resistance associated with MI and HF.

In the present study, we examined the effects of aliskiren on the insulin resistance and the insulin signaling in the skeletal muscle from post-infarct HF mice, mainly focusing on its effects on the (pro)renin receptor-mediated tissue RAS and oxidative stress in the skeletal muscle.

2. Materials and methods

All procedures and animal care were approved by our institutional animal research committee and conformed to the Guidelines for the Care and Use of Laboratory Animals of the Hokkaido University Graduate School of Medicine.

2.1. Experimental animals

Male C57BL/6J mice, 8–10 weeks old and 20–21 g body weight (BW), were maintained on a normal diet (CE-2; CLEA Japan, Tokyo) containing 4.2% fat and 54.6% carbohydrate. MI was established by ligating the left coronary artery as described previously (Fukushima et al., 2014; Kinugawa et al., 2000). Sham operation without ligation of the coronary artery was also performed. Each group of mice was then randomly divided into 2 groups, a group with and a group without aliskiren (10 mg/kg BW/day; Novartis Pharmaceuticals, Basel, Switzerland) administered subcutaneously for 4 weeks using an osmotic minipump (model 2004; Alzet, Palo Alto, CA). The non-depressor concentration of aliskiren was chosen on the basis of our preliminary data of blood pressure measurement by using the indirect tail-cuff method (MK-1030; Muromachi Kikai Co., Ltd., Tokyo, Japan) (Supplementary material). Experiments were performed at 4 weeks after operation in the following 4 groups: Sham (n=10), Sham+Aliskiren (n=10), MI (n=11), and MI+Aliskiren (n=11).

2.2. Echocardiographic and Hemodynamic measurements

Echocardiographic and hemodynamic measurements were performed under light anesthesia with tribromoethanol/amylene hydrate (avertin; 2.5% wt/vol, 8 μl/g BW ip), which has short duration of action and modest cardiodepressive effects and spontaneous respiration, as described previously (Fukushima et al., 2014; Ohta et al., 2011). Standard echocardiographic short- and long-axis views were obtained at the levels of the papillary muscles. Left ventricular function, ventricular size and wall thickness were measured from M-mode frames at a paper speed of 50 mm/s. To perform hemodynamic measurements, a 1.4 Fr micromanometer-tipped catheter (Millar Instruments, Houston, TX) was inserted into the right carotid artery and then advanced into the left ventricle (LV) to measure LV pressures.

2.3. Tissue preparation and organ histology

Heart, lung, and hindlimb skeletal muscle including the quadriceps, gastrocnemius, and soleus were excised 3 min after intraperitoneal injection of saline, with or without human regular insulin (1.0 U/kg BW) and weighed under deep anesthesia with avertin (2.5% wt/vol, 10 μl/g BW, ip). To determine the infarct size, myocyte cross-sectional area, and total collagen volume in cardiac tissue, ventricular tissue was fixed in 6% formaldehyde, cut into three transverse sections–the apex, middle ring, and base–and stained with hematoxylin-eosin or Masson's trichrome as described previously (Matsushima et al., 2009; Sobirin et al., 2012).

2.4. Plasma biochemical measurement

After the animals were fasted for 8 h, blood samples were collected from the inferior vena cava, the blood glucose level was determined using a glucometer (Glutest Ace R; Sanwa Kagaku Kenkyusho, Nagoya, Japan) and the plasma insulin was measured by an ELISA kit (Morinaga Institute, Kanagawa, Japan). The homeostasis model assessment index (HOMA-IR) was calculated using the formula of fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5. Total cholesterol, triglyceride, and nonesterified fatty acid (NEFA) were measured by a commercial ELISA kit (Wako Pure Chemical Industries, Osaka, Japan). The plasma renin activity level was determined using a SensoLyte 520 Renin Assay Kit (AnaSpec Inc., San Jose, CA). The plasma angiotensin (Ang) II level was measured by using an enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals Inc., Burlingame, CA) as previously described (Fukushima et al., 2014).

2.5. Intraperitoneal insulin tolerance test

For the insulin tolerance test, mice were injected intraperitoneally with human regular insulin (0.5 U/kg BW) and blood samples were collected before and 15, 30, 45, 60, 90, and 120 min after the injection. Blood glucose levels were determined using a glucometer (Glutest Ace R; Sanwa Kagaku Kenkyusho, Nagoya, Japan) (Takada et al., 2014). Data are shown as a percent change in blood glucose levels after insulin load.

2.6. Western blot analysis

Forty milligrams of frozen quadriceps skeletal muscle tissue was homogenized for 30 s with a Polytron homogenizer in a homogenization buffer containing 20 mM $NaHCO_3$, pH 7.0, 0.25 M sucrose, 5 mM NaN_3, 1 mM leupeptin, 1 mM aprotinin, and 1 mM pepstatin) at 4 °C. Twenty μg of denatured proteins was subjected to 8–12% SDS-PAGE on a polyvinylidene difluoride (PVDF) membrane as previously described (Takada et al., 2013). After blocking in 5% fat-free milk for 1 h, the membranes were probed with the following antibodies: ATP6P2/(pro)renin receptor (Abcam Inc., Cambridge, MA), Akt, phosphoserine Akt (Ser473), and glucose transporter 4 (GLUT4) (Cell Signaling Technology, Beverly, MA). The membranes were then incubated with the appropriate secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h. These bands were visualized by enhanced chemiluminescence and quantified with Image J software (NIH, Bethesda, MD). The resulting values were expressed as the ratio of target band intensity to total protein or internal control intensity. GAPDH (Cell

Signaling Technology) was used as an internal control to normalize the results and to control for blot-to-blot variation. Translocation of GLUT4 was also measured by the methods described previously (Fukushima et al., 2014; Mohammad et al., 2006). In short, subcellular membrane fractions were prepared by sequential differential centrifugation. The homogenate of quadriceps skeletal muscle was centrifuged at 1200g for 10 min to remove debris. The supernatant was then centrifuged at 9,000g for 10 min to allow mitochondria and nuclei to sediment. The resultant supernatant was centrifuged at 190,000g for 2 h at 4 °C, yielding a pellet of total membrane fraction and the remaining supernatant, which was designated the cytosol fraction. The membrane and the cytosol fractions (20 μg each lane) were subjected to immunoblot analysis as described above. GLUT4 translocation was assessed by the ratio of the membrane fraction to the cytosol fraction of total GLUT4 protein.

2.7. Quantitative reverse transcriptase PCR

Total RNA was extracted from hindlimb skeletal muscle tissues including the quadriceps and soleus in the 4 groups of mice with QuickGene-810 (FujiFilm, Tokyo, Japan) according to the manufacturer's instructions. The extracted total RNA (2 μg) was reverse-transcribed with a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Real-time quantitative RT-PCR was performed by using a 7300 real-time PCR system (Applied Biosystems) to amplify samples for angiotensinogen (Mm00599662_m1), ACE (Mm00802048_m1), Ang II type1 receptor (Mm00507771_m1), cathepsin D (Mm00515586_m1), ATP6AP2/(pro)renin receptor (Mm00510396_m1), $p47^{phox}$ (Mm00447921_m1), $p22^{phox}$ (Mm00514478_m1), and Nox2 (Mm01287743_m1) cDNA in the skeletal muscle. Relative mRNA was analyzed using a comparative $_2{}^{-\Delta\Delta_{CT}}$ method, and normalized to GAPDH as the internal control.

2.8. Immunohistochemistry for (P)RR

Quadriceps skeletal muscle tissues were fixed with 4% paraformaldehyde at 4 °C, and were immersed in 1% H_2O_2 in methanol to inhibit endogenous peroxidase. The sections were incubated with anti-ATP6P2/(pro)renin receptor antibody (Abcam Inc.) as a primary antibody, and then reacted with biotin-conjugated anti-rabbit IgG as the secondary antibody (DAKO, Carpinteria, CA). Immunohistochemical reactions were visualized by using a standard kit [3,3′-diaminobenzidine tetrahydrochloride (DAB); DAKO, CA, USA] as previously described (Fukushima et al., 2014; Satofuka et al., 2007).

2.9. Measurement of Ang II content in the skeletal muscle

Ang II content in the skeletal muscle tissues was measured as previously described (Fukushima et al., 2014). Briefly, homogenates of quadriceps skeletal muscle tissues were centrifuged at 15,000g for 20 min, and the supernatant was loaded on an equilibrated Sep-Pak C18 cartridge (Millipore, New York, NY), eluted with buffer (60% acetonitrile in 1% trifluoroacetic acid), and collected in a centrifuge tube. The eluant was then evaporated by using a centrifugal concentrator (Savant Speedvac, Thermo Scientific, Japan). Ang II content was determined using an EIA kit (Phoenix Pharmaceuticals Inc.) (de Resende et al., 2006).

2.10. Superoxide (O_2^-) production and NAD(P)H oxidase activity

The chemiluminescence elicited by O_2^- in the presence of lucigenin (5 μmol/l) was determined in quadriceps skeletal muscle tissues using a luminometer (AccuFLEX Lumi 400; ALOKA, Tokyo, Japan), as previously described (Takada et al., 2013; Yokota et al., 2009). The measurements were also performed in the presence of tiron (20 mmol/l), a cell-permeant, nonenzymatic scavenger of O_2^- to validate the chemiluminescence signals. NAD(P)H oxidase activity was examined in the homogenates from quadriceps skeletal muscle by the lucigenin assay after the addition of NAD(P)H (300 μmol/l).

2.11. Statistical analysis

Data are represented as the means ± S.E.M. Comparisons were performed using a one-way ANOVA followed by the Tukey's multiple-comparison test whenever differences were detected. Survival analysis was performed by the Kaplan-Meier method, and between-group differences in survival were tested using the log-rank test. In the intraperitoneal insulin tolerance test, differences between groups were determined with repeated-measures ANOVA. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of Aliskiren on mortality rates and cardiac dysfunction after MI

The mortality rate up to 4 weeks after operation was significantly lower in the MI + Aliskiren group compared to the MI group (16.8% vs. 46.2%, $P < 0.01$). No mice died after sham operation. Table 1 shows animal characteristics in the 4 groups of mice. The heart weight and lung weight/BW were significantly higher in MI compared to Sham mice. There was no difference in the weights of skeletal muscle, including the quadriceps, gastrocnemius, and soleus, among the 4 groups. The echocardiographic and hemodynamic data are also shown in Table 1. There were no significant differences in heart rate and mean aortic pressure among the 4 groups. MI mice exhibited greater LV diameters and lower LV fractional shortening than Sham mice. LV end-diastolic pressure (LVEDP) was significantly elevated, and both LV + and − dP/dt were decreased in MI mice compared to Sham mice. Consistent with the hemodynamic data, histopathological analysis revealed that the infarct size, myocyte cross-sectional area, and collagen volume fraction were all higher in MI compared to Sham mice (Table 1 and Fig. 1A, B). Treatment with aliskiren tended to attenuate LV dilatation and infarct size, to elevate end-diastolic pressure, and to improve systolic function in MI mice. However, there were no statistically significant differences in these parameters between the MI and MI + Aliskiren groups. In addition, aliskiren treatment did not affect these parameters in Sham mice (Table 1).

3.2. Effect of Aliskiren on insulin resistance in post-infarct HF mice

Fasting blood glucose levels were not different among the 4 groups, but the fasting plasma insulin levels were higher in MI mice than Sham mice and were normalized in the MI + Aliskiren group (Table 2). The HOMA index, an index of insulin resistance, was therefore greater in the MI than the Sham group and was also normalized in the MI + Aliskiren group (Table 2). Consistent with our previous study (Fukushima et al., 2014), the percent decrease of blood glucose 30 and 45 min after insulin load, as well as the area under the curve (AUC) of glucose response after insulin load, was significantly smaller in MI mice than Sham mice (Fig. 2A, B). Importantly, aliskiren administration significantly enhanced the decrease in blood glucose after insulin load in MI. Collectively, these results suggest that insulin resistance occurs in MI mice and can be ameliorated by aliskiren treatment. There were no significant differences in the percent change of blood glucose after glucose load and the AUC among the 4 groups (Supplementary

Table 1
Animal characteristics.

	Sham	Sham+Aliskiren	MI	MI + Aliskiren
N	10	10	11	11
Body and organ weight				
BW, g	25.9 ± 0.3	26.3 ± 0.4	25.8 ± 0.3	26.0 ± 0.5
Heart weight, mg	135.7 ± 2.3	138.9 ± 2.4	215.2 ± 6.2[a]	204.6 ± 12[a]
Lung weight/BW, mg/g	5.3 ± 0.0	5.3 ± 0.1	8.2 ± 0.9[a]	7.8 ± 0.9[a]
SKM weight/BW, mg/g	15.9 ± 0.2	15.9 ± 0.2	16.7 ± 0.2	15.9 ± 0.3
Echocardiography				
Heart rate, beats/min	427 ± 15	410 ± 26	476 ± 19	474 ± 30
LV EDD, mm	3.7 ± 0.1	3.5 ± 0.1	5.1 ± 0.1[a]	4.9 ± 0.1[a]
LV ESD, mm	2.4 ± 0.1	2.3 ± 0.1	4.4 ± 0.1[a]	4.2 ± 0.1[a]
Fractional shortening, %	33.8 ± 1.1	33.1 ± 2.8	14.1 ± 0.5[a]	17.2 ± 2.6[a]
AWT, mm	0.78 ± 0.01	0.75 ± 0.01	0.65 ± 0.02[a]	0.71 ± 0.02
PWT, mm	0.83 ± 0.02	0.78 ± 0.03	1.08 ± 0.03[a]	1.05 ± 0.03[a]
Hemodynamics				
Heart rate, beats/min	448 ± 19	465 ± 19	448 ± 12	460 ± 20
Mean aortic pressure, mmHg	78 ± 2	72 ± 2	78 ± 2	72 ± 2
LV EDP, mmHg	0.7 ± 0.1	0.9 ± 0.1	6.4 ± 1.1[a]	5.4 ± 1.6[a]
LV +dP/dt, mmHg/s	10,409 ± 830	10,344 ± 956	7382 ± 596[a]	7045 ± 750[a]
LV −dP/dt, mmHg/s	−7222 ± 571	−7503 ± 439	−4560 ± 273[a]	−4632 ± 419[a]
Histology				
Infarct size, %	NA	NA	55.5 ± 2.5[a]	52.8 ± 5.1[a]

Values are means ± S.E.M.; *N*, number of animals. MI, myocardial infarction; LV, left ventricle; EDD, end-diastolic diameter; ESD, end-systolic diameter; AWT, anterior wall thickness; PWT, posterior wall thickness; EDP, end-diastolic pressure; +dP/dt, positive change in pressure over time; −dP/dt, negative change in pressure over time; BW, body weight; SKM, skeletal muscle.

[a] $P < 0.05$ vs. Sham.

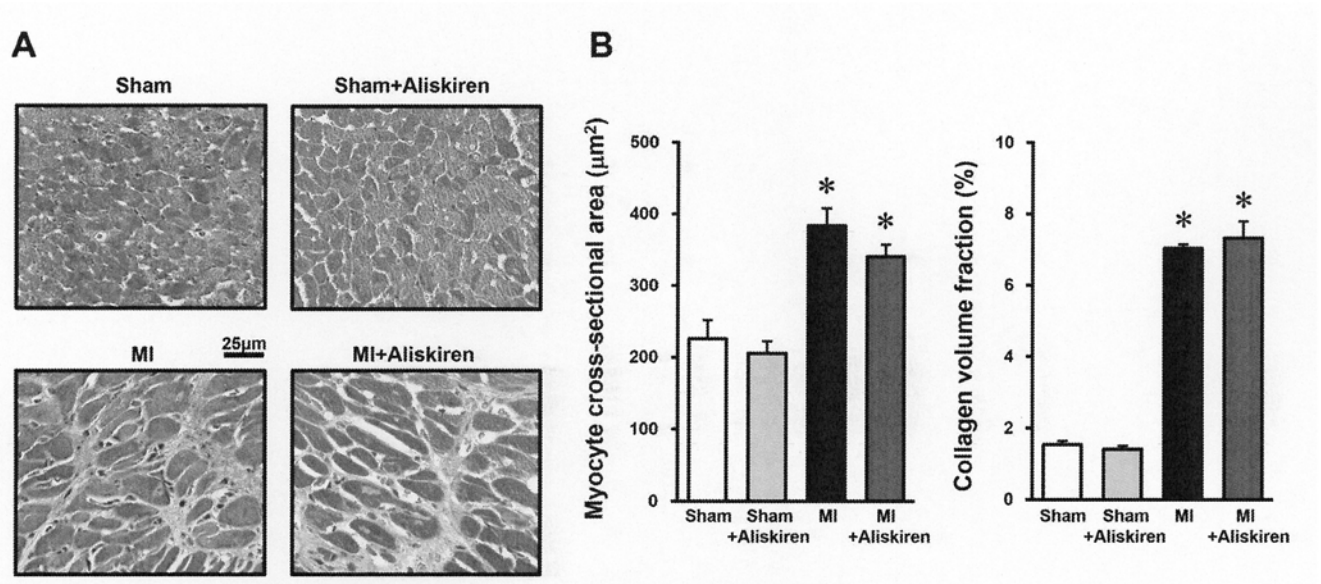

Fig. 1. (**A**) Representative high-power photomicrographs of LV cross-sections stained with Masson's trichrome from Sham, Sham+Aliskiren, MI, and MI+Aliskiren mice, and summary data of myocyte cross-sectional area and collagen volume fraction (**B**) in the 4 groups of mice (n=5). Scale bar, 25 μm. Data are expressed as means ± S.E.M. *$P < 0.05$ vs. Sham.

material). In addition, aliskiren did not affect these parameters in Sham mice (Fig. 2A, B). The other biochemical measurements are shown in Table 2. There were no differences in total cholesterol or triglyceride among the 4 groups. NEFA was higher in the MI than the Sham animals, and this difference was normalized by aliskiren treatment. Furthermore, the mean adipocyte area in epididymal adipose tissue was not significantly changed among the 4 groups (Supplementary material).

3.3. *Effect of Aliskiren on insulin signaling in the skeletal muscle*

There were no significant differences in the total or tyrosine phosphorylated protein levels of IR-β, IRS-1, and PI3-kinase in insulin-stimulated skeletal muscle among the 4 groups (Supplementary material). In contrast, serine-phosphorylation of Akt in insulin-stimulated skeletal muscle was lower in the MI than the Sham mice, and was improved in the MI+Aliskiren group (Fig. 3A). Consistently, GLUT4 translocation from the cytosol to

Table 2
Biochemical data.

	Sham	Sham+Aliskiren	MI	MI+Aliskiren
N	8	8	8	8
Blood glucose, mg/ml	90 ± 8	98 ± 8	92 ± 11	98 ± 10
Plasma insulin, ng/ml	0.43 ± 0.09	0.44 ± 0.05	0.98 ± 0.18[a]	0.52 ± 0.08[b]
HOMA index	1.5 ± 0.4	1.8 ± 0.2	3.6 ± 0.5[a]	2.0 ± 0.5[b]
Total cholesterol, mg/dl	69 ± 10	57 ± 7	57 ± 10	66 ± 11
Triglyceride, mg/dl	61 ± 18	81 ± 23	88 ± 32	88 ± 40
NEFA, meq/l	0.46 ± 0.07	0.48 ± 0.05	0.88 ± 0.15[a]	0.46 ± 0.05[b]

Values are means ± S.E.M.; *N*, number of animals; HOMA, homeostasis model of assessment; NEFA, nonesterified fatty acid.

[a] $P < 0.05$ vs. Sham.

[b] $P < 0.05$ vs. MI.

plasma membrane was significantly lower in MI mice compared to their Sham counterparts, and was normalized in the MI+Aliskiren mice (Fig. 3B). No significant differences in the total or phosphorylated protein levels of insulin signaling were observed in insulin-unstimulated skeletal muscle among the 4 groups (data not shown).

3.4. Effect of Aliskiren on circulating RAS

Both plasma renin activity and Ang II levels were significantly higher in MI mice than Sham mice and were inhibited in MI+Aliskiren mice (Fig. 4A, B).

3.5. Effect of Aliskiren on (Pro)renin receptor in the skeletal muscle

Cathepsin D (a surrogate marker for renin), angiotensinogen, ACE, and Ang II type1 receptor mRNA levels in the skeletal muscle were all comparable among the 4 groups (Fig. 5). In contrast, the gene expression and protein levels of (pro)renin receptor were both higher in the skeletal muscle of MI compared to Sham mice, and these increases were inhibited in the MI+Aliskiren group (Fig. 5 and Fig. 6A). Similarly, positive immunohistochemical staining for (pro)renin receptor was higher in skeletal muscle cells from MI than Sham mice, and was attenuated in the MI+Aliskiren group (Fig. 6A). Since upregulated (pro)renin receptor expression has been shown to potentiate local RAS activation, we next examined Ang II content in the skeletal muscle. As we expected, Ang II content was also higher in the skeletal muscle from MI mice compared to Sham mice, and the increment was significantly suppressed by aliskiren treatment (Fig. 6B).

3.6. Effect of Aliskiren on oxidative stress in the skeletal muscle

Both O_2^- production and NAD(P)H oxidase activities were significantly higher in the skeletal muscle of MI than Sham mice, and these increases were inhibited in MI+Aliskiren mice (Fig. 7A). Consistent with these activities, the NAD(P)H oxidase subunits, NOX2, $p22^{phox}$, and $p47^{phox}$ mRNA levels were all higher in the MI than the Sham group, and these increases were significantly inhibited by aliskiren (Fig. 7**B**).

4. Discussion

The major findings of present study are that the administration of aliskiren into post-infarct HF mice ameliorates both systemic

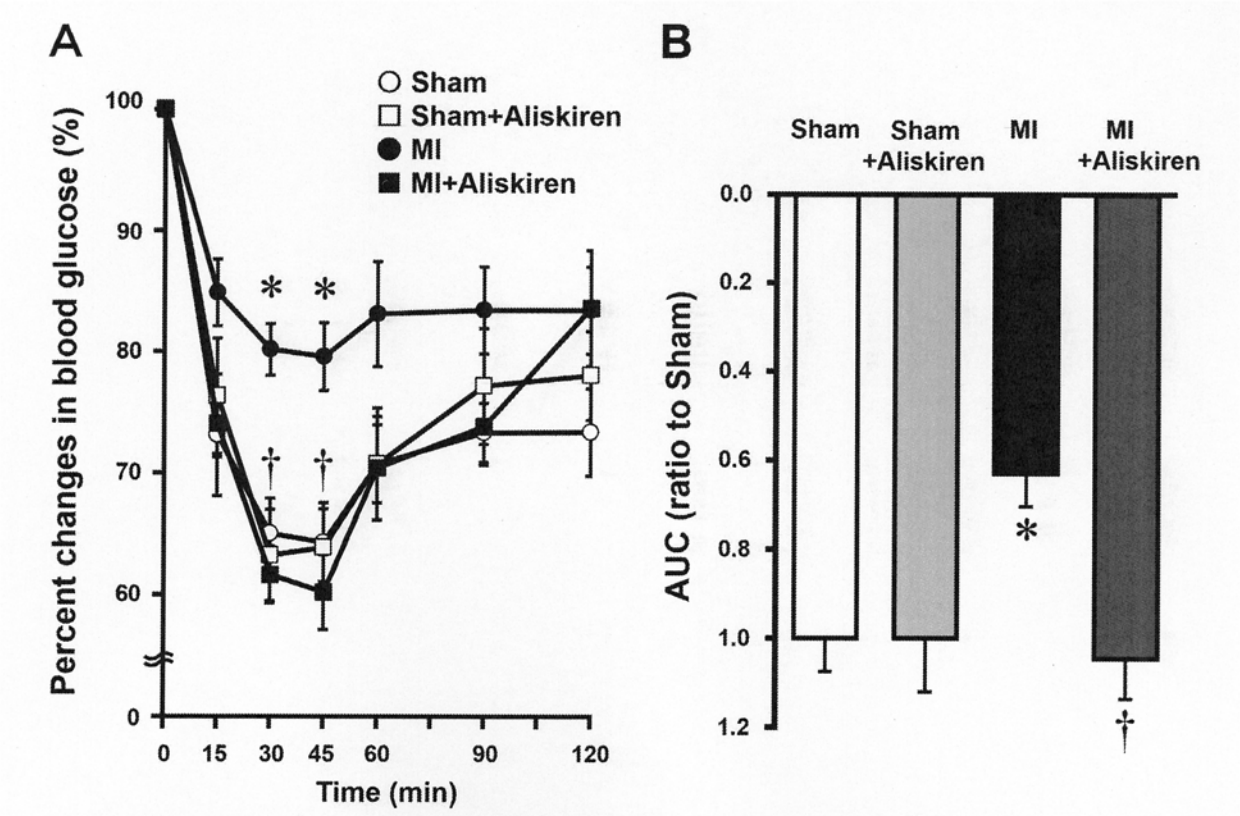

Fig. 2. (**A**) Percent changes in blood glucose during intraperitoneal glucose tolerance test in Sham (open circles, n=10), Sham+Aliskiren (open squares, n=8), MI (closed circles, n=10), and MI+Aliskiren (closed squares, n=9) mice and (**B**) area under the curve (AUC). Data are expressed as means ± S.E.M. AUC, area under the curve. *P < 0.05 vs. Sham, †P < 0.05 vs. MI.

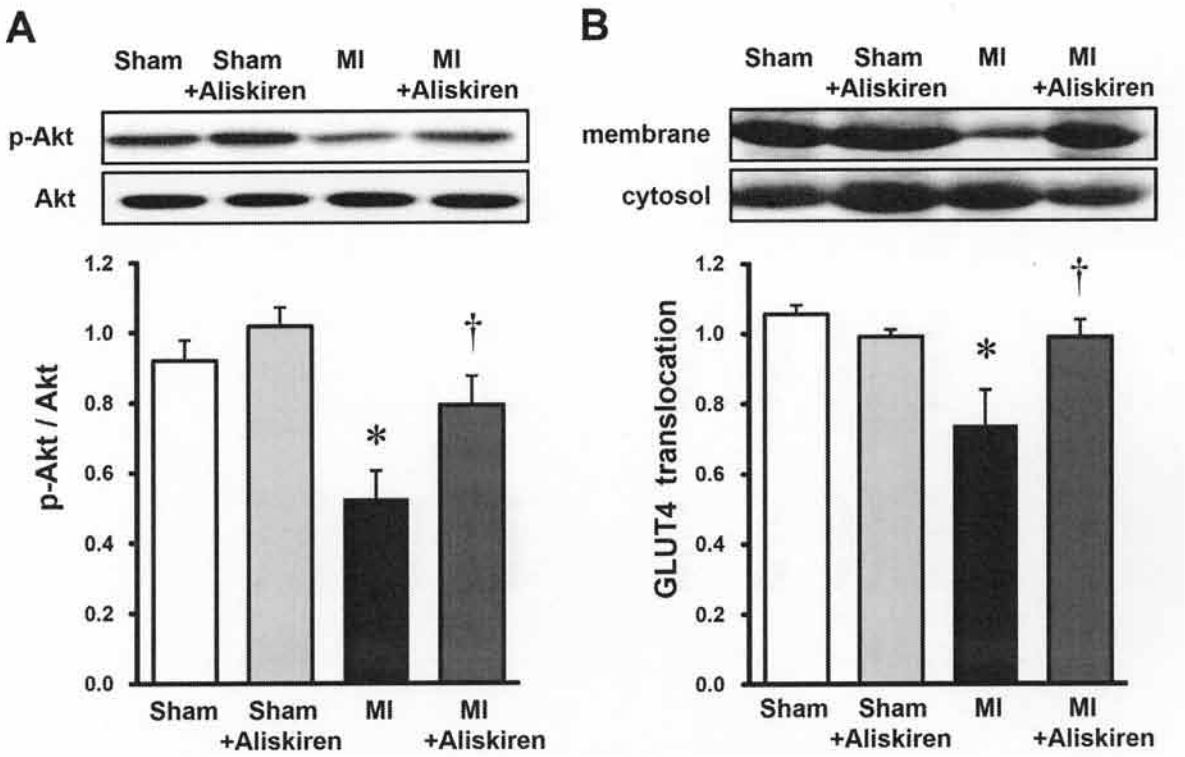

Fig. 3. Representative Western blot analysis (**upper panels**) and the summary data (**lower panels**) for p-Akt/Akt (**A**), and membrane fraction/cytosolic fractions of GLUT4 (**B**) in insulin-stimulated skeletal muscle from Sham, Sham+Aliskiren, MI, and MI+Aliskiren mice (n=4–6 each). Data are expressed as means ±S.E.M. p-Akt, serine-phosphorylation of Akt; GLUT4, glucose transporter 4. *P < 0.05 vs. Sham, †P < 0.05 vs. MI.

insulin resistance and impaired insulin signaling in the skeletal muscle, concomitant with an inhibition of NAD(P)H oxidase–induced O_2^- production. Moreover, aliskiren treatment attenuates an increase in (pro)renin receptor expression and subsequent Ang II production in the skeletal muscle, in addition to attenuating the increases in circulating renin activity and Ang II levels. Therefore, the salutary effect of aliskiren treatment on the insulin resistance in HF mice is likely due to the inhibition of both systemic RAS and (pro)renin receptor-mediated local RAS activation.

Despite the significant inhibition of the increase in plasma renin activity and Ang II in MI mice, aliskiren treatment did not ameliorate LV remodeling or failure after MI, with no changes being observed in infarct size, cardiomyocyte hypertrophy, or interstitial fibrosis. In contrast, a previous study demonstrated that 50 mg/kg/day of aliskiren prevented ventricular remodeling, hypertrophy, and apoptosis after MI (Westermann et al., 2008). However, for our experiments we selected a non-depressor concentration of aliskiren (10 mg/kg/day) in order to observe its anti-

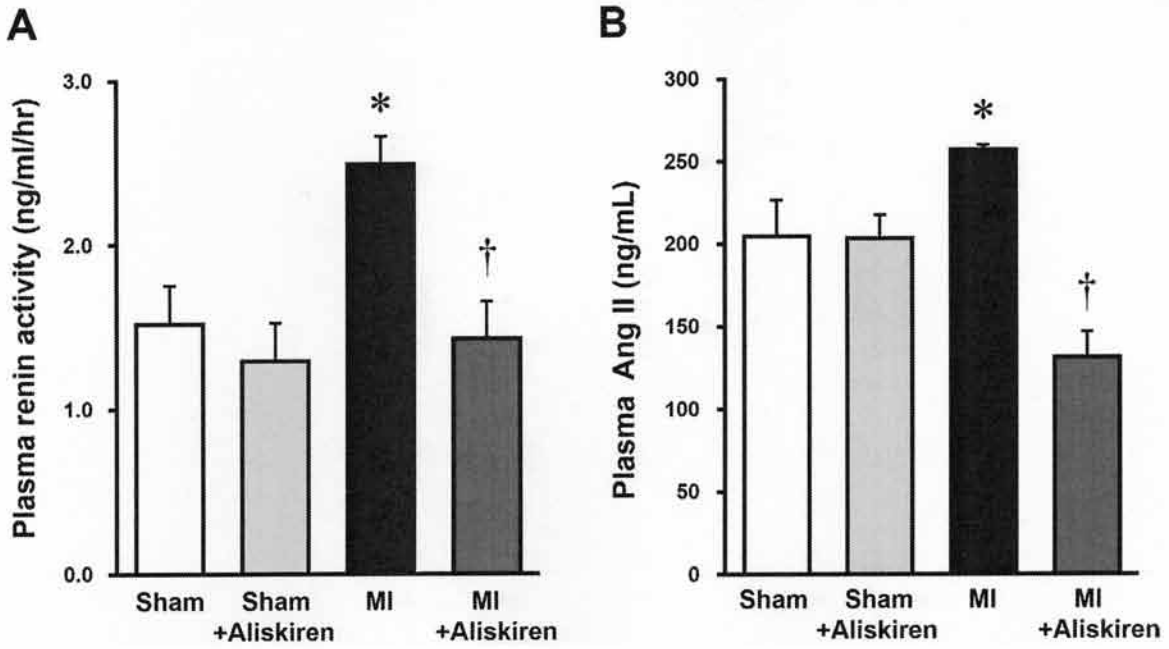

Fig. 4. Plasma renin activity (**A**) and plasma Ang II (**B**) from Sham, Sham+Aliskiren, MI, and MI+Aliskiren mice (n=5–7 each group). Data are expressed as means ±S.E.M. Ang II, angiotensin II. *P < 0.05 vs. Sham, †P < 0.05 vs. MI.

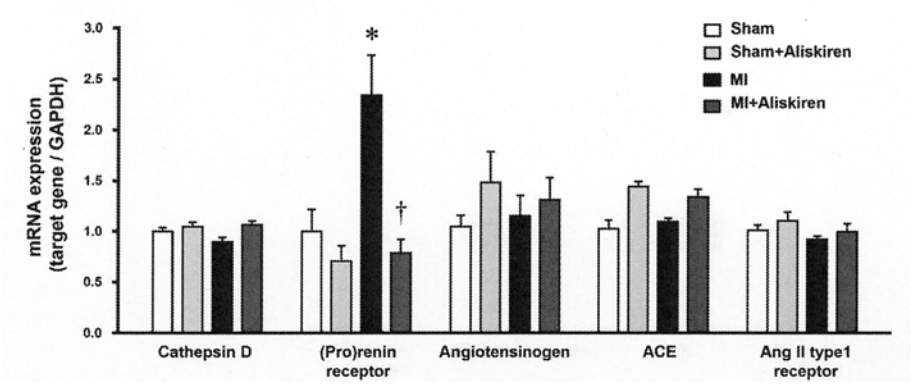

Fig. 5. Gene expression of Cathepsin D, (pro)renin receptor, angiotensinogen, angiotensin converting enzyme (ACE), and angiotensin II (Ang II) type1 receptor in the skeletal muscle obtained from Sham, Sham+Aliskiren, MI, and MI+Aliskiren mice (n=6–8, each group). Data are expressed as means ± S.E.M. ACE indicates angiotensin converting enzyme; Ang II, angiotensin II. *P < 0.05 vs. Sham, †P < 0.05 vs. MI.

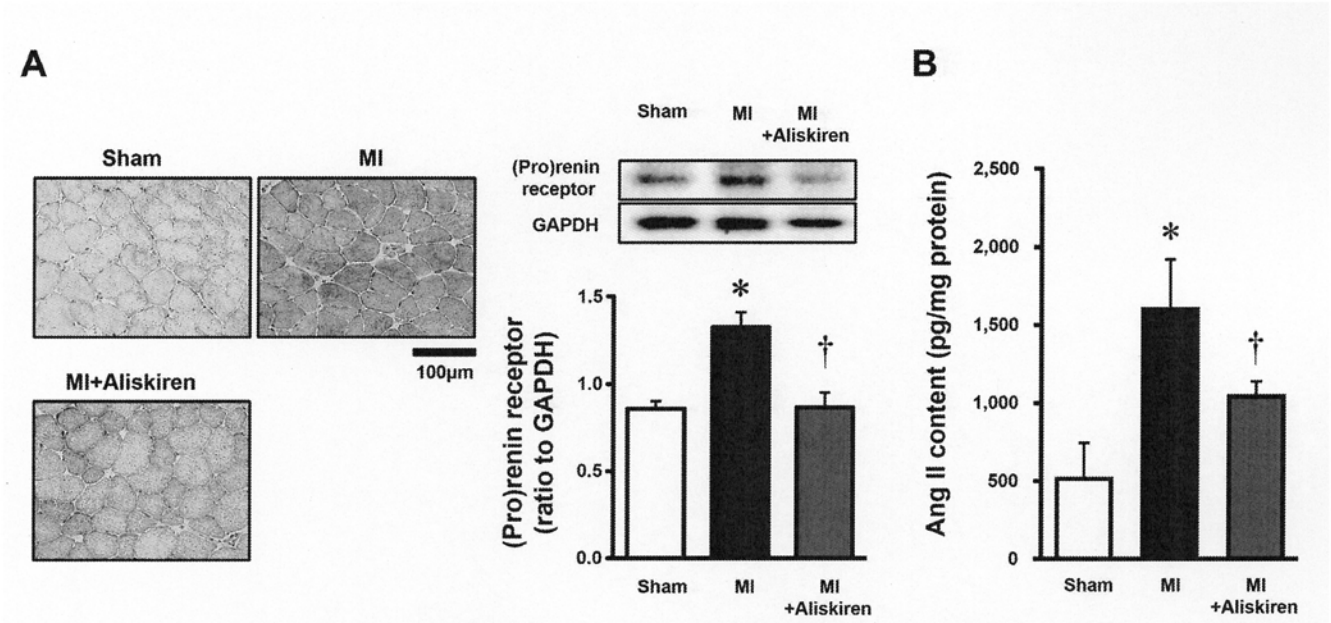

Fig. 6. (**A, left**) Representative immunohistochemical staining for (pro)renin receptor in the skeletal muscle tissue from the Sham, MI, and MI+Aliskiren groups. (**A, right**) Representative Western blot analysis (**upper panel**) and the summary data (**lower panel**) for (pro)renin receptor in the skeletal muscle from the Sham, MI, and MI+Aliskiren groups (n=10 each). (**B**) Angiotensin (Ang) II content in the skeletal muscle from the Sham, MI, and MI+Aliskiren groups (n=8 each). Data are expressed as means ± S.E.M. Ang II, angiotensin II. *P < 0.05 vs. Sham, †P < 0.05 vs. MI.

hypertensive action on insulin resistance in isolation, and thus the minimal effect of aliskiren on cardiac function might have been due to the relatively small amount used. On the other hand, the mortality rate in MI mice was significantly improved by the low-dose administration of aliskiren, suggesting that the favorable effect of aliskiren on insulin resistance may lead to an improvement of outcomes in HF after MI.

In a scientific statement from the American Heart Association, ischemic heart disease has been recommended as a model of acquired dilated cardiomyopathy and HF (Houser et al., 2012). Ischemic heart disease is the most common underlying cause of HF in humans, and the MI model exhibits the structural and mechanical alterations and shares many of the neurohormonal, cellular, and molecular features of HF in humans. The role of HF in the promotion of insulin resistance has been demonstrated by several experimental models, including a pacing-induced HF model, a model of pressure-overloaded HF by transverse aortic constriction (TAC), and a model of post-MI HF by permanent ligation of the left coronary artery (Nikolaidis et al., 2004; Shimizu et al., 2012). In the present study, a major advantage of the MI model is the capacity for studying the pathological impact of ischemic etiology on insulin resistance in HF, because it is still a matter of debate whether insulin resistance enhances the risk for development of HF exclusively in patients with ischemic heart disease (Das et al., 2004; Swan et al., 1997). The present study demonstrates that HF mice exhibit systemic insulin resistance, which is characterized by increases in the plasma insulin level, HOMA index, and percent change in blood glucose after insulin load. Moreover, impaired insulin signaling also develops in the skeletal muscle of MI mice, concomitant with a decrease in insulin-stimulated serine phosphorylation of Akt and GLUT-4 translocation to the membrane. Skeletal muscle is the major organ responsible for glucose utilization, with insulin-stimulated Akt phosphorylation and GLUT4 translocation being the rate limiting steps for these processes (Krook et al., 1997). A previous study reported that the level of GLUT4 protein was decreased in the biopsied skeletal muscle of non-diabetic HF patients, in association with decreases in insulin resistance and functional severity (Doehner et al., 2010). In support of this finding, we previously reported that reduced Akt phosphorylation and GLUT4 translocation to the membrane in the

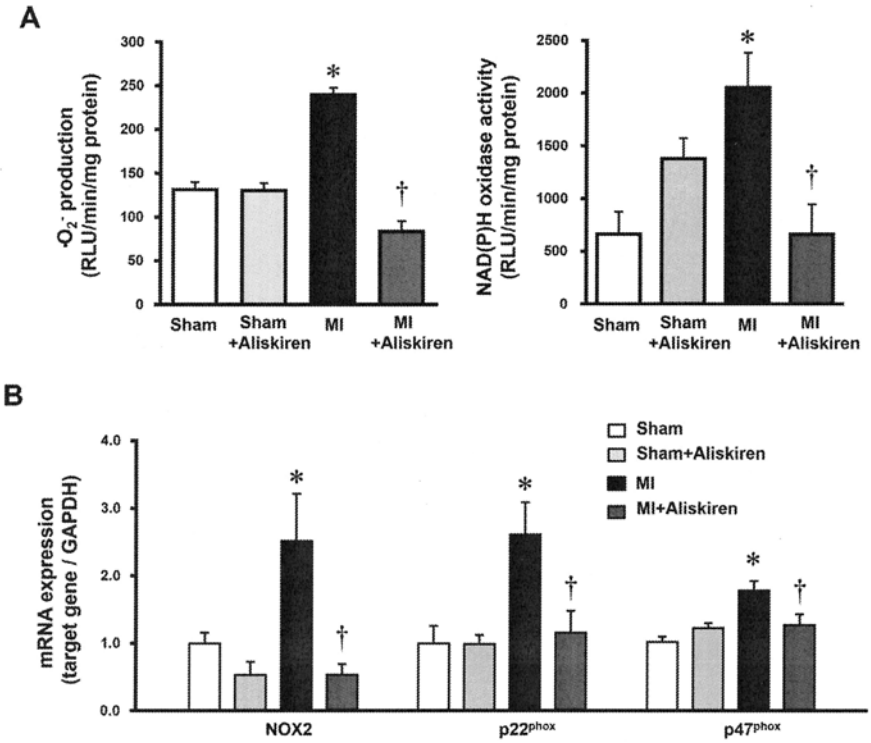

Fig. 7. (**A**) O_2^- production (**left**) and NAD(P)H oxidase activity (**right**) in the skeletal muscle obtained from Sham, Sham+Aliskiren, MI, and MI+Aliskiren mice (n=5–7 for each group). (**B**) Gene expression of NAD(P)H oxidase subunits including Nox 2, $p22^{phox}$, and $p47^{phox}$ in the skeletal muscle obtained from Sham, Sham+Aliskiren, MI, and MI+Aliskiren mice (n=5–7 for each group). Data are expressed as means ± S.E.M. RLU, relative light unit. *P < 0.05 vs. Sham, †P < 0.05 vs. MI.

skeletal muscle resulted in systemic insulin resistance in post-infarct HF mice (Fukushima et al., 2014; Ohta et al., 2011). Thus, impairments in this signaling in the skeletal muscle are likely to contribute to the systemic metabolic derangements seen in HF patients (Kinugawa et al., 2015). Importantly, treatment with aliskiren ameliorated insulin resistance in parallel with normalization of insulin signaling and NAD(P)H oxidase-derived oxidative stress in the skeletal muscle in MI mice. Aliskiren blocks the activation of RAS at the initial step of renin inhibition, and has been shown to improve glucose transport in the skeletal muscle tissue from renin transgenic rats (Lastra et al., 2009). Since Ang II is known to directly impair insulin signaling through NAD(P)H oxidase activation in skeletal muscle cells (Wei et al., 2006), aliskiren is considered to be an attractive therapeutic approach to ameliorate insulin resistance associated with HF. On the other hand, NEFA was increased in MI mice and was normalized by aliskiren, suggesting that HF-induced lipolysis of adipose tissue is alleviated by aliskiren. Although there was no significant change in mean adipocyte area among the 4 groups, we cannot completely exclude the effect of aliskiren on other insulin-sensitive organs such as adipose tissue, because aliskiren was systemically administered by using an osmotic mini pump.

Another important finding is that local (pro)renin receptor expression rather than classical local RAS components is upregulated in the skeletal muscle in MI mice, which is normalized by aliskiren treatment. Activation of (pro)renin receptors has been shown to enhance the enzymatic activity of prorenin, in association with the pathogenesis of cardiovascular and renal injuries in hypertension, diabetes, and heart failure (Fukushima et al., 2013; Ichihara et al., 2006a, 2006b). In addition, we recently reported that upregulated (pro)renin receptor expression enhances Ang II production and oxidative stress, resulting in insulin resistance in HF mice after MI (Fukushima et al., 2014). Interestingly, the present study clearly demonstrates that administration of aliskiren to MI mice attenuates the increase in both (pro)renin receptor expression and the resulting Ang II content in the skeletal muscle. Since aliskiren can bind not only to circulating activated renin, but also to (pro)renin receptor-bound renin and prorenin (Biswas et al., 2010), the binding of aliskiren to renin and prorenin may influence both local levels of (pro)renin receptor as well as Ang II in the skeletal muscle. Indeed, a recent study has shown that aliskiren inhibits the prorenin-induced increase in intracellular Ang II of human podocytes (Sakoda et al., 2010). Therefore, these results suggest the potential impact of aliskiren on (pro)renin receptor-mediated local RAS activation and subsequent oxidative stress, rather than systemic or classical local RAS components. However, it was difficult to clarify the sites of aliskiren activity in the present study due to the dual efficacy of this agent. A major limitation in the present study was thus the lack of more definitive mechanistic experiments to delineate the causal effect of aliskiren on (pro)renin receptors, oxidative stress, and insulin resistance. Further studies on the modulation of (pro)renin receptors under aliskiren treatment are needed.

In conclusion, aliskiren ameliorated insulin resistance associated with HF by improving insulin signaling, at least in part by inhibiting systemic and (pro)renin receptor-mediated local RAS activation and subsequent NAD(P)H oxidase-induced O_2^- production in the skeletal muscle. The current study provides further support for targeting systemic RAS and (pro)renin receptor-mediated local RAS as a therapeutic intervention to improve insulin action in the skeletal muscle in the setting of HF-associated insulin resistance.

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Disclosures

No conflicts of interest.

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Appendix A. Supporting information

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9-2　心不全における骨格筋萎縮予防

Prevention of muscle wasting in heart failure

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〔心臓リハビリテーション（JJCR）22（2・3）：132-135，2016〕

Key words：心不全，慢性疾患，骨格筋萎縮，運動耐容能，リハビリテーション

はじめに

心不全のみならず慢性閉塞性肺疾患，慢性腎疾患，糖尿病，がんおよびエイズなどの慢性疾患において，併発する骨格筋障害/萎縮は予後を左右する重要な病態である．骨格筋は，運動器としてだけではなく，growth factor や myokine を分泌する内分泌臓器としても機能し，心身の健康と生命を支える．ゆえに今日，骨格筋障害/萎縮の機序をターゲットにした様々な治療法/薬が考案されている．各種病態に共通する主要な骨格筋障害は，筋線維型の変化（遅筋から速筋へのシフト）と筋萎縮である．遅筋は萎縮に抵抗性であり，また重要な myokine である脳性神経栄養因子などは，主に遅筋に発現する．一方，抗酸化物/酵素の含有は遅筋と速筋でそれほど差はなく，肥大しやすいのは速筋である．さらに筋力は QOL に強く影響する．従って，筋線維変性の改善と筋量/筋力増加が治療の目標になる．有酸素運動は主に質的に，レジスタンス運動は量的に骨格筋障害を改善する．潜在的な薬剤にも筋ミトコンドリア生合成に作用して速筋から遅筋へシフトさせるものと，筋量増加作用を主とするものがある．今回は，現在考案されている主な骨格筋障害/萎縮の予防および改善方法について概説したい．

骨格筋と疾患・生命予後

握力や膝伸展筋力が健常者の生存率に関連することが報告されている[1]．特に握力は，簡便に測定できるため近年注目されている[2]．また心疾患，高血圧症，慢性閉塞性肺疾患および慢性腎疾患などの疾患群においても骨格筋量・筋力が，生存率と関連することが報告されている[3~7]．骨格筋が生命予後に関わる理由，疫学研究，臨床研究および基礎研究から推測される機序を図1に示した．

心不全にみられる骨格筋障害/萎縮と機序

心不全にみられる主要な骨格筋障害は，骨格筋量・筋力の減少（萎縮）と筋線維型の変化（遅筋から速筋へのシフト）である．遅筋は萎縮に抵抗性であるから，速筋化は易萎縮性を意味する．骨格筋障害/萎縮が生じる原因として，末梢循環不全に起因する神経液性因子の活性化，炎症・酸化ストレスの亢進を発端に成長ホルモン抵抗性，異化・同化不均衡，骨格筋蛋白分解亢進，アポトーシスなどが起こり，これらを身体不活動，栄養障害が助長するというような多面的機序が考えられている（表1）．これまで，特に神経液性因子であるレニン・アンギオテンシン系（RAS）および交感神経系の亢進が重要な役割を果たしていると考えられてきたが[8]，最近では，これらに関連してユビキチン・プロテアソーム系による骨格筋蛋白融解亢進機序が注目されている[9,10]．

骨格筋障害/萎縮に対する治療

1）運動療法

有酸素運動は質的に，レジスタンス運動は量的に骨格筋障害を改善する．有酸素運動により，ミトコンドリア生合成の増加と速筋型から遅筋型へミオシン重鎖型の改善が起こる[8]．前述のように遅筋型は萎縮抵抗性を示す．同じ有酸素運動においても，トレーニング強度（intensity）はミトコンドリア呼吸能の向上に，トレーニング量（volume）はミトコンドリア量の増加に寄与しやすい可能性が示唆されている[12,13]．一方，レジスタンス運動は，高強度の負荷で速筋を動員し，筋肥大・筋力増加をもたらす．臨床現場におい

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ては通常の高強度レジスタンストレーニングの適応が難しい症例も多いが，負荷強度は相対的であり，低強度であっても無効ではない[14]．一方，血流制限を併用することで低強度負荷であってもトレーニング刺激を高める方法が脚光を浴びているが[15]，我々は，この手法が心不全患者においても有効であることを明らかにしている[16]．

2）薬物治療の可能性（臨床研究↔基礎研究）

骨格筋機能改善を示す薬剤には，筋ミトコンドリア生合成に作用して速筋から遅筋へシフトさせるものと，筋量増加作用を主とするものがある．早期の臨床研究において，心不全治療薬であるアンギオテンシン変換酵素阻害薬（ACEi）やアンギオテンシン受容体拮抗薬（ARB）が，心不全患者の骨格筋障害を部分的に改善し，運動耐容能を増加させることが示されているが[8]，後の基礎研究により，RAS活性化により筋萎縮や速筋化が起こることが証明され，この機序においてアンギオテンシンⅡが骨格筋障害の主因であることが示唆されている[17,18]．心不全および糖尿病モデルマウスでは，RAS系活性化がみられ，類似した骨格筋障害（速筋化）が起こり，運動耐容能が低下するが[19]，我々は，糖尿病モデルにおいてARB投与により骨格筋障害および運動耐容能が改善することを証明している[20]．さらに心不全モデルにおいて直接的レニン阻害薬も同様の効果を有する可能性を明らかにしている[21]．

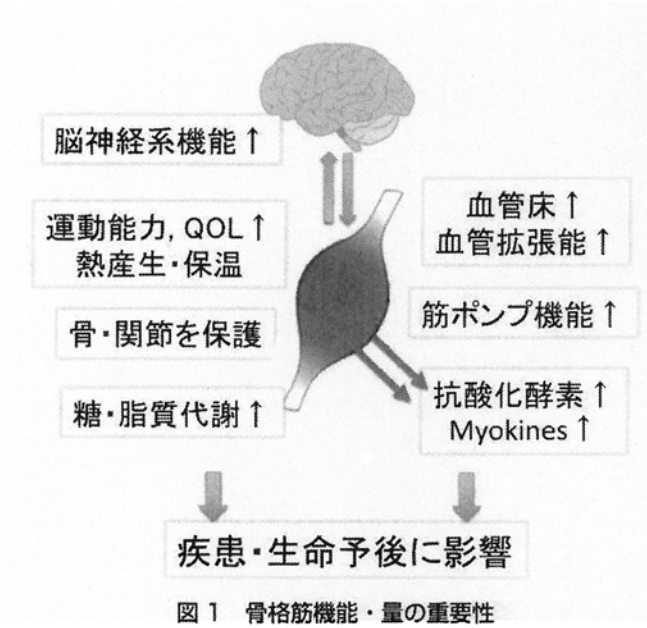

図1　骨格筋機能・量の重要性

RAS系阻害薬については，健常者のRAS遺伝子多型にてRAS活性が弱い型において持久力が高いことが知られていることもあり[22]，心不全以外の患者に対する運動能力向上効果が期待された．しかしながら，現時点では有効性が証明されるに至っていない[23]．このように病的な状態において良好に作用する様々な薬剤も健常な状態では，無効あるいは悪化に傾かせる可能性も考えなければならない．今後さらに詳細な研究が必要であると思われる．

骨格筋量増加作用を主とする薬剤では，成長ホルモンやテストステロンが心不全に使用され，有意な骨格筋量・筋力の増加および運動耐容能改善効果が示された[24]．しかしながら，成長ホルモンについては，副作用およびnon-responderの問題などから適応が限られている[25]．一方，テストステロンは複数の無作為比較試験で有効性が証明されたが，長期使用については，前立腺肥大/がんのリスク，女性における男性化，行動異常などの副作用が問題となるため，現在では非ステロイド系アンドロゲン受容体作動薬が代替薬としてフレイル高齢者などを対象に治験段階にある[26,27]．

ミオスタチンは，TGF（transforming growth factor）-βスーパーファミリーの一つであり，強力な骨格筋の増殖抑制因子として知られているが，ミオスタチンの活性が低下した例では，著しい筋肥大を呈する[28,29]．近年，心不全において，myostatinの増加が筋萎縮に関わっていることが示され，ミオスタチンを阻害する抗ミオスタチン抗体が骨格筋量・筋力を増加させることが基礎研究において示された[30]．

表1　骨格筋異常/萎縮の成因（8～11）

1. Deconditioning（身体不活動）
2. Malnutrition（栄養障害）
3. Hypoperfusion and Hypoxia（低灌流，低酸素）：低灌流が直接骨格筋障害を起こすかどうかは厳密には証明されていない．一方，COPDでは，心不全に類似した骨格筋障害を示す．
4. Neurohumonal Factors（神経液性因子：RAS, SNA, aldosterone-PTHなど）：AT-Ⅱは，骨格筋萎縮を惹起し，また筋線維を遅筋型から速筋型に変える．一方，ARBおよびACEiは，筋線維変化を改善する（速筋型→遅筋型）．
5. Catabolic/Anabolic Imbalance（同化異化不均衡）：心不全では，異化系が亢進し，同化系ホルモンが減少している．また，GHが増加しているにも関わらずIGF-1低下を示す成長ホルモン・IGF-1抵抗性を呈することも知られている．
6. Aggravated Muscle Protein Wastage（筋蛋白分解亢進）：腎不全，がん悪液質，飢餓等に見られる筋蛋白分解の亢進であり，近年，カヘキシー（cachexia）に関連して注目されている．ユビキチン・プロテアソーム系が主要な系として重要視されている．
7. Inflammation（炎症），Oxidative Stress（酸化ストレス）：急性時おけるprimary damageと末梢循環不全に起因する神経・液性因子を介する慢性的機序が考えられる．
8. Cell-cycle dysregulation（apoptosis, autophagy）

COPD：chronic obstructive pulmonary disease, RAS：renin-angiotensin system, SNA：sympathetic nerve activation, PTH：parathyroid hormone, AT：angiotensin, AR：angiotensin receptor antagonist, ACEi：angiotensin-converting enzyme inhibitor, IGF：insulin-like growth factor.

以後，虚弱高齢者，慢性閉塞性肺疾患，がん患者などを対象に臨床治験が進んでいる[31]．その他，プロテインやアミノ酸などによる栄養療法，Megestrolなどの食欲促進薬，ghrelin/ghrelin agonists，IGF-1アナログ，抗TNF-α他複数の薬剤が各種疾患に伴う筋萎縮の予防・改善のために有効性が検討されている[9,32,33]．

おわりに

心不全のみならず慢性閉塞性肺疾患，慢性腎疾患などの慢性疾患において，予後に深く関わる骨格筋障害/萎縮の重要性が認識され，病態解明および治療法開発を目的とした臨床研究と基礎研究が盛んに行われているが，両者は互いに相補しながら着実に進んでいるように思える．

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ミニ特集

2 知りたい！ フレイル患者さんの運動療法

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押さえておくべきポイント

Point 1 心臓リハビリテーション（以下、心リハ）患者さんでは、基礎疾患、合併症、加齢のため、フレイルリスクが高まる。

Point 2 心リハ患者さんでは、骨格筋障害やフレイルが予後を左右する。

Point 3 フレイルには、運動療法が有効！（有酸素、筋トレなど、フレキシブルに行う。）

Point 4 とにかく動かすことが大切：強度アップにこだわらず、継続を第一に考える。

心リハ現場におけるフレイル患者さん

フレイル（Frailty）は筋量・筋力の低下、歩行速度の低下だけではなく、その原因となる低栄養や体重減少、気分や精神・心理的問題、社会的問題まで、多数の要因が複雑に関連して起こります。概念として、①生体機能（身体能力、移動能力、筋力、バランス能力、持久力、栄養、活動性、認知機能、気分）に障害が起きた結果生じる状態、もしくは、②加齢に伴う恒常性保持能の低下や、肉体的・精神的負荷に対する受容力の低下により生理的機能の障害を起こしやすい状態とされていますが、共通のコンセンサスは得られていません[1,2]。

図1[3]に示すように、加齢に加え、分子異常および基礎疾患により身体機能異常が生じ、臨床的症状が現れ、それらの症状がフレイルサイクルを形成し、悪循環に陥ると考えられています（図2）[1]。

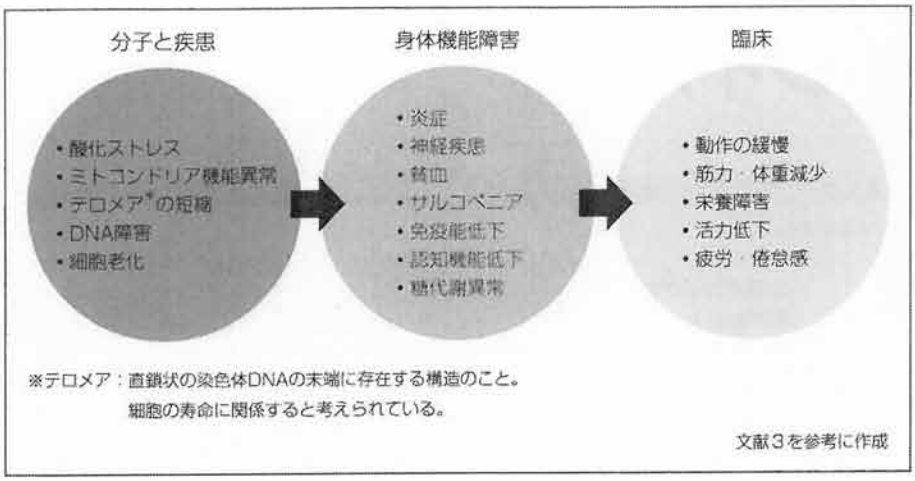

図1 ■フレイルの機序

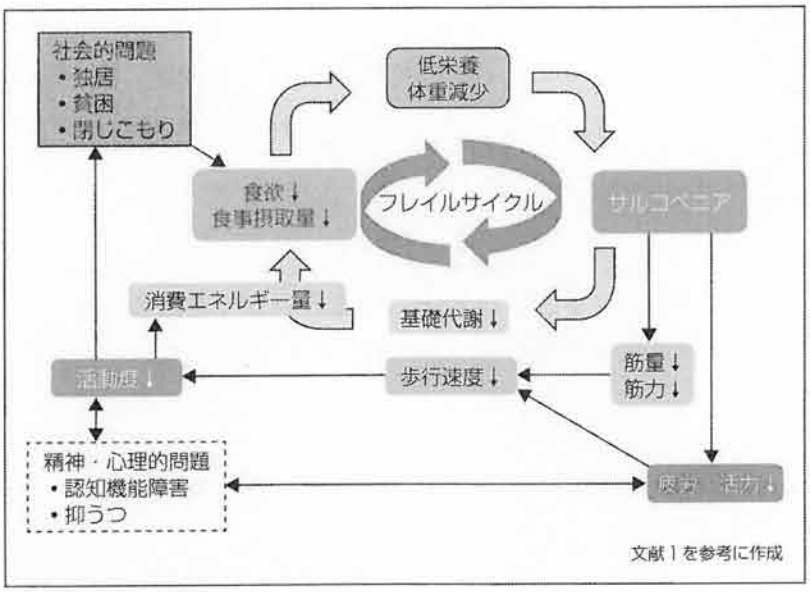

図2 ■フレイルサイクル（心疾患ではサイクルが加速！）

海外報告におけるフレイル高齢者の頻度は7～10％[2]、75歳以上の高齢者におけるフレイルの頻度は20～30％であり、年齢とともにその頻度は増加することが示されています[3]。心リハ患者さんでは、高齢化に加え、各種基礎疾患、心不全、さらにCKD（慢性腎疾患）、COPD（慢性閉塞性肺疾患）などの併発により、サルコペニアおよびフレイルの頻度が極めて高くなります。従って、心リハ現場では、標準的な運動ガイドラインを適用できないフレイル患者さんなどへの対策が大き

表1 ■心リハ患者さんの運動方法例

区分	Phase Ⅰ	Phase Ⅱ	Phase Ⅲ
場所	ベッドサイド	ベッドサイド、運動療法室	運動療法室
目的			全身持久力維持・向上
	ADL低下予防		ADL維持・向上
	廃用性筋力・筋量低下予防		
トレーニング内容	・筋力トレーニング（30% 1 RM以下） ・握力（上肢） ・スクワット、カーフレイズ（下肢） ・タンデム肢位・片足立ち（立位バランス）	・筋力トレーニング（30～40% 1 RM以下） ・握力（上肢） ・スクワット、カーフレイズ（下肢） ・タンデム肢位・片足立ち（立位バランス） ・歩行（持久力）	・筋力トレーニング（40～60% 1 RM以下） ・スクワット、カーフレイズ（下肢） ・タンデム肢位・片足立ち（立位バランス） ・歩行（持久力）

な課題となっています。

心リハ患者さんの骨格筋機能低下とフレイル

特に心不全では、萎縮、筋線維型の変化（遅筋から速筋へのシフト）、筋代謝酵素の変化（好気的酵素の減少）、骨格筋エネルギー代謝異常、ergoreflexの亢進などの骨格筋障害が生じることが知られており、これらはサルコペニア、フレイルを相乗的に悪化させ、また予後に影響を及ぼします[4, 5]。骨格筋障害が生じる原因として、末梢循環不全に起因する神経体液性因子の活性化、炎症・酸化ストレスの亢進を発端に、成長ホルモン抵抗性、異化・同化不均衡、骨格筋蛋白分解亢進、アポトーシスなどがあり、これらを身体不活動、栄養障害が助長する多面的機序と考えられています[4, 5]。一方、運動療法は骨格筋障害・フレイルの最も有効な治療方法ですが、フレイルは多面的な要因で発症するため、それに応じた慢性疾患の管理、栄養管理、うつ・認知機能低下を含む精神心理面への対応も必要です（図1）[3]。最近、各種慢性消耗性疾患における骨格筋障害・萎縮に特異的に焦点を当てた新しい薬物療法も臨床応用されつつあり[6]、近未来には運動、栄養、薬物療法の併用が一般化することが考えられます。

心リハの実際（表1）

フレイルに対する運動療法に関する論文は蓄積しつつあります[7, 8]。有酸素運動、レジスタンス運動のほか、振動板を利用したトレーニングなど[8]、さまざまな運動方法の有効性が示されていますが[7]、統合されたガイドラインが確立されるには至っていません。そのため心リハの現場では、

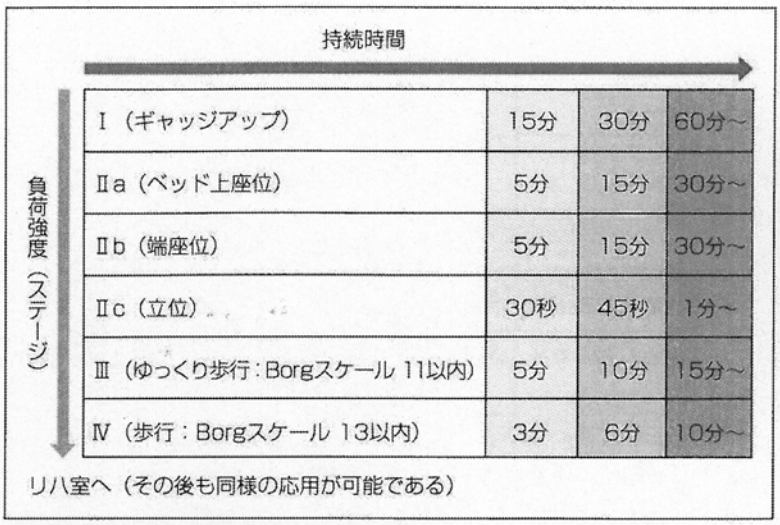

図3 ■運動の持続・継続性および執着性に着目したプログラムの概要
強度アップができない例は、持続時間を延長、繰り返し行うことで運動を継続する。

以下のような基本的な心リハガイドラインを踏襲しています[9]。

1. Phase Ⅰ（急性期）

ADL低下予防を目的に、臥位または座位より可能な運動療法を開始します。また、可能な限り早期から抗重力活動を増やし、起立・立位の機会を設けます。これは筋力トレーニングとしてのみならず、認知機能低下やせん妄予防として、また血圧など自律神経系の廃用を予防する目的があります。上肢は、食事や洗面など座位でできるADLを早期に開始し、実施可能な範囲でADLの低下を予防します。運動療法としてはプレトレーニングとして、起立動作を意識した下肢の屈伸運動などを行い、筋肉の協調的な活動を促します。

2. Phase Ⅱ（回復期）

ADL自立度を向上させるために、特に移動を伴う身体活動を段階的に増やしていきます。また、筋力向上を目的に筋力トレーニングの負荷量を増加させます。歩行を意識して、立位バランスなどの課題も併せて行います。

3. Phase Ⅲ（維持期）

Phase Ⅰ・Ⅱに加えて、筋力向上・筋肥大を目的として筋力トレーニングの負荷量を中等度に増やします。また、全身持久力向上のために自転車エルゴメーターなどの有酸素運動も取り入れます。

フレイル・心リハ患者さんに対する応用運動プログラム

通常の心リハでは、医師による運動処方の後、

リハ室におけるトレッドミルや自転車エルゴメーターでの運動が主体であり、患者さんは一定以上の運動能力を求められます。また、従来の心筋梗塞後の心リハプログラムでも運動強度の増強と持続力の延長が混在し、ステージアップできない症例ではプログラムへの執着性（adherence）が低いのが現状です。大切なのは運動させることであり、必ずしも“型に当てはめる”必要はありません。さらに執着性、継続性と精神的充足度を意識し、症例に応じた適切な心リハを選択していくことが重要です。

図３の横軸は運動持続時間、縦軸は負荷強度を表しています。従来のように一つの運動強度のステージアップをもって直前のステージを終了するのではなく、前段階も並行して持久力を高めることで、運動を継続し、運動耐容能の維持・向上を図ります。ステージの違ういくつかの段階の運動を同時に行うこともあります。特にステージアップできない場合では、持続時間を延ばし、運動を積み重ねることで、骨格筋への刺激が持続し、プログラムへの執着性も高めることになります。すべての段階を終え、図が長方形に近づくことが理想ですが、症例によって院内リハビリ終了パターンは異なってもよいです。この考え方は、長期臥床中から退院間近の患者さんまで、幅広い段階に応用できます。

おわりに

心リハ領域におけるフレイルの意義と問題点および対策について概説しました。運動機能障害のほか、合併疾患、栄養不良、精神・認知機能低下などを含めた症候群としてのフレイルを理解して、有効な治療法である運動療法を広い視野から応用し、さらに新しい治療戦略との組み合わせなど、臨床現場において、より有効で斬新な包括的心臓リハビリテーションが実践されていくことに期待したいと考えます。

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第22回日本未病システム学会学術総会
■ シンポジウム5 運動部会立ち上げ記念シンポジウム「運動の臓器連関と未病対策」

心疾患における運動の意義

髙田 真吾 沖田 孝一

はじめに

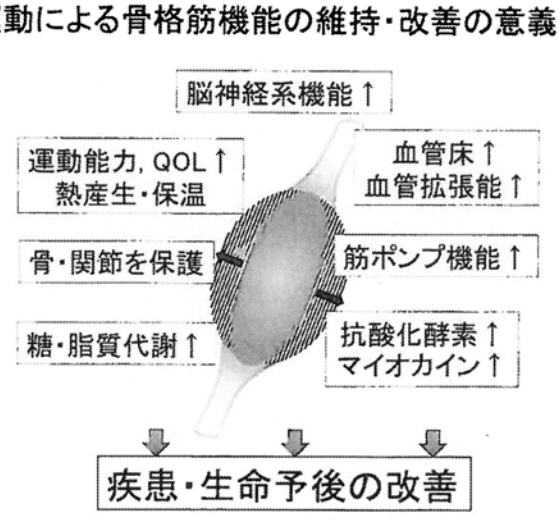
心疾患における運動療法の目的は，病態の改善，QOLおよび生命予後の改善・向上である．運動療法により虚血性心疾患，慢性心不全の生命予後が改善することが，多くの研究において証明されている．また運動耐容能あるいは骨格筋量・筋力そのものが，生存率や死亡率に影響を与えることも明らかにされている．ゆえに，運動は，心臓リハビリテーションの最も重要な要素となっている．運動による疾病予防効果については，言うまでもないが，特に心疾患に罹患している患者においては，その後の運動療法によって大きく生存率や再発率が改善する．さらに，近年の研究において，運動（筋活動）により，抗酸化防御物質・酵素やマイオカイン（骨格筋由来生理活性物質）の産生が亢進することが示されている．生体内の抗酸化システムは，老化，動脈硬化，腫瘍発生を抑制し，一方，マイオカインは脂肪組織由来のアディポカインとは逆に様々な善玉作用を発揮する．これらの知見は，運動の極めて多面的な健康保持・増進効果の説明になるように思える．

1. 心疾患における運動耐容能と予後

心疾患，特に心不全を呈する患者では，運動耐容能が低下しており，その指標である最大酸素摂取量（持久力の指標）は予後に強く関連している[9]．同様に健常者においても最大酸素摂取量や筋力が生存率に影響することが疫学研究にて明らかにされている[7,12]．最大酸素摂取量，筋力は，体力の指標であり，それらは骨格筋の質および量に関連している．骨格筋が生命予後に関わる理由，疫学研究，臨床研究および基礎研究から推測される運動効果の機序を図1に示した．

2. 心疾患（心不全）における運動耐容能低下の機序

心不全患者は運動耐容能が低下しており，労作性呼吸困難や易疲労性などの特徴的症状を呈する．過去には，心機能低下がその主因と考えられていたが，強心薬，血管拡張薬あるいは高濃度酸素吸入により血行動態（心拍出量，下肢血流）や酸素供給が改善するにもかかわらず運動耐容能は改善しないことが明らかになり[10,16]，また運動耐容能の指標と左室駆出率（LVEF）が相関しないことが多数の研究で示された[3]．1980年以降の集約的な研究により，運動耐容能低下の主因は左室機能低下によ



❑図1

運動による骨格筋機能の維持・改善の重要性．さらに筋活動により抗酸化酵素およびマイオカインの産生が誘導される．抗酸化酵素は，老化，動脈硬化，腫瘍発生を抑制し，動物種の寿命に関連し，一方，マイオカインも，糖・脂質代謝の改善，筋肥大，血管増生，抗炎症，抗酸化ストレス，抗動脈硬化，抗腫瘍性など多様な作用を示すことが示されている．

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る循環不全ではなく，骨格筋量・筋力の減少，筋内代謝異常，筋線維型のI型（酸化系）からII型（嫌気性）へのシフト，ミトコンドリア機能・量の低下，血管拡張能低下などの末梢因子であると考えられるようになった[4,11,13]．これらの機序に過度の安静によるdeconditioningや栄養障害が様々な程度に加わり，病態を悪化させる[13]．

3. 心疾患における運動療法

1) 身体活動・運動療法の効果（表1）

運動疫学において最も有名な研究は，身体活動量が多いほど心臓発作の発生数が少ないことを明らかにしたPaffenbargerらの報告であろう[14]．運動は，多面的な治療効果を示し（表1），心臓リハビリテーションにおいて中心的な役割を担っている．心筋梗塞後患者における運動療法は，再発による入院や死亡を減少させ[18]，安定した狭心症患者においては，カテーテル治療を上回る予後改善効果を示す[8]．近年，心不全患者においても運動療法が病状増悪や心臓死を減少させることが明らかになり[1]，慎重な管理が必要ではあるが，過去には禁忌であった運動が重要な治療法となっている[5]．さらに特筆すべきことに，冠動脈疾患および心不全患者における長期的運動療法は，全死因による死亡率を改善する[6]．

❑ 表1　心疾患における運動療法の効果[5]

1）運動耐容能，ADL（日常生活動作）の改善
2）血管機能改善（内皮機能↑）
3）脂質・糖質代謝の改善
4）心筋虚血改善（側副血行↑，Preconditioning）
5）心機能改善（一回拍出量↑）
6）自律神経バランス改善（交感神経↓，副交感↑）
7）心室細動域値上昇
8）血小板凝集抑制
9）炎症性サイトカイン，酸化ストレスの低下
10）精神状態，QOL改善
11）生活習慣改善
12）骨格筋量・質の改善

2) 運動療法の実際

①有酸素性トレーニング[5,15]

心疾患における有酸素運動トレーニングは，すでに多くの施設で実地されている．概要は表2の通りである．わが国では，心肺負荷試験に呼気ガス分析を導入している施設が多く，嫌気性代謝閾値（Anaerobic threshold, AT）を目安にした運動処方が行われている．AT以下で虚血や不整脈が発生する場合は，それより10心拍以下の強度で運動を行う．ガイドラインにおける運動頻度と持続時間は，AT前後で30分以上とされているが，それ以下（12～20分，2～3/週）でも効果は得られるので[15]，病状（disease status）とトレーニング・ステイタスを考慮したメニューを考えることが重要である．

②レジスタンス・トレーニング

骨格筋量・筋力低下が，生存率や死亡率に影響を与えることが報告され，それらを改善するためのレジスタンス運動が推奨されている[2]．アメリカ心臓病会議とヨーロッパ心臓病学会では，運動強度に関して若干のコンセンサスの違いはあるが，以下のような概要である（表3）．レジスタンス運動による介入では，筋力・量増加の他，運動耐容能増加，血圧低下，耐糖能改善，骨強度増加などの有効性が示されている．

③高強度インターバル・トレーニング

最近，心臓リハビリテーション現場において高強度インターバル・トレーニング（High-intensity interval training, HIIT）が応用されてきている[17]．HIITとは，高強度と低強度の運動を交互に繰り返すトレーニング方法である．一般的には，高強度（最大負荷の90%前後）と低強度運動（有酸素運動）の繰り返しであるが，AT（前述）より高い強度の負荷を用いることにより，機械的ストレスのみならず筋内代謝的ストレスが高まり，運動耐容能の増加のみならず，筋力増加などのレジスタンス運動に類似した効果も得られる[17]．

おわりに

今日では諸疾患における運動療法のエビデンスが確立されており，またoverall healthを支える分泌因子産生臓器としての骨格筋機能が明らかにされつつある．紀元前にヒポクラテス（Hippocrates, BC 460年- 紀元前370年推定）は，歩くことの重要性を唱えた（“Walking is man's best medicine.”）．さらに “Everyone has a doctor in him or her.”（人はみな身中に名医を持つ）との名言を残して

❑ 表2 有酸素運動トレーニングのガイドライン[5)]

	一般的ガイドライン（AHA）	これまでの文献より
頻度	5日/週～	2日/週でも効果あり（Hambrecht R）[15)]
強度	予測最大心拍数の55～90% 最大酸摂取量の40～80% 予備心拍数（HRR）の40～80% 自覚的運動強度12～16	最大酸摂取量の40%でも有効 （Belardinelli R）[15)]
方法	歩行，トレッドミル，自転車等	単一の負荷方法では，特定の筋や関節に負担がかかるので，運動方法を変えるcross-trainingも推奨[5)]
持続時間	30～60分	20分でも有効（Coats A）[15)]

AHA，アメリカ心臓協会．
*予測最大心拍数は，220—年齢で算出．HRR（heart rate reserve）は，（最大心拍—安静時心拍）×係数＋安静時心拍．例えば，安静時心拍が60/分で最大心拍160/分の患者が50%で行う場合は，心拍数（HR）=(160-60) ×0.5+60=110となる．自覚的運動強度は，ボルグ・スケールである．なお，心拍数は，β遮断薬非服用下である．

❑ 表3 レジスタンス・トレーニングのガイドライン[2)]

	NYHA I	NYHA II-III	健常初心者
頻度	2-3日/週	1-2日/週	2-4日/週
運動時間	15-30分	12-15分	15-30分
強度	50-60% 1-RM	40-50% 1-RM	60%～1-RM
収縮速度	3秒短縮，3秒伸張	3秒短縮，3秒伸張	3秒短縮，3秒伸張
間隔	60秒以上	60秒以上	60秒以上
対象部位	4-9部位	3-4部位	9部位～
セット数	2-3	1-2	2, 3～
繰り返し	6-15	4-10	6-12

*片側性，部位ごとに行うのが望ましい．
*柔軟性，バランス運動は，毎日行う．
1RM（single-repetition maximal lift），最大挙上重量．

いる．その“一人”は骨格筋なのではないか．心疾患者では，病態による骨格筋機能低下，さらに廃用性の問題も起こりやすく，その結果，自立性の喪失と生命予後の著しい悪化を招く．疾患を持たない健常者は，運動した方がよいだろう．一方，疾患者は運動をしなければならない．運動により身体に秘める名医である骨格筋の崇高な健康保持・増進機能（図1）を呼び起こし，“治療”してもらうことが必要なのではないか．

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【筋肉代謝の基礎】

運動による筋肉の酸素利用効率の改善
—CKDにおける運動耐容能低下との関連*—

沖田孝一** 髙田真吾***

はじめに

骨格筋は運動器として身体活動を掌るばかりではなく，人体最大の内分泌器官でもあることが示され，全身的な健康を支えるための重要な器官であることが認識されてきている。慢性腎臓病（CKD）を含む慢性消耗性疾患においても，運動耐容能および骨格筋の質と量が予後を左右することが明らかにされつつあり[1,2]，併発するサルコペニア，フレイルおよびカヘキシー（cachexia：悪液質）に対する対策が必要となっている。CKD患者では，原因疾患に加え心機能低下，血管機能低下，さらに骨格筋障害の併発から，病初期より運動機能が低下し，CKDの進行に伴い増悪する[3,4]。CKD患者における運動能力の低下は患者の活動度を低下させ，生活の質（QOL）および日常活動動作（ADL）を悪化させるのみならず，心血管疾患などの発症にも関与し[5]，独立した生命予後規定因子となっている[2]。一方，運動トレーニングは，CKDを含む各種病態における運動耐容能低下に対する有効な治療法である[6]。本稿では，運動耐容能を規定する重要な因子である酸素利用能の視点から運動トレーニングの効果を解説し，さらにCKDにみられる運動耐容能低下および骨格筋障害とその対策について言及する。

運動耐容能と酸素利用能

1．運動耐容能を規定する骨格筋と呼吸・循環系の連関（図1）[7~9]

運動は，骨格筋の収縮により行われる。骨格筋の収縮にはATPの絶え間ない供給が必要であり，そのATPは筋細胞内ミトコンドリアが酸素と基質から作り出す。ゆえに，運動能力（運動耐容能）は骨格筋に対して酸素が十分に供給されるか，あるいは骨格筋（ミトコンドリア）にどれほど酸素を利用する能力があるかということで決まる。重度の慢性呼吸不全のように，呼吸による酸素取り込みが障害されていれば，たとえ心臓と骨格筋が頑丈であっても，骨格筋への酸素供給不足から嫌気性代謝が亢進し（乳酸蓄積），運動が制限される。同様に心ポンプ機能が障害されていれば，肺と骨格筋が頑丈であっても運動が制限され，骨格筋障害があれば，心肺機能に問題がなくても運動耐容能が低下することになる。ただし，これら3つの系をつなぐのは血液であり，貧血や血流障害が存在すれば，それが運動耐容能規定因子となりうる。

* Exercise improves skeletal muscle O_2 delivery and utilization—Exercise intolerance in CKD—
key words：酸素利用，運動耐容能，CKD，骨格筋障害
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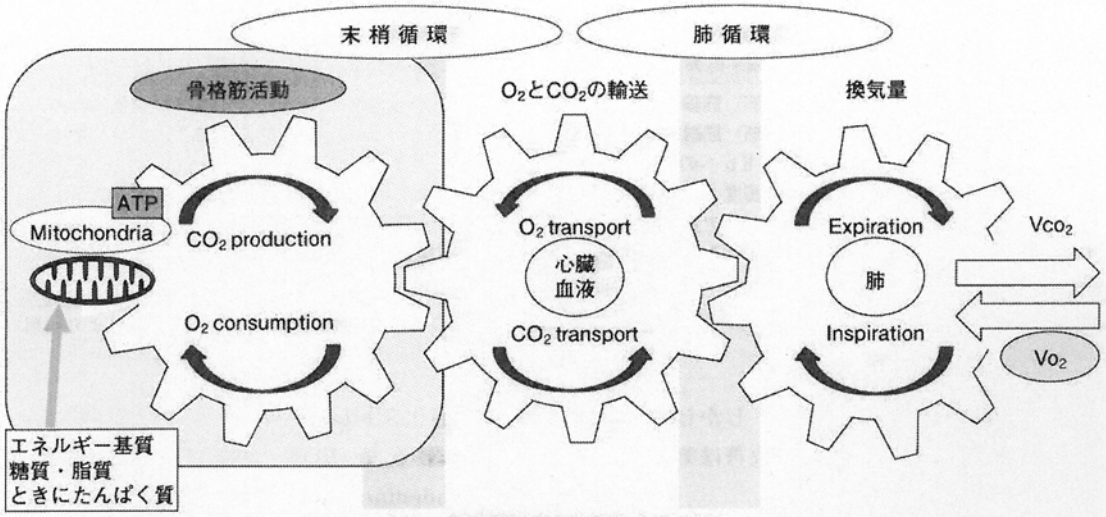

図1　運動耐容能を規定する因子

肺呼吸により酸素を取り込み，酸素化した血液を心臓から骨格筋へ供給し，細胞内小器官であるミトコンドリアが酸素を受けとり，エネルギー基質を利用して，ATPを産生する。ATP分解をエネルギーとして骨格筋が収縮し，運動が行われる。

Vo_2：酸素摂取量，Vco_2：二酸化炭素排出量

骨格筋の酸素摂取量（$\dot{V}mo_2$）は運動時に増大するが，それは換気量増加，肺血管拡張，心拍出量増加，末梢血管拡張などによる筋への酸素輸送（$\dot{Q}mo_2$，delivery）の増加と筋による酸素抽出（extraction）増加の結果である。肺胞からの酸素摂取量（$\dot{V}o_2$）は，呼気ガス分析により比較的容易に測定できる指標であり，定常状態では，$\dot{V}o_2 = \dot{V}mo_2$となる。

$\dot{V}o_2$は，動脈血酸素含量（Cao_2）と混合静脈血酸素含量（Cvo_2）の差（動静脈酸素含量較差）と心拍出量（CO）の積で表される（Fickの原理）。

$\dot{V}o_2 = CO\ (Cao_2 - Cvo_2)$

動静脈酸素含量較差の増大は，主に筋による酸素抽出の増加による。

2．骨格筋への酸素輸送と利用に影響する因子[7~9]

骨格筋の酸素輸送は，換気量，肺血流，心拍出量などの中心性因子と，末梢性因子である血管拡張能，毛細血管機能（microcirculation），酸素拡散能（diffusion），さらに血液性因子によって規定される。血液性因子とは，ヘマトクリット，ヘモグロビン濃度，ヘモグロビン酸素親和性，Cao_2，血液粘度，血漿粘度などである。酸素の物理的溶解はわずかであり，大部分は赤血球中のヘモグロビンによって運ばれるため，Cao_2は動脈血酸素分圧（Pao_2）とヘモグロビン濃度で規定される。一方，骨格筋は輸送された酸素を利用するが，その程度は筋に存在するミトコンドリアの量と質に規定される。十分な酸素が届いていても，ミトコンドリア機能が低下していれば，それを利用できない。

3．有酸素トレーニングによる骨格筋における酸素利用の改善[7~10]

運動トレーニングにより，主に呼吸循環系の改善〔1）~4）〕により酸素輸送能が改善し，また骨格筋の酸素利用能も増大する〔5）〕。さらに酸化ストレスの軽減〔6）〕も加わり，酸素利用効率は改善する。

1）呼吸系

肺活量の増加は必ずしも得られないが，最大分時換気量は増大する。酸素拡散能は改善し，また換気血流比が適正化され，効率が改善する。

2）中心循環系

心ポンプ機能および陽性変時作用が改善し，1

表　CKDにみられる骨格筋障害

形態的異常	組織学的異常	生化学的異常	そのほか
筋萎縮	Ⅰ型（遅筋）筋線維↓	酸化系酵素（CS）↓	インスリン抵抗性
筋線維径（Ⅱb）↓→	Ⅱ型（速筋）筋線維↑		RAS↑
	（ⅡaからⅡbへのシフト）		
	毛細血管密度↓→	ヘモグロビン（Hb）↓	炎症↑，酸化ストレス
	ミトコンドリア量↓	ミトコンドリア呼吸能↓	尿毒素↑
	アポトーシス↑	ミトコンドリア複合体Ⅰ-Ⅲ活性↓	
	オートファジー↑		

CS：クエン酸合成酵素，RAS：レニン-アンジオテンシン系　　（文献8，11～15，19）より引用）

回拍出量，心拍出量が増加する。しかしながら，基礎心疾患によっては心機能の改善は望めない。

3）末梢循環・微小循環系

eNOS（endothelial nitric oxide synthase）活性増加によるNO bioavailability（生体利用能）の改善，細胞間接着分子の減少，エンドセリンの減少などによる血管内皮機能の改善，交感神経活性低下，血管リモデリングなどにより末梢血管拡張能が改善し，活動筋への血流が増加する。一方で，血管運動神経の調節により非活動筋への血流が減少する（血流の再配分）。また毛細血管網の増加によりガス交換面積が増大し，酸素拡散距離が短縮する。

4）血液性因子

PAI-1（plasminogen activator inhibitor 1）やフィブリノーゲンの減少および線溶系の亢進などによる血液粘度の低下は，血流をスムーズにする。ヘモグロビン酸素解離曲線は，2,3DPG（diphosphoglycerate）増加などの影響で右にシフトし，酸素を解離しやすくなり，輸送能が増大する。

5）骨格筋

筋量の増加，筋線維系のシフト（遅筋/速筋比↑），ミトコンドリアの増加・機能の改善，酸化系酵素の増加，エネルギー代謝の改善などにより酸素利用能が増大する。特に慢性心不全では健常者と異なり，骨格筋レベルの障害が$\dot{V}mo_2$および運動耐容能に強く影響することが知られており，骨格筋障害を呈する慢性消耗性疾患では，酸素輸送能以上に重要な問題かもしれない。

6）酸化ストレス

活性酸素種（ROS）およびNADPH（nicotinamide adenine dinucleotide phosphate）oxidase活性が減少する一方で，抗酸化酵素および抗酸化物質の産生増加，マイオカインの産生・分泌などにより運動時の酸化ストレスおよび炎症が軽減される。特に活性酸素は，scavengingによりNOを不活化するため，その低下によりNO bioavailabilityが改善し，酸素利用能が増大する。そして，この抗酸化防御系の強化は，さらに高強度の運動を可能にする。

Ⅱ CKDにおける運動耐容能低下と骨格筋障害

CKDにおける運動能力低下の原因として，併存する心血管病や腎性貧血に伴う骨格筋への酸素供給の減少などのほかに，骨格筋障害の存在が報告されている[11]。これまでに明らかにされたCKDにおける骨格筋障害は，筋代謝酵素の変化（好気的酵素の減少），ミトコンドリア量・機能の低下，筋線維型の変化（遅筋から速筋へのシフト），筋萎縮などである（表）[12～15]。その成因として，尿毒素の増加，神経体液性因子の活性化，炎症・酸化ストレスの亢進，インスリン抵抗性，筋蛋白分解亢進，アポトーシスに加え，これらを身体不活動，栄養障害が助長するというような多面的機序が考えられている（表）。近年，CKD患者や動物モデルにおいて，骨格筋ミトコンドリアの量・機能が低下することが報告された[12,14,15]。同様に，遅筋（赤筋）が減少し，速筋（白筋）が増加することで運動耐容能が低下する[15]。

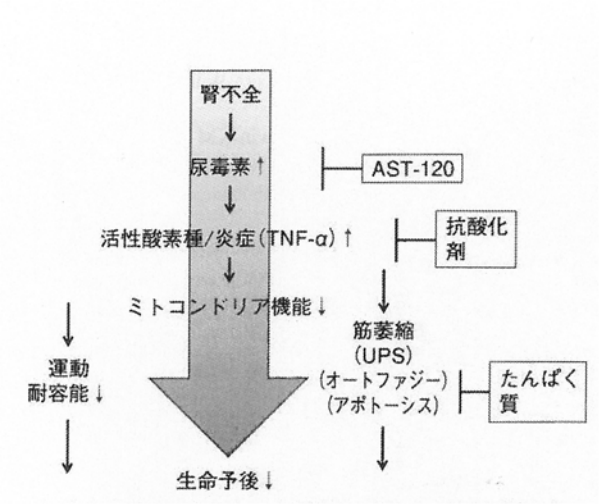

図2　腎不全により生命予後が低下する機序（仮説）
腎不全による尿毒素の増加を起因とし，活性酸素種の増加，炎症が亢進する。それらは骨格筋ミトコンドリア機能を低下させ，運動能力の低下，筋萎縮を促進することで，腎不全の予後を悪化させる。
TNF-α：腫瘍壊死因子アルファ，UPS：ユビキチン・プロテアソーム系

CKD患者における骨格筋量・筋力の減少は骨格筋蛋白合成能の低下，もしくはカヘキシーによって生じる[13,15]。骨格筋量の減少はQOLやADLレベルを低下させ，肥満，インスリン抵抗性，メタボリックシンドロームなどの代謝異常に関連し，CKD患者における死亡率の強力な予測因子となることが報告されている[1]。また，最大酸素摂取量低下にカヘキシーが加わると，さらに予後が不良である[16]。骨格筋量・筋力の減少には，これまで特に神経体液性因子であるレニン・アンジオテンシン系（RAS）がきわめて重要な役割を果たしていると考えられてきたが[17,18]，最近では，これらに加えてユビキチン・プロテアソーム系，オートファジーの亢進を介した骨格筋蛋白融解亢進の関与が注目されている（図2）。

Ⅲ 運動耐容能低下および骨格筋障害への対策

1．腎臓リハビリテーション・運動療法

すべてのステージのCKD患者において，定期的な運動療法が骨格筋機能・運動能力の改善に有用であることは示されており，推奨されている[6]。運動療法は筋内ミトコンドリア量・機能を増進させ，エネルギー代謝を改善する[20]。特に有酸素トレーニングは，酸化ストレスおよび炎症を減弱させることで，病態・予後の改善に寄与する[20]。

レジスタンストレーニングは，CKD患者においてもインスリン様成長因子の発現を増加させ，インスリンシグナルを活性化することで，骨格筋線維を肥大に導く[20]。このように運動療法の有効性は明らかであり，透析患者を含めたCKD患者に対する運動処方ガイドラインの確立が今後の課題である。

2．薬物療法

近年，Tamakiらによって，臨床研究と同様にCKD動物モデルにおいても骨格筋障害および運動能力の低下が起こることが報告された[15]。CKD早期（モデル作製後20週）では，炎症および酸化ストレスの亢進によって，運動能力および骨格筋ミトコンドリア量・機能が低下し，骨格筋線維型も遅筋から速筋へシフトする。さらにCKD後期（モデル作製後48週）では，早期でのphenotype（表現型）に加えて骨格筋量減少および筋力低下もみられようになる。

われわれは，CKDモデルに生じる運動能力低下および骨格筋ミトコンドリア量・機能障害を改善すべく，尿毒症性毒素の1つであるインドキシル硫酸（IS）に着目した。CKDモデル作製直後からAST-120（インドールの腸管吸収を抑制し，ISの血中濃度を低下させる）を投与し，早期（20週間後）の運動能力および骨格筋ミトコンドリア量・機能を検討した。血中IS濃度はCKDモデルで上昇するが，AST-120の投与で抑制された。さらに，CKDモデルでは運動能力，骨格筋ミトコンドリア量・機能が低下し，骨格筋酸化ストレスが亢進したが，それらはAST-120投与で改善された[14]。CKDにおけるAST-120による尿毒素の抑制は骨格筋内の酸化ストレス亢進を減弱し，ミトコンドリア量・機能の低下を防ぐのではないかと推察された[14]。

今日，骨格筋障害/カヘキシーが，心不全や

CKDのみならず，癌，慢性呼吸不全，HIVなど慢性消耗性疾患の予後を規定する共通の病態であると認識され，骨格筋萎縮・融解を予防・改善する方法および薬剤の開発が急速に進んでいる。CKDにおいては抗ミオスタチン抗体，miR-27，miR-486，C188-9などが治療薬として検討されている[21]。

おわりに

近年，健常者のみならずCKDを含む慢性消耗性疾患において，各病態に併発する運動耐容能の低下が予後を左右する重要な因子であることが明らかになってきている。そして運動トレーニングは，それらを改善する有効な治療法である。しかしながら，運動能力低下の背景には酸素運搬・利用能の低下のみならず，骨格筋障害など複雑な病態が絡んでおり，治療方法の選択あるいは薬物療法も含む介入効果の判定においては，より細かな知識が必要とされる。本稿がその理解の一助となり，広い視野からの診療，新しい治療戦略を考える契機になってもらえれば幸いである。

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平成27年度月形健康づくり・体力づくり推進事業実施報告

A Report with Regard to Tsukigata Health and Fitness Promotion Projects

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Ⅰ．はじめに

北翔大学生涯スポーツ学部は，月形町教育委員会，保健福祉課と連携し，平成24年度から開始した月形町民を対象とした健康づくり・体力づくり推進事業を実施している。本事業の目的は，月形町民が健康的で心にゆとりのある生活をおくることができるようになるために，町民自らが意識を高め，健康増進と体力増強に努める態度を培うことである。また，自分の身体に対して意識を高め，自分の健康は自分で守り，さらに町民自ら行う運動やスポーツ活動を通して地域コミュニティを形成しそれを充実させるという目標を掲

表１．ヘルシーアカデミーの展開について

	開催日時	場　所	内容及び担当
第１回	７月11日（土） 10時～12時	月形町総合体育館	チャレンジスポーツレク 【担当】本多理沙，小川裕美
第２回	10月12日（月・祝） ９時～13時	多目的アリーナ 町内	ノルディック・ウォーキング講習及び町民歩け歩け大会 【担当】本多理沙，他
第３回	11月15日（日） 10時～12時	総合体育館	がたリンピック 【担当】本多理沙，小川裕美
第４回	１月24日（日） 10時～13時	月形小学校グラウンド	ゴルボッカ 【担当】小田史郎，他
第５回	３月５日（日） 予定	総合体育館	年次まとめ 【担当】本多理沙，井出幸二郎

１）北翔大学生涯スポーツ学部スポーツ教育学科
２）北翔大学生涯スポーツ学部健康福祉学科
３）北翔大学北方圏生涯スポーツ研究センター
４）月形町教育委員会

げ，本事業は展開されている。展開内容は，第一ステップでは町民を対象とした体力測定，第二ステップでは測定結果の公表，町民に対する健康・体力づくりに関する講話，運動プログラムの紹介，第三ステップでは運動プログラムの実施とした。

本報では，平成27年度月形健康づくり体力づくり推進事業の実施内容について報告する。

Ⅱ．第一ステップ　町民体力測定

平成27年５月17日（日）月形町総合体育館にて，月形住民が各々の健康状態や体力を知り，自ら健康・体力づくりに取り組む生活態度を培うことを目的に，20歳以上の月形町民を対象に『町民体力測定』を実施した。体力測定前に血圧，身長，体重を測定し，身長及び体重から肥満度（体重÷身長×身長（m））を算出した。体力測定項目として，握力，長座体前屈，開眼片足立ち，ファンクショナルリーチ，10m障害物歩行，10m全力歩行，30秒立ち座りを行った。また，通常歩行時の動画をデジタルビデオカメラにより撮影記録し，画像解析ソフトウェア（ダートフィッシュ）により連続画像を撮影した。測定・記録を，北翔大学生涯スポーツ学部教員及び学生スタッフが担当した。

体力測定結果を表２に示した。参加者数は19名であった。今年度の参加者の体力は，こ

表２．参加者の年齢，血圧及び身体的特徴

		年齢（歳）	最高血圧（mm Hg）	最低血圧（mm Hg）	身長（cm）	体重（kg）	肥満度（体重/身長^2）
男性	全体(n=7)	67 ± 13	153 ± 20	83 ± 11	161 ± 7	66 ± 11	25 ± 3
	65歳以下(n=3)	54 ± 9	166 ± 8	93 ± 8	166 ± 4	74 ± 9	27 ± 2
	65歳以上(n=4)	77 ± 4	146 ± 23	79 ± 9	157 ± 6	60 ± 8	24 ± 2
女性	全体(n=12)	64 ± 15	134 ± 14	73 ± 7	156 ± 5	51 ± 7	21 ± 3
	65歳以下(n=4)	45 ± 7	137 ± 17	75 ± 10	161 ± 2	51 ± 5	20 ± 3
	65歳以上(n=8)	73 ± 7	132 ± 13	73 ± 6	154 ± 5	52 ± 7	22 ± 3

平均±標準偏差

表３．参加者の身体機能

		握力（kg）	FR（cm）	長座体前屈（cm）	開眼片足立ち（秒）	10m障害物歩行（秒）	10m歩行（秒）	30秒起居（回）
男性	全体(n=7)	40.3 ± 8.4	32.2 ± 4.6	28.2 ± 8.1	70.1 ± 53.0	6.1 ± 1.4	4.8 ± 1.1	24.6 ± 10.5
	65歳以下(n=3)	47.9 ± 6.6	33.3 ± 3.1	33.3 ± 10.3	111.7 ± 14.4	6.0 ± 1.9	4.6 ± 1.2	34.5 ± 3.5
	65歳以上(n=4)	35.2 ± 4.8	31.4 ± 5.8	24.4 ± 3.7	38.9 ± 49.5	6.2 ± 1.3	5.0 ± 1.1	18.0 ± 7.2
女性	全体(n=12)	24.3 ± 3.9	35.4 ± 6.6	33.2 ± 7.2	76.3 ± 46.1	7.2 ± 2.3	5.2 ± 1.0	22.4 ± 7.4
	65歳以下(n=4)	25.5 ± 5.0	40.0 ± 5.0	32.3 ± 6.0	120.0 ± 0.0	6.3 ± 1.3	4.8 ± 0.9	25.4 ± 7.0
	65歳以上(n=8)	23.7 ± 3.4	33.1 ± 6.2	33.6 ± 8.1	54.5 ± 41.3	7.6 ± 2.7	5.4 ± 1.1	20.9 ± 7.5

FR:ファンクショナルリーチ　　平均±標準偏差

れまでと平均値で比べても大きな差は認められなかった[1, 2]。今年度の参加者のうち７名が３年前の平成24年の第１回の測定にも参加しており，その７名の体力変化を図１に示した。体力の経年変化にはstudent t-testを用いた。この７名の内訳は男性３名，女性４名で，平成27年の測定時の年齢は73±７歳であった。血圧に経年変化は認められなかったが，肥満度が有意に改善した（P=0.01）。身体機能について，３年後においても，握力，

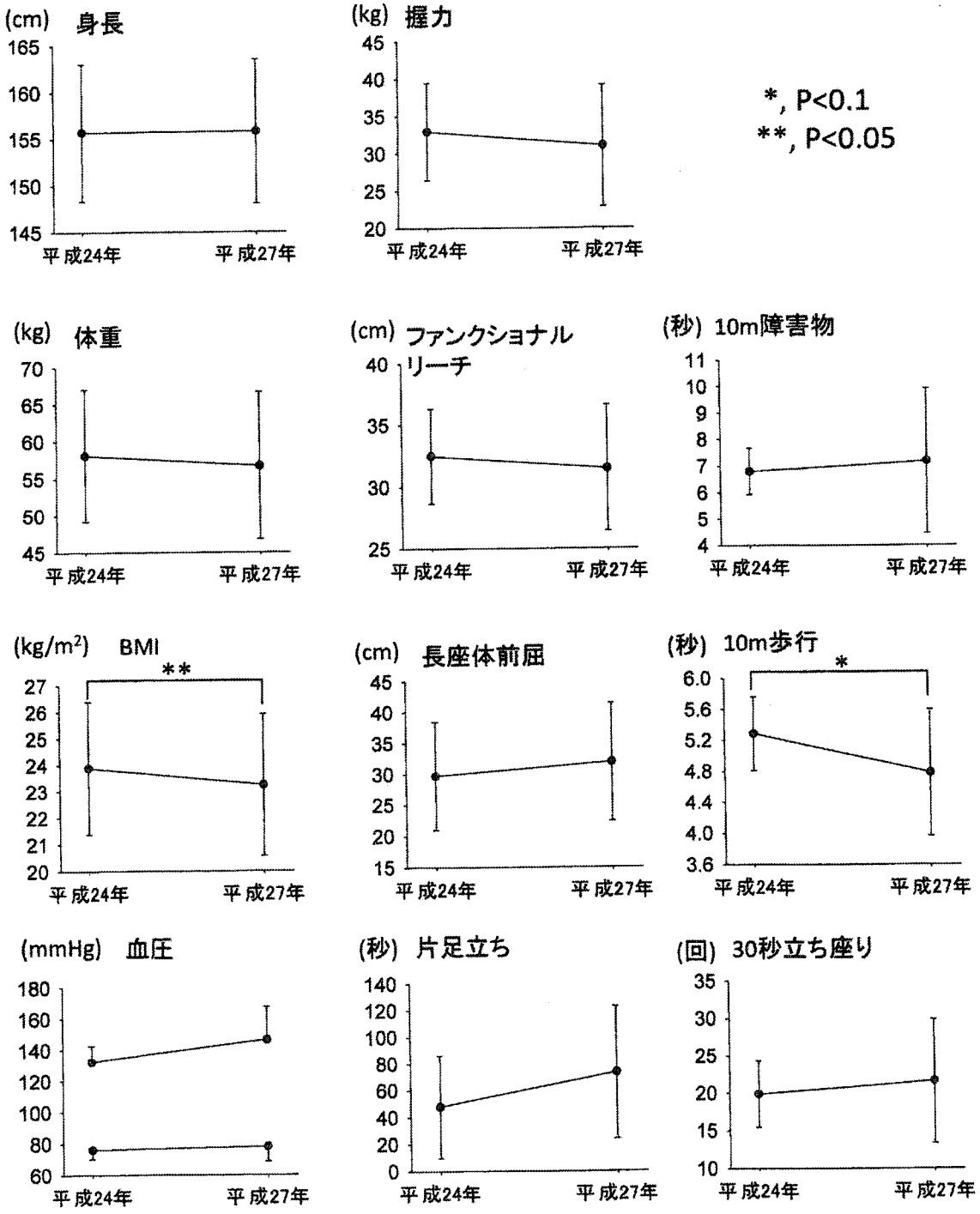

図１　月形町町民体力測定会の参加者の３年後の身体的な変化

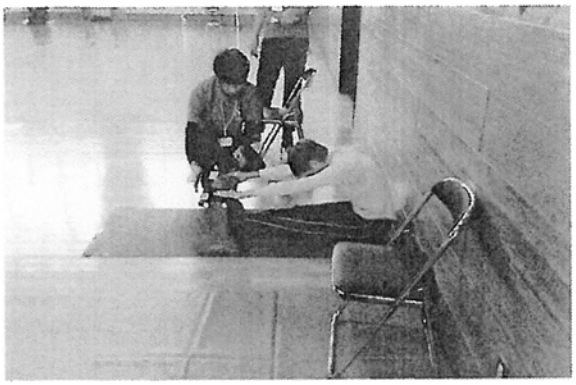

写真１　体力測定会

柔軟性，平衡機能，動的平衡機能，下肢パワーの低下は認められなかったが，10メートル歩行速度から評価した歩行機能は改善傾向が見られた（P=0.05）。月形町ヘルシーミーティングが始まって以来，年々参加者が低下している中で，複数回以上参加している者はヘルシーミーアカデミーにも頻繁に参加しており，そういった健康活動への関心の高さが肥満度の改善や歩行機能の改善傾向へ現れているのかもしれない。

Ⅲ．第二ステップ　ヘルシーミーティング

６月21日（日）月形町総合体育館にてヘルシーミティングを開催し，前回の体力測定結果のフィードバック及び健康・体力づくりの講話を行った。その後，参加者に対して体力

写真２　運動プログラム紹介

アップチャレンジ教室ヘルシーアカデミーで行われる運動を北翔大学ホップ圏生涯スポーツ研究センター研究員，北翔大学生涯スポーツ学研究科院生，北翔大学教員及び学生スタッフが指導した。参加人数は16名であった。

Ⅳ．第三ステップ　体力アップチャレンジ教室

第一ステップ，第二ステップを受け，第三ステップである体力アップチャレンジ教室ヘルシーアカデミーを実施した。展開内容は，第１回「チャレンジスポーツレク」，第２回「元気はつらつウォーキング講座　ノルディックウォーキング及び町民歩け歩け大会」，第３回「がたリンピック」，第４回「ゴルポッカ」，第５回「年次まとめ」とした。

「チャレンジスポーツレク」では，サッカー的当て，ペタンクボーリング，ワンバウンドバスケット，ピンポン飛ばし，玉入れを行った。「元気はつらつウォーキング講座　ノルディックウォーキング及び町民歩け歩け大会」では，歩行時の姿勢や歩幅など，効率よく運動できるウォーキングの方法や，ノルディックウォーキングの紹介及びその効果について説明し，実際にポールを用いた歩行を行い，ノルディックウォーキングを実演した。町民歩け歩け大会では，６㎞と９㎞のコースを設定し，体力に合わせて参加者はウォーキング，あるいは，ノルディックウォーキングを実施した。ノルディックウィーキング講座も町民歩け歩け大会とも参加した本学学生がつきがた町民参加者へのサポートを行った。「がたリンピック；つき“がた”オ“リンピック”」の内容は①ラダーゲッター，②ディスゲッター，③フロアカーリング，④ペタング，⑤スカットボール，

⑥スポーツ吹き矢で，幅広い年齢層で楽しめるレクリエーションスポーツで構成した。がたリンピックには，北翔大学生涯スポーツ学部健康福祉学科の教員及び学生，北翔大学ホップ圏生涯スポーツ研究センター研究員，北翔大学生涯スポーツ学研究科院生が支援した。「ゴルポッカ」は，月形小学校グラウンドにて行った。つきがた町民参加者と北翔大学からの学生参加者がグループを組み一緒に行い，学生は町民とコミュニケーションとサポートを重視して参加した。

ヘルシーアカデミーで展開されている内容は，転倒予防に有効な筋力トレーニングや，心臓循環器系の機能の維持改善に有効であり，高齢者の認知機能やメンタルヘルスの維持・改善に有効と考えられているウォーキング等，各々が習慣的に個としておこなえる運動と，高齢者でも冬期に雪上でもおこなえるゴルポッカ，ペタンクやフロアカーリング等，集団で行うニュースポーツやレクリエーションスポーツにより構成され，「自分の身体に対して意識を高め，自分の健康は自分で守る」という個の目標と，「運動やスポーツ活動を通して地域コミュニティを形成しそれを充実させる」という集団の目標を反映したものとなっている。

写真３　月形町民歩け歩け大会

Ⅴ．まとめ

「自分の身体に対して意識を高め，自分の健康は自分で守り，運動やスポーツ活動を通して地域コミュニティを形成しそれを充実させる」という本事業の目標を掲げ，月形町で健康づくり・体力づくり推進事業をH27年度も引き続き実施した。H24年から始めた月形町民体力測定会への参加者は，年々減少し，今回の参加者は24年度と比べ半数以下となった。体力測定会の参加者の減少は，町民各々の体力に対する関心の低さや体力測定会の面白み等の理由が挙げられる。体力は習慣的な運動習慣や日常の身体活動量を反映し，習慣的な運動習慣や身体活動量は，生活習慣病の危険因子であり，また，高齢者における認知機能の低下を予測する因子とみなされており[3, 4]，生活習慣病や認知症についての住民の関心を高めるにも，対策を講じる必要がある。体力だけではなく，簡単な医学的な検査や認知機能も評価することは，地域住民の心の健康にも関心をもたせることになるため，測定項目の一つとして取り入れることを検討したい。

謝辞

本事業を進めるにあたり，月形町関係者の方々の協力と支援をいただきましたことを，深く御礼申し上げます。

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S町における町民アンケート調査の結果から

The results of the residents survey in S town

上　田　知　行[1)]　　国　枝　　知[2)]
Tomoyuki　UEDA　　Satoshi　KUNIEDA

１．はじめに

S町は，北海道南西部に位置し，東西に14.0km，南北に23.5kmの総面積95.39㎢で日本海に面している。ほとんどが山林や原野に占められており，生活空間の多くは海岸線で形成されている．S町の主要な産業は，漁業であり，その歴史は古い。1600年代に豊富なニシンを背景に集落が形成され，町の始まりともされている。人口は2017年１月末現在で，男1553人，女1613人の3166人（寿都町発表），世帯数1750世帯である。出生数の減少と平均寿命の延伸により，高齢化率は年々高まっている。

平成26年９月，S町と北翔大学は，包括連携協定を締結し，北翔大学の掲げる地域貢献をS町で実現することとなった。平成27年４月に竣工したS町総合体育館の健康づくりへの活用に関するプログラム作成および実施をはじめ，S町有志による総合体育館活用プランの作成，すでに3者連携協定を結んでいる，NPO）ソーシャルビジネス推進センター・コープさっぽろとともに「地域まるごと元気アッププログラム」のS町での実施に取り組み始めた。

これらの取り組みの中で，住民の健康づくりや運動実施に関するニーズ分析をする目的で，平成27年９月にアンケート調査を実施した。本報告はこのアンケート調査の結果に基づき，成人住民の志向する健康づくり運動プログラムについて，考察をすすめる。

２．S町総合体育館活用プランの概要

S町と包括連携協定を締結した北翔大学は，生涯スポーツ学部を中心として，S町総合体育館活用プランを策定した。総合体育館を健康づくり施設として町民の活用を促すため，運動に対して「つらい」「きつい」といった負のイメージを変容するために「健康スポーツマインド」を形成することを目的とした。概要は以下のとおりである。

１）S町運動促進委員会の設立

特に運動習慣がない，または運動を実施していない住民をよく理解している住民に対して，「S町運動促進委員」を公募した。５名の公募があり，S町教育委員会教育長から同委員が委嘱された。おおむね月に一度の会議を持ち，健康づくりに取り組む町の方向性の作

１）北翔大学生涯スポーツ学部スポーツ教育学科
２）寿都町教育委員会

案，体育館を活用する事業案，町民アンケートの作成，アンケート結果の分析，町民の志向に見合う体育館活用に向けたプログラムの作成・試行・評価・修正を行うこととした。

２）健康づくりのための運動プログラム「S町総合体育館活用プログラム」の実施

S町総合体育館が竣工された2016年５月から2017年３月にかけて「さわやか元気塾」と称し，以下の運動教室を実施した。第１期は，2016年５月11日から2016年７月27日の11回にわたる「歩くを極める！」で，ウォーキングや筋力トレーニング・軽体操の内容を実施し，延べ268名の参加があった。第２期は，2016年８月10日から11月30日の14回にわたる「男の筋トレ」で，男性を対象に，筋力トレーニングを中心とするプログラムを実施し，延べ158名が参加した。第３期は，2016年12月７日から2017年３月21日の15回にわたる「ぱわふる運動教室」で，サーキットトレーニングを中心とするプログラムを実施している（2017年２月22日現在の延べ参加者数282名）。他に，各種運動プログラムを体験する40回のプログラムを実施した。

３）運動を敬遠する住民層に向けた体験型イベントの開催

S町総合体育館が竣工された2016年５月から2017年３月にかけて，総合体育館の健康づくりへの活用促進を促すため，運動を敬遠している住民層が気軽に参加できるレクリエーションイベントとして，「あそび場！（ASO-VIVA）」と称し，22回実施した。けん玉やコマなどの昔遊びの他，ニュースポーツやレクリエーションスポーツの実施，多世代交流が可能な集団型ゲームなどを実施し，2017年２月14日現在で，延べ995名の参加があった。その他講座型の健康講演会を５回開催した。

４）「地域まるごと元気アッププログラム」運動教室の開催

高齢者の介護予防につなげることを目的とした継続的な運動教室として2015年10月に始まった「地域まるごと元気アッププログラム」（以下，「まる元」）の開催会場を，これまでの文化センターからS町総合体育館に移し，25名定員４クラスの教室として平成27年度は各クラス48回実施した。

３．S町町民アンケート調査の方法

S町アンケート調査は，S町運動促進委員会により，アンケート項目が検討され，次の項目とし2016年９月に実施した。

①健康づくりへの興味や関心，②具体的な健康づくりのための運動への参加状況，③町内で実施されている運動プログラムの認知程度や参加状況，関心の程度，④総合体育館活用のための自由意見，⑤家族や友人などのかかわりと社会活動。

19歳以上の町民を無作為に1000名抽出し，郵送法にて調査を実施した結果，各年代から50％前後の回答があった（表１）。また，小学校高学年および中学生のすべての町民に調査を実施したところ，116名から回答が得られた（表２）。

質問項目の概要は以下のとおりである。

１）健康づくりに関する興味や関心について

（１）自分の健康に興味はありますか

表１：Ｓ町アンケート調査の回答者数（成人）

年代区分	実人口	割合	抽出人数(割合×1000)	回答者(男性)	回答者(女性)	性別不明	回答者合計	回収率
19歳～29歳	197	7.1%	71	14	21		35	49.3%
30歳～39歳	331	12.0%	120	30	40		70	58.3%
40歳～49歳	348	12.6%	127	30	26		56	44.1%
50歳～59歳	375	13.6%	136	29	42		71	52.2%
60歳～69歳	564	20.4%	205	59	55		114	55.6%
70歳～79歳	468	16.9%	170	29	59		88	51.8%
80歳以上	472	17.1%	171	23	41	1	65	38.0%
年齢不明				1	1	7	9	
合計	2755	99.7%	1000	215	285	8	508	50.8%

（２）定期的に健康診断は受けていますか

（３）健康づくりのためにどのようなことを行っていますか(または心がけていますか)

２）運動習慣や身体活動について

（１）運動教室に参加したことがありますか

（２）１回30分以上の運動を週２回以上行っていますか

（３）１回５分以上の簡単な体操を週４回以上行っていますか

３）健康づくりのために役立つ運動プログラムについて

（１）Ｓ町で行なわれている健康づくりのプログラムについて

（２）Ｓ町で行なわれている運動サークルを教えてください

（３）運動にはどのようなイメージがありますか

（４）参加してみたい運動プログラムはありますか

（５）多くの町民が総合体育館を利用するには何が必要であると思われますか

３）家族や友人との関わりと社会活動について

（１）家族や親せき

（２）近くに住んでいる人を含む友人全体について

表２：Ｓ町アンケート調査の回答者数（子ども）

年齢区分	回答者(男性)	回答者(女性)	回答者合計
小学５年生	16	5	21
小学６年生	12	16	28
中学１年生	14	9	23
中学２年生	9	13	22
中学３年生	9	13	22
合計	60	56	116

（３）この１年間で行なった社会活動について

４．Ｓ町アンケート調査の結果

１）健康づくりへの興味や関心について

「自分の健康に興味はありますか」の質問に関しては，90.7％が「かなりある」「多少ある」と答え，また「定期的に健康診断」を受けている人の割合は70.0％で，いずれも性別や年代に大きな差はなかった。

「健康づくりのためにどのようなことを行っていますか（または心がけていますか)」の質問では，性別や年代に関わらず「食事に気をつける」「運動をする」ことが上位となった。特に70歳代以上では，それぞれ77.1％，74.5％と高い割合であった。他に「休養をと

表3：「健康づくりのためにどのようなことを行っていますか（複数回答）」

	人数	食事	体重管理	飲酒を控える	休養をとる	運動をする	喫煙をしない	健康食品を買う	健康器具を買う	ストレスをためない	特に何もしない
合計	508	65.4%	48.4%	17.9%	32.3%	56.1%	18.5%	9.6%	4.1%	28.7%	11.0%
男性	215	57.2%	42.8%	22.3%	29.8%	56.3%	23.7%	8.8%	5.1%	24.7%	13.0%
女性	285	71.6%	53.3%	15.1%	35.1%	56.1%	14.7%	10.2%	3.2%	31.9%	9.5%
20～30歳代	105	57.1%	40.0%	21.9%	36.2%	41.0%	25.7%	6.7%	4.8%	24.8%	14.3%
40～60歳代	241	61.8%	49.4%	18.7%	27.0%	51.0%	19.9%	8.7%	3.3%	29.5%	11.2%
70歳代以上	153	77.1%	53.6%	15.0%	39.9%	74.5%	11.8%	13.7%	5.2%	30.1%	7.8%

表4：「1回30分以上の運動を週2回以上行っていますか」

	人数	行うつもりはない	行わなければならないと思う	ときどき行っている	最近はじめた	6か月以上行っている
合計	508	10.6%	42.1%	23.4%	4.3%	12.2%
男性	215	10.7%	37.7%	27.4%	2.8%	14.9%
女性	285	10.5%	46.0%	20.7%	5.6%	10.2%
20～30歳代	105	12.4%	49.5%	24.8%	1.9%	8.6%
40～60歳代	241	9.5%	49.8%	20.3%	3.3%	11.6%
70歳代以上	153	10.5%	26.1%	28.8%	7.8%	15.0%

表5：「運動にはどのようなイメージがありますか」

	人数	楽しい	ストレス解消	健康になれる	生きがい	達成感	疲れる	面倒くさい	きらい	きつい	つらい	体をこわす	合計
行うつもりはない	54	8	16	21		2	18	15	10	13	8	2	113
行わなければならないと思う	214	76	107	145	15	57	61	38	10	31	17	5	562
ときどき行っている	119	57	66	1	14	29	22	9	1	9	5		213
最近はじめた	22	5	9	13	2	3	1	2		1	1		37
6か月以上行っている	62	40	45	51	17	26	9	3		4	2		197
合計		186	243	231	48	117	111	67	21	58	33	7	1122

る」「ストレスをためない」も30％程度の回答となり，健康の三要素である「栄養」「運動」「休養」に関しての興味や関心がある回答者が多いことがわかった（表3）。

2）運動習慣や身体活動について

「運動教室に参加したことがありますか」の質問について，「ない」と答えた者が全体の75.0％であった。一方，「1回30分以上の運動を週2回以上行っていますか」の質問について，「ときどき行っている」「最近はじめた」「6カ月以上行っている」と答えた者が，全体では40.0％おり，年齢層があがるほどに多くなっていた。また，「行わなければならないと思う」と答えた者を含めると90％程度が運動について肯定的な態度を示していた（表4）。

3）健康づくりのために役立つ運動プログラムについて

表5は，「運動にはどのようなイメージが

ありますか」の質問に対する回答を「１回30分以上の運動を週２回以上行っていますか」の回答者ごとに区分して集計したものである。運動習慣を継続している者ほど否定的イメージは少なかった。「行わなければならないと思う」という運動実践にはいたらないものの肯定的な態度を示している者は，運動に対するイメージも肯定的にとらえている一方で，「疲れる」「面倒くさい」「きつい」という負担感を持つ者も多かった。

「参加してみたい健康づくり活動プログラムはありますか」の質問に対する回答を「１回30分以上の運動を週２回以上行っていますか」の回答者ごとに区分して集計した（表６）。全体では「ウォーキング」「ストレッチ」筋力トレーニング」「マシントレーニング」「ヨガ」などといった内容が理解しやすく，手軽に実施できそうなプログラムに対する回答が多かった。また，「行わなければならないと思う」「ときどき行っている」「６カ月以上行っている」と答えた者は，複数回答が多く，簡単に実施できるプログラムを用意することで，参加してみたいと考えていることが示唆された。一方「最近はじめた」と答えた者は，回答率が少なく，始めだした運動プログラムへの関心が高いことが推察された。「行うつもりはない」と答えた者は，回答者が少なかった。

「多くの町民が総合体育館を利用するには何が必要であると思われますか」の質問は自由記載で回答を求めた。508名の回答者のうち，142名から192件の積極的な意見が出された。出された意見を10種類に分類した。①「団体に所属していなくても個人で気軽に利用できる雰囲気があるとうれしいと思います」など個人利用に関する意見が12件あり，自由に利用できる仕組みづくりの必要性が考えられた。②「ポイントカード制」などインセンティブに関する意見が４件あり，きっかけづくりにより利用を促進できる可能性が示唆された。③「どのような活動が行われているのか，どのようなことが可能かなど情報発信機能を高め，興味をもってもらう」など情報発信に関する意見が30件あり，総合体育館の存在を一般化させることが必要であることがわかった。④「面白いメニューを考える必要がある」など運動プログラムに関する意見が40件，また⑤「トレーナーが付いた指導が受けられるスポーツジム的なことが出来たら利用したい」など指導者に関する意見が12件あり，ソフト面での活用促進が考えられた。⑥「使い方などをきちんとガイドラインとしてつくる」など使用上のルールに関する意見が11件あり，誰もが気持ちよく利用できるルール作りの必要が示唆された。⑦「簡単なトレーニングマシンがあればよいと思います」などトレーニングマシンの設置や設備に関する意見が23件，また⑧「送迎バスがあるとうれしい」など交通利便性や送迎に関する意見が24件あり，施設内外でのインフラを整備するニーズがあることがわかった。他に⑨「集うと楽しい場」など仲間づくりに関する意見が８件，⑩「参加する気持ちと意志が必要である」など町民の意識に関する意見が６件などであった。

５．まとめ

2015年４月に竣工したＳ町総合体育館が町民の健康づくり施設として活用されるための取組のうち，町民への健康づくりに対するニ

表６：「参加してみたい健康づくり活動プログラムはありますか」（複数回答）

	人数	ウォーキング	マシントレーニング	ダイエット	ヨガ	ジョギング	エアロビック	メタボ対策プログラム	太極拳	ストレッチ	転倒予防プログラム	介護予防プログラム	筋力トレーニング	合計
行うつもりはない	54	5	4	1	3			2	5	3	1	1	1	21
行わなければならないと思う	214	70	46	44	42	15	14	39	13	67	16	8	59	363
ときどき行っている	119	37	26	18	26	12	6	8	9	30	15	4	26	180
最近はじめた	22	7	4	3	2			1		3	2	1	5	21
6か月以上行っている	62	24	13	13	13	7	4	5	4	15	9	5	10	98
その他	21	4	2	2	4	1	1	1	3	4	6	1	1	26
合計		147	95	81	90	35	25	56	34	122	49	20	102	709

ーズを探るためアンケート調査を行った。

健康づくりへの関心は高いものの，行動を起こすまでは至っていない町民の存在が明らかになり，適切な運動プログラムの提供により，多くの町民が総合体育館を活用する可能性が高いことがわかった。また，健康づくりのための運動が行いやすいように，ルールやプログラム，マンパワーなどのソフト面や，施設や交通機関などのハード面に関する整備を行うことで，総合体育館を活用しても良いと考える住民の利用が促進することが示唆された調査結果となった。

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Sex-differences in age-related grip strength decline: A 10-year longitudinal study of community-living middle-aged and older Japanese

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Abstract The purpose of this study was to estimate sex differences in age-related grip strength decline and describe the course of decline in grip strength from age 40 to 89 years by a longitudinal epidemiological study. Participants were randomly selected community-living men (n = 648) and women (n = 598) aged 40 to 79 years at baseline. Grip strength was measured with standard techniques every other year over a 10-year period. The preservation rate of grip strength was calculated as the 10-year follow-up value divided by the baseline value. The relationship between the preservation rates of grip strength and age group (by decade) at baseline by sex was analyzed using Two-Way Analysis of Variance and the Tukey-Kramer method. The trajectories of grip strength over 10 years were plotted for both men and women. The mean grip strength preservation rates of participants in their 40s, 50s, 60s and 70s over 10 years was 0.90, 0.88, 0.84 and 0.79 in men, and 0.89, 0.89, 0.89 and 0.88 in women, respectively. There were significant differences in sex and age group at baseline in the preservation rate of grip strength. Among men, the preservation rate of grip strength for the 70s group was significantly lower than that of younger groups ($p < 0.05$); however, no significant difference was observed among age groups in women. The trajectories of grip strength decline year by year were steep in men, but even in women. Age-related decline in grip strength markedly increased in older men, but remained constant throughout middle and late adulthood in women.

Keywords : sex difference, grip strength, aging, longitudinal data

Introduction

By 2035, it is estimated that people aged 65 years and older will comprise more than one third of the Japanese population[1]. The number of older people who depend on health care services is thus set to grow. In addition, about 70% of older people are expected to be living alone or with their spouse[2]. In order for older people to maintain independence in the community, it is important to perform daily tasks without difficulty.

Poor muscle strength has proven to be one of the strongest indicators of impairment, activity limitation and mortality among older people[3-6]. The term dynapenia was coined to explain the loss of physical function and increased risk of disability among older adults[7]. Grip strength, which represents hand strength, as well as lower extremity muscle strength, has been adopted as a useful indicator[8]. Recently the term sarcopenia, which was initially defined as the age-related loss of muscle mass, has been redefined as the loss of muscle mass in combination with loss of muscle strength (grip strength) and/or physical performance (walking speed)[9,10]. Frailty is a major health problem for aging populations as it makes older people vulnerable to poor recovery after a stressor event[11]. According to Fried et al.[12], the definition of physical frailty, which is one of the most well-known criteria of the frailty phenotype, includes weakness (grip strength) with weight loss, self-reported exhaustion, slow walking speed, and low physical activity.

Because grip strength is established and simple to measure, it has been used across wide age groups[13] and in institutional settings to assess older people[14]. However, most nationwide surveys and functional assessments of grip strength in institutions have typically not addressed

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the aging process. Although longitudinal studies show that grip strength in adults declines with age[15-19], little research has been conducted on changes in grip strength using measure-based longitudinal data and well-balanced large subject groups comprising both sexes.

The aim of the present study was to estimate sex differences in grip strength decline and describe the course of this decline from 40 to 89 years of age for men and women using longitudinal data from community-living middle-aged and older people.

Methods

Study Population. Participants included 648 men and 598 women who participated in both the baseline study and the subsequent 10-year follow-up study of the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA). The NILS-LSA was based on data obtained from interviews and laboratory examinations of medical, nutritional, psychological and physical fitness variables. Details of the study can be found elsewhere[20]. The initial survey of the NILS-LSA involved 2267 participants 40 to 79 years of age, including almost 300 men and 300 women from each group categorized by age decade (40s, 50s, 60s and 70s). The participants were gender and age-stratified random samples of the residents of Obu-shi and Higashiura-cho, Aichi Prefecture, in central Japan. Participants were drawn from residence registrations in cooperation with local governments. All participants lived or had lived at home in the community. Those who lived in nursing homes or long-term care homes were excluded. The NILS-LSA involves biennial examinations; therefore, the participants had a maximum of six examinations (baseline and 2nd, 4th, 6th, 8th and 10th year) during the 10-year follow-up period. By the end of the 10-year follow-up, some participants had dropped out due to death (n = 248), moving to another area (n = 9), or refusal to participate or no response (n = 748) (Fig. 1). Participants who could not take part in the grip strength test during the 6th wave survey were also excluded (n = 16). The final numbers of participants in each age group by decade (40s, 50s, 60s and 70s) were 210, 219, 156 and 63 men, and 203, 204, 140, and 51 women, respectively; and the participation rate for the grip strength test in the 10-year follow-up was 72.2%, 77.7%, 55.1% and 22.3% for men and 72.0%, 73.1%, 49.1%, and 18.1% for women, respectively. As expected, the participants aged 70–79 years had the highest number of dropouts. The mean number of examinations per participant was 5.8 ± 0.5. No significant differences were observed in the participation rate of each age group or the number of examinations by sex. All NILS-LSA procedures were approved by the Ethical Committee of the National Center for Geriatrics and Gerontology, and all participants provided written informed consent.

Measurements. A handgrip dynamometer (Takei Co., Niigata, Japan) was used to assess grip strength in kg. The participants held a handgrip dynamometer while standing with their arms at their sides and their elbows extended and squeezing with maximum force, alternating the left and right hands. The average of two readings from each hand was used as the measurement result. The safety of the participants was closely monitored during all tests. The examiners carefully measured grip strength while monitoring the participants' blood pressure and fa-

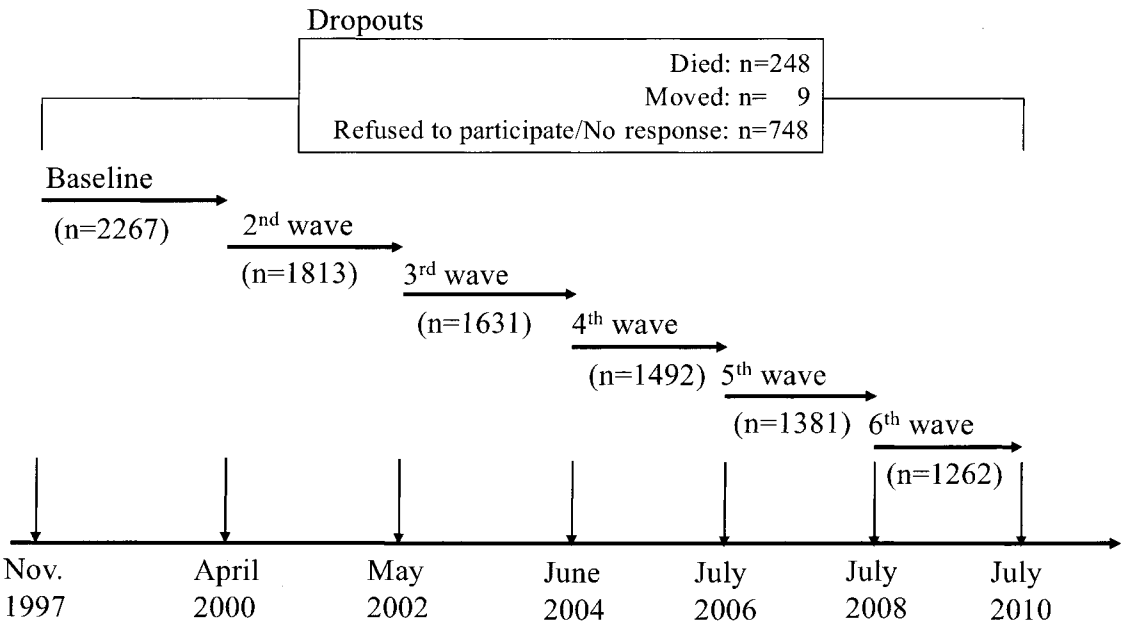

Fig. 1 Time frame and number of participants at baseline and at each follow-up survey.

tigue level. They advised the participants to exhale while squeezing during the grip strength measurements and to perform the repetitions at their own pace. A medical doctor asked the participants about their health condition before starting the grip strength tests. If any participant had any serious pain, physical injury, or illness of the orthopedic or cardiovascular systems, they did not take part in the tests.

Statistical Analysis. The baseline characteristics of the participants in the 10-year follow-up and the dropouts were analyzed for differences using Student's-*t* test for continuous variables, and the Cochran-Mantel-Haenszel test for categorical variables by sex. Student's-*t* test was also used to compare grip strength at baseline between the participants and the dropouts by sex and age group. The preservation rate of grip strength for evaluating changes regardless of the absolute value was calculated by dividing the 10-year follow-up value by the baseline value. The relationship between the preservation rate of grip strength and age group by decade at baseline and sex was analyzed using Two-Way Analysis of Variance (ANOVA), and the Tukey-Kramer method was used for multiple comparison analysis by sex. Effect sizes were calculated to evaluate the magnitude of differences in grip strength by sex, age group and sex*age group. To observe the age-related changes in grip strength throughout middle and late adulthood, the mean grip strength for each year within the age groups was calculated at baseline and at each follow-up for both men and women. The mean grip strength was plotted for the age span of 40 to 89 years. Statistical testing was performed using the Statistical Analysis System version 9.1 (SAS Institute Inc., Cary, NC, USA). Significant probability levels were considered to be less than 0.05.

Results

The baseline characteristics of the participants in the 10-year follow-up and the dropouts are shown in Table 1. The participants in the 10-year follow-up were significantly younger, taller, heavier, had higher incomes, more education, better self-rated health and less prevalent diseases than dropouts in men. The results for women were similar. The participants in the 10-year follow-up had greater grip strength than the dropouts in the 50s, 60s, and 70s age groups among men and in the 60s age group among women (Table 2).

The mean grip strength preservation rates of participants in their 40s, 50s, 60s and 70s for the 10 years at baseline were 0.90, 0.88, 0.84 and 0.79, in men, and 0.89, 0.89, 0.89 and 0.88 in women, respectively (Fig. 2). The skewness and kurtosis of the grip strength preservation rate by sex were 0.00 and 3.02 in men, and 1.20 and 8.83 in women, respectively. The results of the two-way ANOVA indicated that there were significant main effects and interaction of sex and age group at baseline in the preservation rate of grip strength (sex, $df = 1$, $F = 8.87$, $p = 0.003$, $\eta^2 = 0.007$; age group at baseline, $df = 3$, $F = 10.54$, $p < 0.0001$, $\eta^2 = 0.024$; sex*age group at baseline, $df = 3$, $F = 8.52$, $p < 0.0001$, $\eta^2 = 0.020$). Power analysis using the GLM-POWER procedure showed that this model had more than 99% power to detect differences in grip strength by sex (99.4%), age group (99.5%) and sex*age group (99.2%).

The grip strength preservation rate in older age groups was significantly lower than that of younger groups among men (40s, 50s, 60s > 70s, 40s > 60s; $p < 0.05$); however, no significant difference was observed among age groups in women. The average annual decline rate in grip strength in men was -1.0% per year in the 40s, and gradually increased with age to double the reduction rate (-2.0% per year) in the 70s. In contrast, among women, grip strength declined at a constant rate (-1.0% per year) among all age groups.

Yearly changes in grip strength using the mean grip strength for each year of age are shown in Fig. 3. Each line joins six values for every two years from baseline to the 10-year follow up. Trajectories of grip strength for the age span of 40 to 89 years illustrated that the course of decline in grip strength with age was steep among men, whereas it remained the same among women. Although sex differences in grip strength tended to be less obvious in the older age groups, women had much lower grip strength than men throughout the age span of 40 to 89 years.

Discussion

We demonstrated an age-related decline in grip strength across middle and late adulthood for men and women using 10-year longitudinal data. Our main finding was that the decline in grip strength differs between sexes in middle and late adulthood. Among men, age-related decline in grip strength was greater in late adulthood, whereas among women, age-related decline in grip strength was constant across middle and late adulthood.

The age-related decline in grip strength among men was associated with age at baseline. It is well-known that women have less muscle strength than men at every stage of their adult life[21]; however, sex differences in the decline of muscle strength remain unclear because few longitudinal studies of muscle strength for men and women in the same population have been reported[17]. Previous studies support our findings that the decline in grip strength accelerates with age among men[15] and that, among the oldest women, a horizontal plateau in grip strength decline is observed[16]. However, no sex differences in the rate of decline in grip strength were reported in a previous 10-year prospective study[22]. Different age ranges and cohort sizes between the previous and current study may have led to the varied results. This study, which was designed for the same follow-up period and

Table 1. Baseline charactaristics of the participants in the 10-year follow-up and the dropouts for men and women

		Men			Women		
		follow-up n=648	dropout n=491	*p*-value	follow-up n=598	dropout n=530	*p*-value
Age	years	**55.5 ± 9.2**	**64.1 ± 11.1**	**<0.01**	**54.8 ± 9.2**	**64.3 ± 10.6**	**<0.01**
Height	cm	**165.9 ± 5.9**	**162.7 ± 6.6**	**<0.01**	**152.9 ± 5.3**	**149.5 ± 6.3**	**<0.01**
Weight	kg	**63.7 ± 8.5**	**60.1 ± 9.6**	**<0.01**	**53.2 ± 7.7**	**51.6 ± 8.7**	**<0.01**
BMI	kg/m^2	**23.1 ± 2.6**	**22.7 ± 3.1**	**<0.01**	22.8 ± 3.1	23.0 ± 3.5	0.16
Body fat	%	21.2 ± 4.1	21.5 ± 4.7	0.17	**31.1 ± 4.8**	**32.0 ± 5.5**	**<0.01**
Education	years	**12.6 ± 2.5**	**11.6 ± 2.5**	**<0.01**	**11.8 ± 2.1**	**11.0 ± 2.0**	**<0.01**
Annual income	%						
6,500,000 yen and higher		**61.9**	**34.8**	**<0.01**	**53.7**	**30.2**	**<0.01**
Smoking	%			0.10			0.53
Never		23.9	18.7		89.4	90.2	
Former		39.7	41.1		2.7	3.2	
Current		36.4	40.1		7.9	6.6	
Self-rated health	%			**<0.01**			**<0.01**
Excellent		**3.3**	**2.7**		**4.2**	**1.7**	
Very good		**28.9**	**17.6**		**24.1**	**11.9**	
Good		**59.5**	**64.6**		**63.9**	**71.6**	
Fair		**8.2**	**14.3**		**7.9**	**13.2**	
Poor		**0.2**	**0.8**		**0.0**	**1.5**	
Prevalent diseases	%						
Stroke		**1.6**	**6.0**	**<0.01**	**0.5**	**3.4**	**<0.01**
Hypertension		**20.1**	**30.0**	**<0.01**	**19.2**	**35.7**	**<0.01**
Heart diseases		**8.7**	**16.1**	**<0.01**	**7.9**	**14.2**	**<0.01**
Diabetes		**7.5**	**14.1**	**<0.01**	**3.0**	**8.3**	**<0.01**

BMI, Body mass index. Continuous variables are presented as means ± standard deviation (SD), and categorical variables are presented as percentages. The differences between groups were analyzed by Student's-*t* test for continuous variables and by Cochran-Mantel-Haenszel test for categorical variables. Bold represents significant *p*-value (<0.05).

Table 2. Baseline grip strength of the participants in the 10-year follow-up and the dropouts in each age group for men and women

	Men							Women						
	follow-up			dropout				follow-up			dropout			
age groups	n	Mean	SD	n	Mean	SD	*p*-value	n	Mean	SD	n	Mean	SD	*p*-value
40-49	210	46.2	± 6.4	81	45.8	± 6.6	0.56	203	27.5	± 4.8	79	26.7	± 4.7	0.21
50-59	**219**	**42.7**	**± 6.3**	**63**	**40.9**	**± 7.4**	**0.05**	204	25.3	± 4.4	75	24.3	± 4.5	0.08
60-69	**156**	**38.9**	**± 5.7**	**127**	**37.1**	**± 6.4**	**0.01**	**140**	**23.5**	**± 4.1**	**145**	**22.2**	**± 4.3**	**0.01**
70-79	**63**	**35.2**	**± 5.6**	**220**	**33.4**	**± 6.0**	**0.04**	51	20.7	± 3.7	231	19.8	± 3.9	0.13

SD, standard deviation. The differences between groups were analyzed by Student's-*t* test. Bold represents significant *p*-value (<0.05).

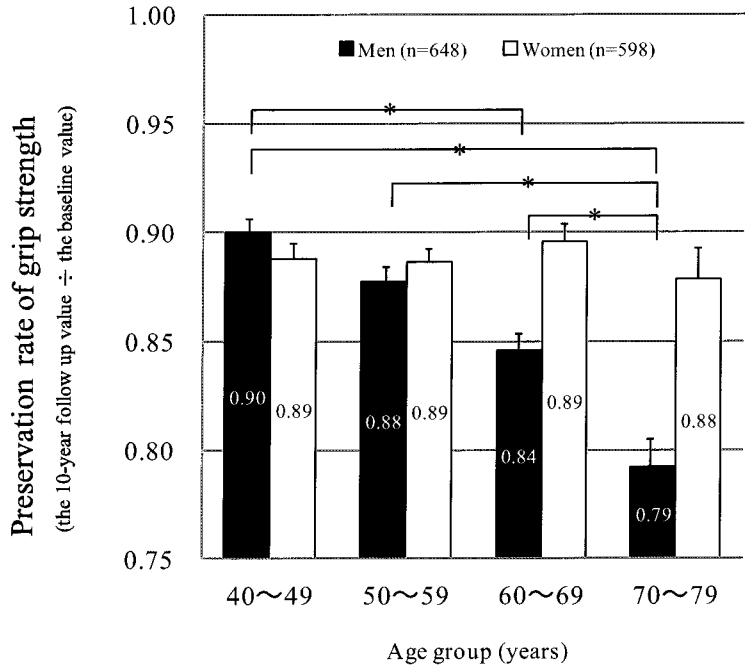

Fig. 2 Preservation rate of grip strength by sex and age group at baseline. Means and standard errors are presented. *; Tukey-Kramer test; $p < 0.05$.

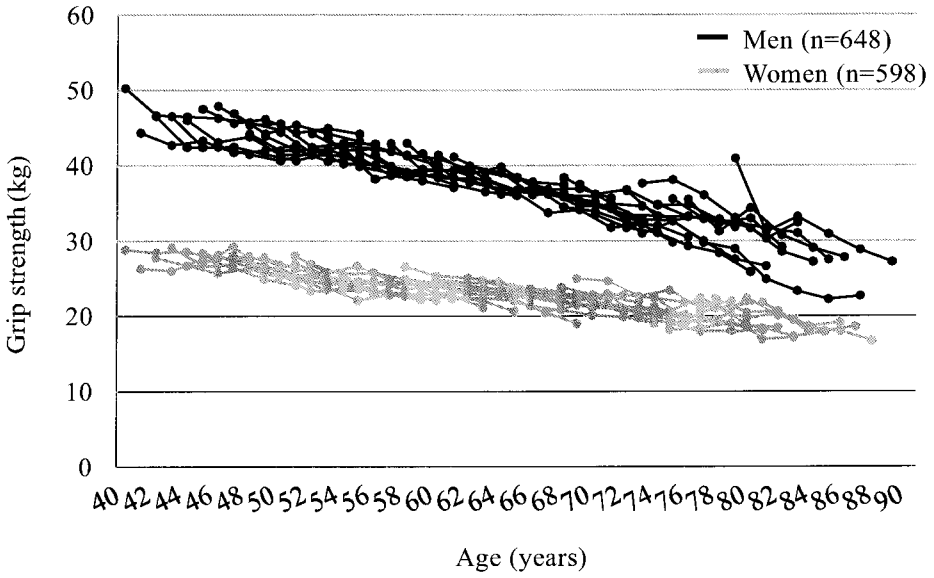

Fig. 3 Longitudinal changes in grip strength for ages 40 to 89 years in men and women.

age-stratified numbers for both men and women, clearly demonstrated that the decline in grip strength throughout middle and late adulthood was different for men and women.

Muscle mass is directly associated with muscle strength[23]. Age-related decreases in muscle mass have been reported as trivial; however, age-associated decreases in arm muscle quality (muscle strength/muscle mass) were lower among women than among men in our previous study[24]. Generally, women assume the main role of housework and continue this role until old age. This may help women maintain the same level of grip strength over time. However, Goodpaster et al. suggested that muscle weakness leads to diminished physical activity, consequently leading to secondary muscular disuse atrophy[25]. To prevent further loss of muscle strength among women in late adulthood, it is important to activate muscles and increase muscle quality throughout their lifetime.

For men, both muscle mass and muscle quality steeply decrease with age[24]. Additional physiological declines such as decreasing insulin-like growth factor-1 and testosterone levels[26] and loss of social roles at work and in

the community may cause the steep decline in muscle strength among men. Forrest et al. suggested that older adults who have adequate muscle strength may experience more noticeable declines in strength with age[19]. Rapid declines in muscle strength could have an impact on the ability of older adults to function in daily life as well as cause a sense of loss, particularly among men. Further studies are needed to examine the effects of large declines in muscle strength later in life among men.

We illustrated longitudinal changes in grip strength for the age span of 40 to 89 years using 7252 observational data points (1246 persons × 2~6 biennial values). We also demonstrated the trajectories of grip strength decline year by year in both men and women. This data provided a detailed course of decline in grip strength with age throughout middle and late adulthood for both sexes.

Previous studies have shown that the relationship between age and grip strength in men is both linear[16,27,28] and curved[17,29]. Our 10-year follow-up data decreased among older people, and thus the variance of the mean grip strength of participants in their 70s was wider than that of the other age groups. Therefore, whether the decline in grip strength among men in their 70s and older is linear or curved remains uncertain. However, our data of grip strength indicated that grip strength among men declined markedly year by year during the age span of 40 to 89 years.

Among women, previous studies also reported conflicting results with horizontal slopes in grip strength[16] and quadratic changes in grip strength[30]. However, grip strength among women was considerably lower than among men in middle and late adulthood. Grip strength in women may be considered the threshold of grip strength for community living. Although the cut-off point for sarcopenia is 20 kg of grip strength in women[9], grip strength among women in hospitals and nursing homes was reported to be below this level[14]. Recently, the Foundation for the National Institutes of Health Sarcopenia Project defined less than 16 kg of grip strength in women as weakness[31]. In our study, most women in their 70s reached the level of 20 kg of grip strength and the average grip strength among women in their late 80s was 17 kg, which is near the threshold level of grip strength for geriatric syndromes associated with functional limitations and disability. Although the age-related decline in grip strength seen among women was less than that of men, the available capacity of grip strength for community living was far less among women than men throughout middle and late adulthood.

Sarcopenia is a key component of physical frailty[11], and there is considerable overlap between the two[32]. Both are important concepts in geriatric research, and emphasize grip strength in their definition. Monitoring actual muscle function may be useful in identifying older people at risk of daily living disability and dependency. In addition, characterizing how the aging process affects grip strength over middle and late adulthood may lead to improved methods for preserving muscle strength. Our results may therefore contribute to the development of improved interventions and advice for the prevention of functional limitations and the maintenance of quality of life in late adulthood.

Some limitations of this study need to be addressed. First, there were more dropouts in the older than in the younger age groups in our longitudinal study. About 60% of the dropouts due to death during the study period were in their 70s at baseline. However, this was considered unavoidable, and despite the dropouts, we still had over 300 observations in men and women alike that allowed us to show the effects of the aging process on grip strength among participants in their 70s. Second, the participants in this study regularly attended the examinations in the NILS-LSA. This may have resulted in an underestimation of the decline in grip strength. The main reasons given for dropping out of the study were health problems, inconvenience, and a lack of time. However, it was difficult to examine the details of the dropouts because some did not respond or state any reasons for dropping out. Grip strength at baseline was lower among the dropouts than among the participants in the follow-up, especially in men (Table 2). We also performed a sub-analysis for the entire study population from baseline to the 10-year follow-up. Grip strength among participants who were examined six times was significantly stronger than that of those who were only examined once (at baseline); however, when compared to participants of both sexes who were examined two to five times, no difference in grip strength was found (data not shown). Regarding socio-demographic variables, the dropouts who had been examined only at baseline were significantly older and had poorer health than the participants in the follow-up for both sexes (Table 1). These differences might have led to an underestimation of the decline in grip strength. Finally, although the statistical analyses in this study had high power (more than 99%), the effect sizes were small[33]. Age-related decline in grip strength was clearly different between men and women. However, age-related grip strength decline is associated with numerous factors; therefore the role of sex in explaining this decline may be limited.

The strengths of the present study include the large number of randomly selected community-living participants and the fact that the 10-year longitudinal observation data tracked participants from age 40 to 89. We measured changes in grip strength biennially, and every year of age was assessed. The number of male and female participants in each age group was nearly equal, which allowed for an accurate estimation of sex differences. We were also able to show the effects of the aging process on grip strength using actual measurements for men and women.

Conclusion

Age-related decline in grip strength markedly increased in older men, but remained constant across middle and late adulthood in women. The large decline in grip strength among men and the low level of grip strength among women may indicate progressive declines in health, eventually making it impossible to perform daily tasks independently. It may be important to introduce sex-specific measures to maintain a higher level of grip strength in older individuals.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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ORIGINAL RESEARCH

Arm-Cranking Exercise Training Reduces Plasminogen Activator Inhibitor 1 in People With Spinal Cord Injury

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Abstract

Objective: To investigate the effects of arm-cranking exercise training on plasminogen activator inhibitor 1 (PAI-1) as a risk factor of deep vein thrombosis, along with general physical parameters such as muscle strength, aerobic capacity, and hemodynamics, in individuals with spinal cord injury (SCI) and control subjects.

Design: Longitudinal study.

Setting: Community-based supervised intervention.

Participants: Participants (N=17) comprised individuals with SCI (n=9) who volunteered for this study, and able-bodied individuals (n=8) matched for age, height, and body mass index who were assessed at baseline only.

Intervention: The arm-cranking exercise program was performed for 10 weeks with 4 sessions per week. Sessions consisted of 2 sets of warmup (5min) and arm crank exercises (25min) with a 10-minute recovery at an intensity of 50% to 70% of heart rate reserve.

Main Outcome Measures: Body mass (BM), waist circumference (WC), aerobic capacity (peak oxygen consumption [Vo_2peak]), PAI-1, blood pressure, glucose metabolism, and lipids.

Results: PAI-1, BM, WC, systolic blood pressure, and triglycerides (TG) decreased, and Vo_2peak increased after training ($P<.05$, respectively). Spearman rank-order analysis revealed that changes in PAI-1 were related to changes in Vo_2peak, BM, WC, TG, and high-density lipoprotein cholesterol. Multiple linear regression analysis revealed that WC was the most sensitive factor for predicting changes in PAI-1 ($P=.038$).

Conclusions: These results suggest that 10 weeks of arm-cranking exercise training for people with SCI may help to reduce the risk factors of cardiovascular disease. In addition, changes in abdominal fat may be related to changes in PAI-1 in the SCI population.

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Plasminogen activator inhibitor 1 (PAI-1) has been suggested to be a crucial risk factor for metabolic syndrome,[1-3] indicating that decreases in PAI-1 may prove to be sensitive and clinically relevant for the prevention of cardiovascular diseases (CVDs). The prevalence of CVDs or CVD-related diseases, including diabetes,[4] hypertension,[5] dislipidemia,[6] peripheral arterial disease,[7] stroke,[8] deep vein thrombosis (DVT),[9,10] and obesity,[11] is higher in individuals with spinal cord injury (SCI) compared with the able-bodied (AB) population. Among these, DVT is commonly observed in either acute or chronic SCI because of immobilization of the paralyzed lower limbs; the limb immobilization also leads to complications such as decreased venous return, which is likely due to reduced muscle volume in the muscle pump.[9,12-14] Because PAI-1 is the most important direct inhibitor of the tissue-type of urokinase-type plasminogen activators, as well as a major regulator of the fibrinolytic system, reduction in PAI-1 is considered as a target biomarker in the prevention of DVT.[15]

Aerobic exercise training reduces the risk factors of CVD, and the American College of Sports Medicine has suggested that this effect is likely due to loss of body mass (BM).[16] In ambulatory humans, studies have demonstrated that exercise training reduced PAI-1 in AB people,[17-20] as well as in patients with CVD.[21,22] Additionally, sedentary people with SCI have been reported to show higher PAI-1 levels, compared with highly trained athletes

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with SCI, indicating that increasing the amount of exercise may be a therapeutic strategy to reduce the PAI-1 level in SCI.[23] However, little known is about the positive effects of upper body training on PAI-1 in SCI. Previous interventional training studies[17-22] designed to reduce PAI-1 revealed positive effects by exercise training; conversely, whether detraining may alter PAI-1 is uncertain. It has also been suggested that physical inactivity (eg, unilateral lower limb immobilization, which can exacerbate the effects of localized deconditioning) increases the risk of DVT.[24] Therefore, the observation of both training and detraining effects may offer further insight into changes in the PAI-1 level and related factors.

With this information in mind, we chose to investigate the effects of arm-cranking exercise training on PAI-1 and several other related parameters that can influence CVD risk factors, namely BM, waist circumference (WC), and lipids. We hypothesized that PAI-1 levels would be reduced by arm-cranking exercise training and then would recover to their pretraining values after detraining (ie, equivalent period without training); we also expected that these alterations would be related to BM, WC, and lipids. To test this, we investigated the effects of 10-week training and detraining periods on these biomarkers in individuals with SCI.

Methods

Participants

Nine men with SCI and 8 age-matched AB participants voluntarily participated in this study. Physical characteristics for each of the participants with SCI along with averaged values for the AB participants are shown in table 1. All participants with SCI were engaged in sedentary work to which they commuted using their own vehicle, indicating that their daily wheelchair activity levels were similar. AB participants were relatively sedentary and had not performed any regular exercise for 6 months before the study. AB participants were free of any known CVD, and both groups were taking no medications and did not smoke. Participants were asked to abstain from caffeinated beverages for 12 hours, and from strenuous physical activity and alcohol for at least 24 hours before the study. Participants were familiarized with all measurement techniques in the arm-cranking exercises at 50 revolutions/min. All studies were performed at an ambient temperature of 24°C±1°C with minimal external stimuli. All procedures were approved by the ethical committee of Hokusho University, Japan, and were performed in accordance with the guidelines of the Declaration of Helsinki. After a detailed description and explanation of all study procedures, including the possible risks and benefits, each participant gave written informed consent.

Table 1 Individual characteristics of SCI participants and mean values of AB and SCI participants

Participant No.	Classification	Injury Level	Injury Duration (y)	Age (y)	Height (cm)
1	A	T12	11	30	176
2	A	T10-12	14	34	173
3	A	L1	12	31	162
4	B	T10	20	41	164
5	A	T12	7	26	174
6	A	T8-9	28	55	165
7	B	T12	16	39	170
8	A	L1	24	54	169
9	A	T9-10	9	32	167
SCI	NA	NA	16±7	38±10	169±5
AB	NA	NA	NA	35±7	171±4

NOTE. Values are mean ± SD for SCI and AB subjects. Classification was determined by the standards of the American Spinal Injury Association. Abbreviations: A, complete injury; B, sensory incomplete; L, lumbar; NA, not applicable; T, thoracic.

Study procedures

The study consisted of 3 experimental protocols: (1) pretraining measurements (baseline) for SCI and AB participants; (2) 10-week arm-cranking exercise training for SCI participants only; and (3) posttraining measurements after a 10-week detraining for SCI participants only. At pretraining, the physical characteristics, peak aerobic capacity, upper body muscle strength, and biomarkers were evaluated (for details, see Physical Characteristics Measurement, Aerobic Capacity Measurement, Handgrip Strength Measurement, and Biomarker Outcomes sections below). During the training period, each participant with SCI performed two, 30-minute sets of arm-cranking exercises with a 10-minute resting interval between them, 4 days per week for 10 weeks at an intensity of 50% to 70% heart rate reserve (HRR). During the first 2 weeks, the target heart rate was set at 50% of HRR, and increased 5% every 2 weeks; thus, during the last 2 weeks (weeks 8–10), the target heart rate was set at 70% of HRR. According to the guidelines of the American College of Sports Medicine,[16] this exercise intensity and duration can improve aerobic fitness levels. HRR was obtained from the following equation[16]:

$$\text{HRR}\ (\%) = (\text{Exercising HR} - \text{Resting HR})/(\text{Peak HR} - \text{Resting HR}) \times 100\%$$

where resting and peak heart rate were derived from the arm-cranking exercise at the pretraining evaluation, followed by the same assessment pretraining measurement carried out for participants with SCI only. The researchers carefully supervised the training and carefully monitored the exercise duration and intensity to prevent injury.[25] Notably, in the initial stages, a subset of subjects stated that they did not want to continue the exercises. In these cases, the researchers did not force the subjects to continue exercising but rather increased the recovery interval between sets, so that the subjects became motivated again and finally completed the total exercise protocol. During training, they wore a wireless heart rate

List of abbreviations:

AB	able-bodied
BM	body mass
CVD	cardiovascular disease
DVT	deep vein thrombosis
HDL-C	high-density lipoprotein cholesterol
HRR	heart rate reserve
PAI-1	plasminogen activator inhibitor 1
SBP	systolic blood pressure
SCI	spinal cord injury
TG	triglycerides
Vo_2peak	peak oxygen uptake
WC	waist circumference

monitor. During the detraining period, all participants with SCI were asked not to increase their physical activity (eg, by performing additional upper body training such as arm cranking or resistance exercise), but rather to live as usual (fig 1).

Physical characteristics measurement

Initially, each participant was measured for height; BM, wearing only underwear and after voiding the bladder; and WC at the umbilicus after normative expiration. Height and WC were measured using a nonelastic tape measure while lying in a 30° supine position, as this approximated the method used to measure standing AB participants.[26] BM was measured using a custom scale with an individual's wheelchair weighing approximately 20kg. In AB participants, height and WC were measured in the same positions as for the participants with SCI. BM was measured using a commercial body weight scale.[a]

Aerobic capacity measurement

To determine individual peak oxygen uptake (Vo_2peak) for both groups, each participant performed an incremental arm-cranking test (Rehabi Trainer 881E[b]) in a sitting position until exhaustion (10W/min increase at 50 revolutions/min).[27] After 30 minutes of rest in the supine position, the SCI participants moved to their own wheelchairs. AB participants also used a wheelchair suitable to their body size. The wheelchair was firmly fixed, and the participant's legs and feet were firmly held in place with a strap. The pedal axis was aligned with the participant's shoulder, and the elbow was positioned in a slight flexion position. The participants rested for 10 minutes in the wheelchair before beginning, then performed an incremental arm-cranking exercise test until exhaustion. The criteria for exhaustion were as follows: (1) a score of 19 on the rating of perceived exertion; and (2) failure to maintain 50 revolutions/min despite strong verbal encouragement. The test was terminated when either of these 2 criteria was met. For this measurement, gas-exchange variables were measured using the breath-by-breath method.[c] Heart rate was continuously monitored by an electrocardiogram attached to a metabolic measuring system.

Handgrip strength measurement

Isometric maximum handgrip strength was evaluated. Handgrip was measured 3 times in both arms with participants in a sitting position using a dynamometer.[d] Participants were encouraged to exert the strongest possible force for 3 seconds with 5-minute intervals. The maximal handgrip strength was calculated by averaging the values of the 3 measurements.

Biomarker outcomes

Resting venous blood samples (10mL) were taken from the antecubital vein under fasting conditions and immediately centrifuged at 3000 revolutions/min for 15 minutes at 4°C to separate plasma and serum. Blood samples were frozen at −80°C for further analysis of PAI-1, fibrinogen, blood glucose, hemoglobin A1c, total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol. All analyses were measured by a clinical testing company.[e] Systolic and diastolic blood pressures were measured at the upper left arm by the oscillometric method.[f] Blood pressures were measured at 1-minute intervals at least 3 times, and averaged values of blood pressure were taken.

Posttraining and detraining measurements for participants with SCI

The measurements after the 10-week arm-cranking exercise training and detraining periods were obtained only from the participants with SCI. Our reasoning for this was that the AB group would have continued walking in their daily lives using their lower limbs. It would therefore be impossible to control for the potential effects of this ambulatory activity on the physical and biomarker measurements.

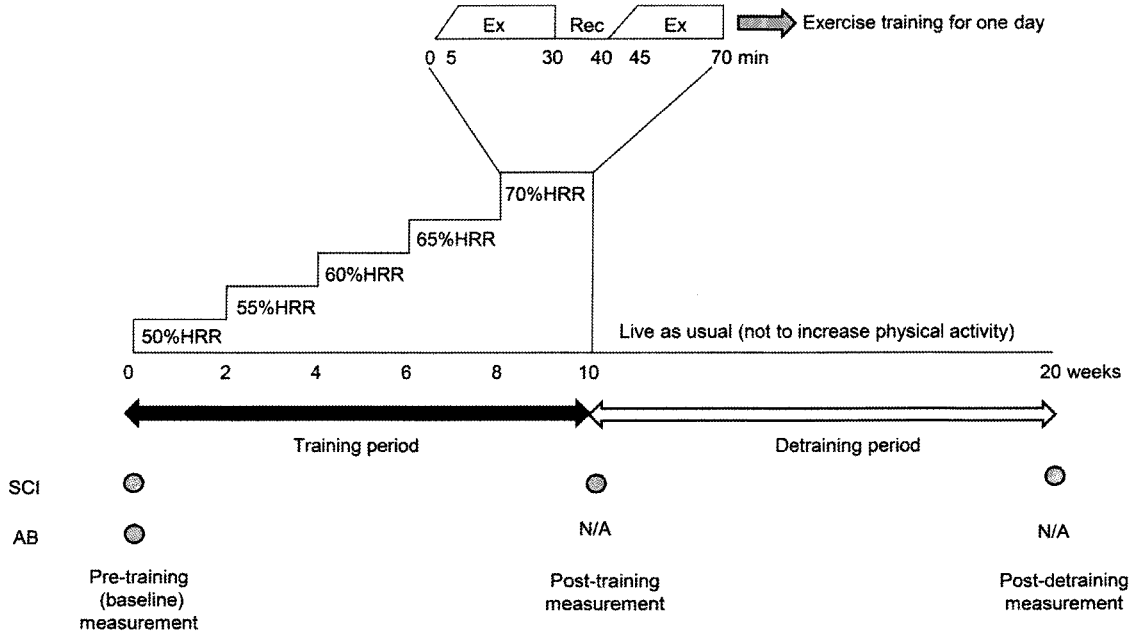

Fig 1 Study protocol. Abbreviations: EX, exercise; NA, not applicable; Rec, recovery.

Statistical analysis

All data are shown as means ± SD. The unpaired t test was used to compare the pretraining (baseline) values between SCI and AB participants. One-way repeated-measures analysis of variance was used to compare all SCI participants at 3 time points: pretraining (baseline), posttraining, and detraining. Spearman rank-order analysis was used to detect influential parameters for changes in PAI-1.[28] A multiple linear regression analysis was conducted to predict changes in PAI-1. A P value <.05 was considered statistically significant.

Results

Pretraining (baseline) values in SCI and AB participants

Physical characteristics, aerobic capacity, blood pressure, and biomarkers in both groups at pretraining (baseline), posttraining, and detraining are shown in table 2. At pretraining, there were significant differences in peak heart rate and PAI-1 between groups. Maximum WC, handgrip, systolic blood pressure (SBP), total cholesterol, TG, and fibrinogen tended to be higher in SCI than AB participants, but without statistical significance. There were no significant differences in other variables between the groups.

Effect of arm-cranking training and detraining

Table 2 also shows changes in the physical characteristics, aerobic capacity, blood pressure, and the biomarkers between pre- and posttraining in SCI participants. These results are summarized in table 3. Maximum WC, BM, Vo_2peak, SBP, TG, and PAI-1 significantly improved with the 10-week arm-cranking exercise training (P<.05). After the 10-week detraining phase, BM, WC, Vo_2peak, SBP, TG, and PAI-1 acutely recovered, with statistical differences between posttraining and detraining (P<.05). However, the postdetraining values of BM, WC, and Vo_2peak continued to be significantly different than the pretraining values (P<.05). Conversely, the values of SBP and TG recovered and were similar to the pretraining values (all P>.05). Moreover, the PAI levels at posttraining tended to be lower than the pretraining values, but without statistical significance (P=.052). The other parameters were not affected by either training or detraining (all P>.05).

Potent factors affecting changes in PAI-1 level

In the present study, we calculated relative changes between the posttraining and pretraining values, and between the postdetraining and posttraining values. Spearman rank-order analysis revealed that these relative changes in PAI-1 levels were related to relative changes in BM, WC, Vo_2peak, TG, and HDL-C; that is, 2 different periods (difference between posttraining and pretraining, and between postdetraining and posttraining) × 9 participants =18 (table 4). Additionally, relative changes in PAI-1 can be explained by following equation with multiple regression analysis. As a result, relative changes in WC were determined to predict the relative changes in PAI-1 level.

Relative changes in PAI-1 = −6.118 + (.952×BM) + (4.582×WC) − (.077×Vo_2peak per BM) + (.220×TG) − (.251×HDL-C); (adjusted R^2=.759, P<.001; P value of WC is .038).

Discussion

Our results have provided several pieces of information with importance for treatment of SCI. First, participants with SCI showed statistically significant differences in PAI-1 and peak heart

Table 2 Physical characteristics, aerobic capacity, muscle strength, and hemodynamic variables at each period in SCI and AB participants

	SCI (n=9)			AB (n=8)	Baseline
Measures	Pretraining (Baseline)	Posttraining	Postdetraining	Baseline	P
BM (kg)	61.0±7.0	59.1±7.5*	60.1±7.0†,‡	62.8±5.2	.563
WC (cm)	85.5±6.2	83.6±5.9*	84.2±5.6†,‡	81.0±2.1	.071
Vo_2peak per BM ($mL \cdot kg^{-1} \cdot min^{-1}$)	28.9±4.1	32.7±4.4*	30.2±4.2†,‡	29.3±4.1	.868
Peak V_E ($L \cdot min^{-1}$)	67.2±11.1	72.5±9.4	67.7±10.4	68.1±12.1	.878
Peak HR ($beats \cdot min^{-1}$)	160±10	163±8	159±6	151±3§	.046
Handgrip strength (kg)	50.4±5.5	52.2±6.3	51.3±6.0	46.4±4.1	.085
SBP (mmHg)	136±5	133±4*	135±5	132±4	.070
DBP (mmHg)	75±8	73±5	74±7	71±6	.276
Blood glucose (mg/dL)	102±25	99±34	101±27	90±10	.231
Hemoglobin A1c (%)	4.9±0.6	4.8±0.8	4.9±0.7	4.7±0.3	.470
Total cholesterol (mg/dL)	203±34	196±36	207±31	178±16	.074
TG (mg/dL)	154±69	116±58*	140±56†	107±24	.087
HDL-C (mg/dL)	56±7	58±7	56±8	60±10	.265
LDL-C (mg/dL)	114±24	110±30	113±27	99±7	.109
PAI-1 (ng/dL)	52±11	38±12*	41±11†	30±6§	<.001
Fibrinogen (mg/dL)	297±57	290±46	303±51	249±50	.087

NOTE. Values are mean ± SD or as otherwise indicated. P values denote the significance of differences between SCI and AB participants at baseline.
Abbreviations: DBP, diastolic blood pressure; HR, heart rate; LDL-C, low-density lipoprotein cholesterol; V_E, pulmonary ventilation.

* P<.05 between values at baseline and after training in SCI participants.

† P<.05 between values after training and detraining in SCI participants.

‡ P<.05 between values at baseline and after detraining in SCI participants.

§ P<.05 between AB and SCI participants at baseline values.

Table 3 Summarized results of statistics in SCI participants

	One-Way Repeated ANOVA			Post Hoc Pairwise Comparisons		
Measures	F	Partial η^2	*P*	Pre- vs Posttraining	Posttraining vs Detraining	Pretraining vs Postdetraining
BM	34.928	0.814	<.001	<.001	.005	<.001
WC	10.177	0.560	.001	.019	.021	.033
Vo_2peak per BM	48.458	0.858	<.001	<.001	<.001	.008
Peak V_E	4.296	0.350	.032	.023	.126	.805
Peak HR	1.818	0.185	.194	.413	.234	.623
HG strength	2.101	0.208	.155	.252	.218	.311
SBP	3.964	0.331	.040	.005	.146	.512
DBP	0.290	0.035	.752	.829	.853	.829
Blood glucose	0.225	0.027	.801	.900	.900	.900
HbA1c	0.548	0.064	.588	.696	.696	.696
TC	1.681	0.045	.458	.221	.256	.634
TG	10.518	0.568	.001	.010	.010	.136
HDL-C	3.225	0.287	.067	.123	.189	.565
LDL-C	1.081	1.135	.363	.457	.457	.600
PAI-1	56.150	0.875	<.001	<.001	<.001	.052
Fibrinogen	1.671	0.173	.219	.326	.326	.326

Abbreviations: ANOVA, analysis of variance; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; HG, handgrip; HR, heart rate; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; V_E, pulmonary ventilation.

rate, and marginally significant differences in WC, muscle strength, and some biomarkers, compared with AB participants. Second, arm-cranking exercise training improved several parameters, such as indices of obesity, biomarkers for CVD risk, and aerobic capacity. Third, Spearman rank-order analysis revealed that relative changes in PAI-1 were related to the relative changes in BM, WC, TG, HDL-C, and Vo_2peak. WC was shown to be especially predictive of changes in the PAI-1 level by multiple regression analysis.

Table 4 Spearman rank-order correlation coefficients between changing rate in PAI-1 and changing rate in other variables

Changes in Variables (%)	*R* Values to Change in PAI-1 (%)	*P*
BM	.609	.007
WC	.654	.003
Vo_2peak per BM	−.696	.001
Peak V_E	−.401	.100
Peak HR	−.324	.189
Handgrip strength	−.152	.548
SBP	.427	.078
DBP	.157	.534
Blood glucose	.174	.491
Hemoglobin A1c	.119	.639
TC	.456	.057
TG	.869	<.001
HDL-C	−.694	.001
LDL-C	.266	.287
Fibrinogen	.174	.491

NOTE. Values are shown as Spearman rank-order correlations and *P* values. Changing rate was taken between posttraining and baseline values, and between postdetraining and posttraining, indicating the total sample size was 18 (9 subjects × 2 periods).
Abbreviations: DBP, diastolic blood pressure; HR, heart rate; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; V_E, pulmonary ventilation.

SCI versus AB participants at baseline

We found a significant difference in the baseline values of PAI-1 between the 2 groups. Maximum WC, SBP, total cholesterol, TG, and fibrinogen tended to be higher in the SCI than in the AB participants, but without statistical significance. These results suggest that the risk factors for CVD may be higher in persons with SCI than in AB individuals. Because many studies have reported that the prevalence of CVDs or CVD-related disease,[4-10] and obesity[11] is higher in persons with SCI compared with AB individuals, our results are reasonable. There were no differences in Vo_2peak or pulmonary ventilation, while peak heart rate was significantly higher in SCI than in AB participants. Since a previous study[29] demonstrated that a higher heart rate in SCI during exercise compensates for a lower stroke volume, the significantly higher heart rate in our subjects with SCI might also have occurred to compensate for a lower stroke volume. We also found a higher trend for handgrip strength in the participants with SCI. This may be related to their daily use of the upper extremities for wheelchair operations.

Effect of arm-cranking training and detraining in SCI

In this study, 10-week arm-cranking exercise training significantly reduced BM, WC, SBP, TG, and PAI-1. Similarly, aerobic capacity improved. These improvements in BM, and WC were maintained even after detraining. Moreover, PAI-1 was acutely increased after detraining, but it still showed a lower trend between baseline and postdetraining ($P=.052$). By contrast, SBP and TG returned toward the postdetraining baseline values. These

results may indicate that exercise training–induced changes in PAI-1 are related to body composition. Several previous interventional studies (eg, exercise training, weight loss, and intense lifestyle programs) have shown that decreases in PAI-1 are associated with reductions in body weight[19] and WC.[30] Although these previous studies were not conducted for patients with SCI, our results may account for these parallel changes among PAI-1, BM, and WC. Indeed, previous studies on patients with SCI have reported that upper body endurance training (ie, arm-cranking exercises or wheelchair ergometer training) significantly decreased WC[31,32] and BM.[32] Other studies of patients with SCI showed that upper body exercise training significantly reduced TG[33,34] and increased HDL-C.[33-35] Our Spearman rank-order analysis results also revealed that changes in PAI-1 were related to BM, WC, Vo_2peak, TG, and HDL-C, but were not related to peak pulmonary ventilation or peak heart rate. These results suggest that exercise training may play a more important role in improving health outcomes than merely improving aerobic capacity. Moreover, WC is the most sensitive factor for predicting exercise-induced changes in PAI-1. Taken together, these findings suggest that exercise training may be clinically relevant for reducing PAI-1 in individuals with SCI, probably via a reduction of WC.

One possible explanation for the relation between the decreases in PAI-1 and WC observed herein may be that they involve the abdominal adipose tissue. In the present study, maximum WC was extracted as the most potent factor for predicting changes in PAI-1. It is known that adipose tissue is an important organ in producing PAI-1.[36] Additionally, adipose gene expression of PAI-1 is elevated with obesity[37] and has been positively linked with the risk factors of CVD.[38] WC measurement is known to be a valid method for identifying obesity in SCI.[39] Similarly, percent body fat is strongly associated with WC but not with body mass index in individuals with SCI,[24] which may imply that reductions in WC cause reductions in body fat in those with SCI. Since we did not directly assess abdominal body fat, future studies would be warranted to clarify the underlying mechanisms and causal relationships that may affect changes in PAI-1 levels.

Future perspectives

After SCI, the body's composition undergoes various changes that might lead to obesity (eg, limited mobility, results in reductions in muscle mass).[11] Of note, the participants with SCI in the present study had average body mass indices (see tables 1 and 2). Nonetheless, WC and PAI-1 were significantly decreased, and WC was deemed to be predictive of changes in PAI-1. In the training sessions, a subset of SCI participants complained that they wanted to discontinue the exercises because they were too difficult; however, this may have been due to a lack of experience with an arm-cranking exercise using an ergometer, since all participants ultimately completed the full training protocol despite increases in exercise intensity. Our results may indicate that exercise is useful not only to reduce the risks of DVT and other CVDs, but also to increase the quality of life in people with SCI.[40]

Study limitations

First, we must acknowledge the relatively small sample size in both groups; thus, we used post hoc power analysis tests to obtain statistical power and the required sample size. We estimated that a sample size of 8 would have been necessary to achieve the appropriate statistical power for the measurement of exercise-induced changes in PAI-1, BM, WC, SBP, TG, and Vo_2peak. This may indicate that the exercise-induced changes in the main outcomes were not strongly affected. Second, we observed the participants for 10 weeks in the present study. In a previous 12-week study investigating the relation between arm-cranking exercise and PAI-1, no changes were observed in PAI-1.[31] However, the training volume in our study (40h) was greater than in this previous study (18h), although the exercise intensities were similar. It is possible that these differential training volumes contributed to the different responses of the PAI-1 levels.

Conclusions

Ten weeks of arm-cranking exercise training significantly decreased PAI-1, BM, WC, and several CVD-related markers. Relative changes in PAI-1 were related to relative changes in BM, WC, HDL-C, TG, and Vo_2peak. Additionally, a change in WC was found to be the factor most predictive of changes in PAI-1, indicating that reductions in visceral fat may provide a sensitive, clinically relevant way to reduce the risk of thrombosis in patients with SCI.

Suppliers

a. TBF 410; Tanita.
b. Rehabi Trainer 881E; Monarch.
c. V-max; Nihon-koden.
d. ES-100; Evernew.
e. SRL Co, Ltd.
f. STBP-680; Nippon Colin.

Keywords

Abdominal fat; Body weight; Lipids; Rehabilitation; Venous thrombosis; Waist circumference

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ORIGINAL ARTICLE

Pioglitazone improves whole-body aerobic capacity and skeletal muscle energy metabolism in patients with metabolic syndrome

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ABSTRACT

Aims/Introduction: Low aerobic capacity is a strong and independent predictor of all-cause mortality in patients with metabolic syndrome (MetS). Here, we investigated the effects of pioglitazone treatment on whole-body aerobic capacity and skeletal muscle energy metabolism in MetS patients.

Materials and Methods: A total of 14 male patients with MetS received oral pioglitazone 15 mg/day for 4 months. To assess whole-body aerobic capacity, exercise testing with a bicycle ergometer was carried out before and after pioglitazone treatment. To assess skeletal muscle energy metabolism, intramyocellular lipid in the resting leg and high-energy phosphates in the calf muscle during plantar-flexion exercise were measured using ^{1}proton- and ^{31}phosphorus magnetic resonance spectroscopy, respectively.

Results: Pioglitazone significantly increased peak oxygen uptake (25.1 ± 4.9 mL/kg/min pretreatment vs 27.2 ± 3.9 mL/kg/min post- treatment, $P < 0.05$) and anaerobic threshold (12.7 ± 1.9 mL/kg/min pretreatment vs 13.6 ± 1.6 mL/kg/min post-treatment, $P < 0.05$), although daily physical activity was comparable before and after the treatment. Intramyocellular lipid content was significantly reduced after pioglitazone treatment by 26%, indicating improved skeletal muscle fatty acid metabolism. Pioglitazone also significantly decreased the muscle phosphocreatine loss during exercise by 13%, indicating improved skeletal muscle high-energy phosphate metabolism. Notably, the increase in anaerobic threshold; that is, submaximal aerobic capacity, closely correlated with the decrease in intramyocellular lipid content after pioglitazone treatment.

Conclusions: Pioglitazone significantly improved the MetS patients' whole-body aerobic capacity and skeletal muscle energy metabolism. The beneficial effect of pioglitazone on whole-body aerobic capacity might be at least in part through improved fatty acid metabolism in the skeletal muscle.

INTRODUCTION

Metabolic syndrome (MetS) is a multifactorial condition characterized mainly by obesity and insulin resistance, which increases the risk of the development of type 2 diabetes and cardiovascular disease. The worldwide prevalence of the MetS is increasing dramatically, leading to both medical and public health crises worldwide. Furthermore, the rapid growth of the numbers of individuals with type 2 diabetes worldwide over the past decade highlights the necessity of early pharmacological intervention to prevent type 2 diabetes and its complications, such as macrovascular and microvascular diseases[1,2].

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Pioglitazone, an insulin-sensitizing thiazolidinedione, is widely used for the treatment of type 2 diabetes. Thiazolidinediones are known to activate peroxisome proliferator-activated receptor-γ, which is distributed not only in adipose tissue but also in skeletal muscle, and plays an important role in fatty acid and glucose metabolism[3]. Among the various antidiabetic drugs, pioglitazone has a strong effect on insulin resistance, and is considered a potential candidate treatment for patients with MetS[4].

Large clinical trials have shown that pioglitazone reduces the risk of the development of type 2 diabetes in patients with impaired glucose tolerance[5], and reduces the risk of stroke and acute myocardial infarction in insulin-resistant patients with a history of ischemic stroke or transient ischemic attack[6].

Low aerobic capacity is a more powerful predictor of all-cause mortality than other established risk factors for cardiovascular diseases in healthy individuals[7]. We previously showed that aerobic capacity is lowered in association with impaired skeletal muscle energy metabolism in MetS patients[8,9]. Low aerobic capacity could independently increase the risks of cardiovascular diseases and death in obese and insulin-resistant MetS patients[10,11]. These findings support the importance of treatment to improve the aerobic capacity in patients with MetS.

The first-line treatments for MetS are lifestyle interventions, including exercise and calorie restriction. However, most MetS patients cannot maintain high physical activity and/or their optimal bodyweight over a sustained period of time, and consequently they remain at high risk for developing type 2 diabetes and cardiovascular disease. Therefore, additional treatment to improve aerobic capacity is clinically beneficial for MetS patients.

We recently showed that pioglitazone increased aerobic capacity with improved skeletal muscle mitochondrial function in diet-induced obese and insulin-resistant mice[12], which raises the possibility that pioglitazone can improve aerobic capacity in patients with MetS. However, to our knowledge, no study showing the effect of pioglitazone on aerobic capacity in humans has been published.

In the present study, therefore, we examined whether pioglitazone could improve the aerobic capacity and skeletal muscle energy metabolism in MetS patients. We also determined whether the effects of pioglitazone on aerobic capacity are associated with improved skeletal muscle energy metabolism.

MATERIALS AND METHODS

Patients

A total of 14 male MetS patients who did not engage in habitual exercise and who were diagnosed with MetS according to the International Diabetes Federation criteria participated in the study. All 14 patients were in good health with no evidence of cardiovascular, hepatic or renal disease, as determined by medical history and physical examination including screening blood tests, electrocardiograms and cardiac ultrasounds before the study. A total of 10 of the 14 patients were treated with antihypertensive drugs: a calcium antagonist, β-blocker, angiotensin-converting enzyme inhibitor, angiotensin II receptor blocker or diuretic. One patient was also receiving statin therapy. These medications were not altered for at least 3 months before enrolment or during the study period. The present report is a part of a large study investigating the impairment of skeletal muscle energy metabolism in MetS, and thus, part of the data are from the same patients whose data were published previously, but in a different context[8,9]. All patients gave written informed consent before the study, which was approved by the Medical Ethics Committee of Hokkaido University Hospital, and all investigations were carried out according to the guidelines in the Declaration of Helsinki.

Study design

The patients underwent blood tests to evaluate their insulin sensitivity and lipid profiles after a 10-h overnight fast, followed by clinical and anthropometric measurements including body composition determined by an air displacement plethysmograph (BOD POD® Body Composition System; Life Measurement Instruments, Concord, California, USA), and ^{1}proton (^{1}H) -magnetic resonance spectroscopy (MRS) studies to measure the intramyocellular lipid (IMCL) content in the resting leg muscle.

The patients also underwent an exercise test with a bicycle ergometer to assess their aerobic capacity. ^{31}Phosphorus (^{31}P)-MRS studies were carried out to assess the high-energy phosphate metabolism in the leg muscle during exercise on another day. Each patient's daily physical activity was monitored by a pedometer with an accelerometry sensor (Lifecorder Plus; Suzuken, Nagoya, Japan) for at least 1 week before and after the pioglitazone treatment. The patients were instructed not to change any aspect of their lifestyle including diet and physical activity during the study period. Each patient received oral pioglitazone 15 mg/day for 4 months. After 4 months of pioglitazone treatment, the patients underwent the same tests to evaluate the effects of pioglitazone.

Systemic oxidative stress

Each patient's level of serum thiobarbituric acid reactive substances, which are lipid peroxides known as a marker of oxidative damage, were measured, as described[13]. We also measured the patients' systemic anti-oxidant defense capacity including serum thiols and enzymatic activities of superoxide dismutase, glutathione peroxidase and glutathione reductase, as described[13].

Whole-body aerobic capacity

Whole-body aerobic capacity was assessed by respiratory gas analysis (Aeromonitor AE-300S; Minato Medical Science, Osaka, Japan) with a bicycle ergometer. A ramp protocol of 25 watts/min (after a 3-min warm-up) was used for the exercise testing. The respiratory exchange ratio was calculated as the ratio of carbon dioxide production/oxygen uptake (VO_2).

For the measurement of their peak VO_2, the patients were asked in advance to attain their symptom-limited maximal point. As an index of perceived effort, the rating of perceived exertion was evaluated with the 10-point Borg scale. The anaerobic threshold (AT) was determined by the V-slope method[14], except in one patient.

IMCL content in skeletal muscle

We measured IMCL content in the patients' resting tibialis anterior muscle at the level of the muscle belly of the calf using ^{1}H-MRS, as described[8,9]. The IMCL content from one of the 14 patients could not be measured because of technical difficulties.

High-energy phosphate metabolism in skeletal muscle

Before the measurement of high-energy phosphate metabolism in skeletal muscle, one-repetition maximum (1-RM) was determined, as described[8,9]. The calf flexor muscle cross-sectional area at the level of the muscle belly was also measured using magnetic resonance imaging. After the patient rested for ≥30 min, the high-energy phosphate metabolism in the calf muscle was measured at rest and during a plantar flexion exercise with the patient in the supine position on the original apparatus equipped with a 1.5-Tesla (T) whole-body scanner system (Magnetom Vision VB33G; Siemens, Erlangen, Germany), using ^{31}P-MRS, as described[8,9]. The exercise protocol was a constant load of 20% 1-RM at the pace of 40 times/min for 4 min. Phosphocreatine (PCr) was standardized as (PCr)/([PCr] + [Pi]) on the basis of the notion that (PCr) + (Pi) is constant at rest and during exercise, where (PCr) indicates the concentration of PCr and (Pi) indicates the concentration of inorganic phosphate (Pi). In addition, the degree of PCr change (i.e., PCr loss) during exercise was calculated as PCr loss = standardized PCr_{rest} − standardized PCr_{lowest}, where PCr_{rest} indicates the PCr level at rest and PCr_{lowest} indicates the lowest PCr level during exercise.

Table 1 | Patient characteristics before and after pioglitazone treatment

	Before (n = 14)	After (n = 14)
Age (years)	52 ± 11	–
Body weight (kg)	77.5 ± 11.1	77.0 ± 10.3
Body mass index (kg/m^2)	26.6 ± 3.3	26.4 ± 3.0
Percent fat (%)	28.0 ± 3.9	28.8 ± 4.2
Lean body mass (kg)	55.2 ± 7.9	54.6 ± 6.3
Waist circumference (cm)	94.1 ± 9.0	93.5 ± 8.0
Systolic blood pressure (mmHg)	143 ± 13	138 ± 15
Diastolic blood pressure (mmHg)	83 ± 10	83 ± 7
Daily steps (steps/day)	7617 ± 3871	6739 ± 2374
MCC (kcal/day)	259 ± 176	221 ± 92

Data are means ± standard deviation. MCC, movement-related calorie consumption.

Table 2 | Blood biochemistry before and after pioglitazone treatment

	Before (n = 14)	After (n = 14)
Fasting blood glucose (mmol/L)	6.4 ± 1.0	5.9 ± 0.7*
Insulin (pmol/L)	70 ± 60	38 ± 17*
HOMA-IR	3.8 ± 3.4	1.7 ± 0.8*
HbA1c (%)	5.7 ± 0.6	5.6 ± 0.4
HDL cholesterol (mmol/L)	1.34 ± 0.25	1.43 ± 0.28
LDL cholesterol (mmol/L)	3.18 ± 0.71	3.52 ± 0.67
Triglyceride (mmol/L)	1.74 ± 0.94	1.14 ± 0.59*
Free fatty acids (g/L)	0.17 ± 0.09	0.16 ± 0.10

Data are mean ± standard deviation. *P < 0.05 vs Before. HbA1c, glycohemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein.

Statistical analysis

Data are expressed as mean ± standard deviation. The values obtained before and after treatment were compared using paired t-tests. We examined correlations by carrying out a linear regression analysis using Pearson's correlation coefficient. Statistical analyses were carried out using GRAPHPAD PRISM v5.01 (GraphPad Software, San Diego, California, USA), and significance was defined as $P < 0.05$.

RESULTS

Patient characteristics

The patients' bodyweight, body mass index, percent fat, lean body mass, waist circumference and blood pressure did not change after the pioglitazone treatment (Table 1). There was no significant change in the cross-sectional area of the calf

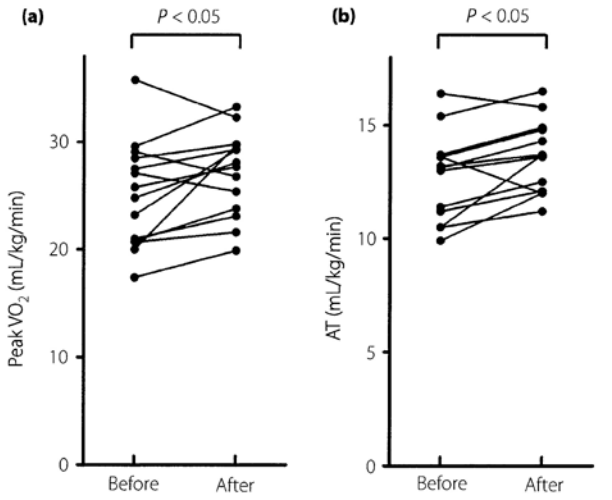

Figure 1 | The metabolic syndrome patients' aerobic capacity before and after pioglitazone treatment. (a) Peak oxygen uptake (VO_2; n = 14). (b) Anaerobic threshold (AT; n = 13).

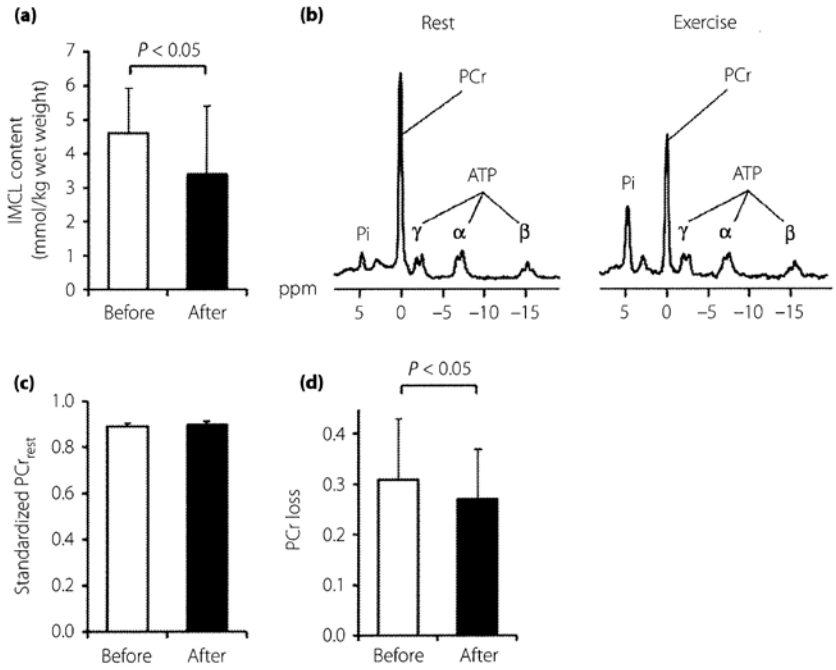

Figure 2 | The metabolic syndrome patients' skeletal muscle energy metabolism before and after pioglitazone treatment. The skeletal muscle energy metabolism was evaluated using (a) ^{1}proton-magnetic resonance spectroscopy and (b–e) ^{31}phosphorus-magnetic resonance spectroscopy. (a) intramyocellular lipid (IMCL) content. (b) Representative spectra of ^{31}phosphorus-magnetic resonance spectroscopy at rest (left panel) and during plantar flexion exercise (right panel) in the calf muscle. (c) Standardized phosphocreatine (PCr) level at rest. (d) Muscle PCr loss during exercise. Data are mean ± standard deviation (n = 14, except for IMCL content, which is n = 13). ATP, adenosine triphosphate; Pi, inorganic phosphate.

muscle after pioglitazone treatment (57.9 ± 9.5 cm^2 before treatment vs 58.2 ± 7.4 cm^2 after treatment).

The patients' daily physical activity was characterized by the number of steps taken and the movement-related calorie consumption, which were both measured by a pedometer. These two parameters' values were comparable before and after pioglitazone treatment (Table 1), suggesting that similar daily physical activity occurred during the study period.

The 4-month pioglitazone treatment significantly reduced the patients' fasting blood glucose, insulin, homeostasis model assessment of insulin resistance and triglyceride levels, whereas it did not change the levels of glycohemoglobin, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol or free fatty acids (Table 2).

Systemic oxidative stress

The pioglitazone treatment did not change the patients' serum thiobarbituric acid reactive substances (16.5 ± 4.4 μmol/L before vs 15.4 ± 3.1 μmol/L after treatment). There were no significant changes in the systemic anti-oxidant defense capacity including total thiols (8.4 ± 1.5 units/g protein vs 7.6 ± 2.5 units/g protein), superoxide dismutase activity (0.86 ± 0.22 units/g protein vs 0.86 ± 0.27 units/g protein), glutathione peroxidase activity (13.1 ± 1.7 units/g protein vs 13.5 ± 1.4 units/g protein) and glutathione reductase activity (0.99 ± 0.18 units/g protein vs 0.99 ± 0.16 units/g protein) from before to after pioglitazone treatment.

Whole-body aerobic capacity

Pioglitazone significantly increased the patients' peak VO_2 (Figure 1a) and AT (Figure 1b), indicating that pioglitazone improved the MetS patients' aerobic capacity. In addition, there were no significant changes in the patients' peak respiratory exchange ratio, peak heart rate or rating of perceived exertion after the pioglitazone treatment (peak respiratory exchange ratio: 1.24 ± 0.10 before treatment vs 1.22 ± 0.11 after treatment; peak heart rate: 144 ± 26 b.p.m. before treatment vs 143 ± 23 b.p.m. after treatment; rating of perceived exertion: 7.3 ± 1.8 before treatment vs 7.8 ± 1.6 after treatment), suggesting that the effort at peak exercise was similar.

IMCL content in skeletal muscle

The IMCL content in the resting leg muscles of the MetS patients was significantly reduced after the pioglitazone treatment (Figure 2a).

High-energy phosphate metabolism in skeletal muscle

We measured 1-RM in MetS patients (42.6 ± 6.1 kg) only before the pioglitazone treatment, assuming that 1-RM would not be changed throughout the study period, which is

supported by the unchanged cross-sectional area of the calf muscle after the treatment. After the initiation of a constant load of 20% 1-RM exercise, the PCr level in the patients' calf muscle started to decrease, and was finally stabilized within a few minutes in all experiments. The representative spectra of ^{31}P-MRS at rest and during the plantar flexion exercise are shown in Figure 2b. The standardized muscle PCr level at rest was similar before and after the treatment (Figure 2c). The pioglitazone significantly decreased the muscle PCr loss during exercise (Figure 2d), suggesting that the MetS patients' intramuscular high-energy phosphate metabolism was improved after the pioglitazone treatment.

Relationships between the changes in aerobic capacity and skeletal muscle energy metabolism after pioglitazone treatment

There was an inverse correlation between the changes in AT and the changes in IMCL content after the treatment (Figure 3b), whereas there was no significant correlation between the changes in peak VO_2 and the IMCL content (Figure 3a). The decrease in muscle PCr loss did not correlate with the increase in peak VO_2 and AT (data not shown).

DISCUSSION

The major finding of the present study was that the 4-month pioglitazone treatment (15 mg/day) improved the aerobic capacity characterized by increased peak VO_2 and AT in patients with MetS. The pioglitazone treatment decreased the resting IMCL content and PCr loss during exercise in the patients' leg muscle, indicating that pioglitazone improved skeletal muscle energy metabolism in these MetS patients. There was also a significant relationship between the increase in AT and the decrease in IMCL content after pioglitazone treatment. The improved skeletal muscle fatty acid metabolism might thus have contributed to the MetS patients' increased aerobic capacity after the pioglitazone treatment.

Low aerobic capacity has been well documented to be a strong and independent predictor of all-cause mortality in patients with obesity and insulin resistance[10,11]. It has also been shown that the increase in maximal aerobic capacity by each 1-metabolic equivalent, equivalent to a 3.5-mL/kg/min increase of peak VO_2, confers a 12% improvement in survival[7]. The modest, but significant, increase in aerobic capacity in the present study's MetS patients after the pioglitazone treatment is thus clinically relevant. In addition, there was no significant difference in the number of daily steps or movement-related calorie consumption between before and after pioglitazone treatment, indicating that the increased aerobic capacity after the pioglitazone treatment was independent of the patients' daily physical activity.

We observed a decrease in the IMCL content in the MetS patients after the pioglitazone treatment, which is consistent with previous studies of patients with impaired glucose tolerance[15] and patients with type 2 diabetes[16]. The content of

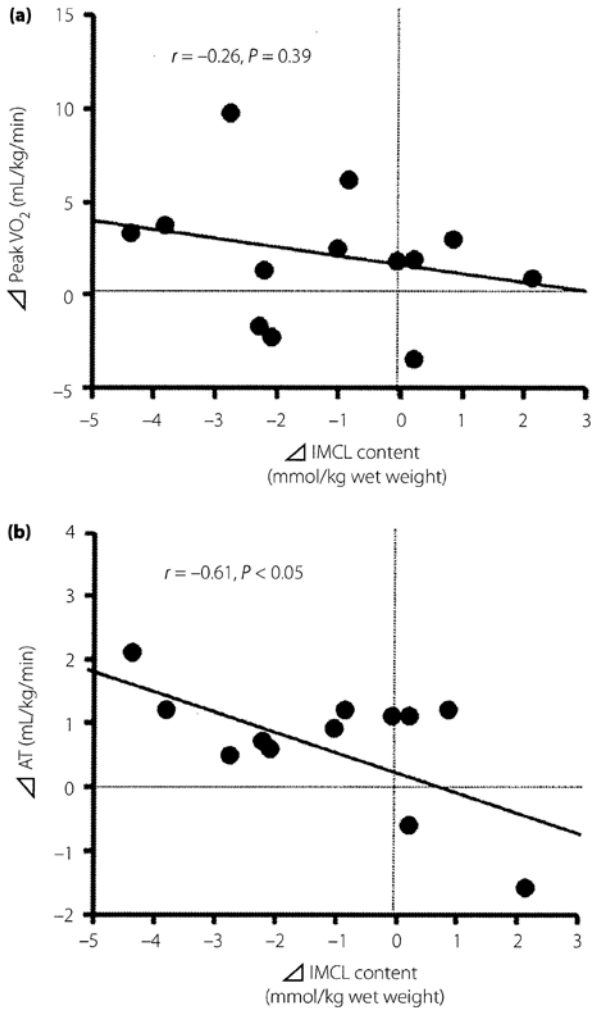

Figure 3 | Relationships between the changes in aerobic capacity and intramyocellular lipid (IMCL) content after pioglitazone treatment. AT, anaerobic threshold; VO_2, oxygen uptake.

IMCL, which is triglycerides within the muscle cells, is regulated by both uptake of fatty acids and fatty acid oxidation in skeletal muscle, and an accumulation of IMCL might be attributable to a reduced capacity for fatty acid oxidation rather than increased fatty acid uptake in skeletal muscle in obese patients with insulin resistance[17]. In addition, it was shown that pioglitazone increases the gene expression involved in fatty acid oxidation in the skeletal muscle in patients with type 2 diabetes[18]. These findings support the hypothesis that pioglitazone might decrease the IMCL content in individuals with MetS through an increased capacity for fatty acid oxidation in skeletal muscle.

The results of one of our previous studies showed that MetS patients had impaired high-energy phosphate metabolism characterized by greater muscle PCr loss during aerobic exercise

with the constant load of 20% 1-RM[8]. Muscle PCr works as an energy reserve in the cytosol and is converted to adenosine triphosphate (ATP) through creatine kinase reaction to keep the ATP level constant in the muscle cell[19].

PCr + adenosine diphosphate → ATP + creatine

When mitochondrial ATP production cannot meet the energy demand under aerobic conditions, the increased amount of PCr is converted to ATP, which might result in greater PCr loss. In the present study, the pioglitazone treatment significantly decreased muscle PCr loss during low-intensity exercise in the MetS patients. It was shown that pioglitazone activates AMP-activated protein kinase, which is known as a key regulator of mitochondrial biogenesis, and that pioglitazone increases the gene expression involved in mitochondrial function in human skeletal muscle[18]. It was also reported that pioglitazone improves mitochondrial respiratory capacity in skeletal muscle in patients with type 2 diabetes[20]. Taken together, all of these findings show that improved mitochondrial function as well as increased substrate utilization in skeletal muscle might contribute to the improvement in skeletal muscle energy metabolism after pioglitazone treatment.

We observed that fasting blood glucose, insulin, homeostasis model assessment of insulin resistance, and serum triglycerides were decreased after pioglitazone treatment without changing waist circumference and body composition in MetS patients, although pioglitazone has been reported to reduce visceral fat volume in patients with impaired glucose tolerance or type 2 diabetes[21]. It has been reported that pioglitazone treatment improves muscle insulin sensitivity as well as adipose tissue insulin sensitivity[22]. Several studies have shown that IMCL content is inversely correlated with muscle insulin sensitivity[23]. Therefore, in the present study, pioglitazone treatment increased systemic insulin sensitivity and decreased serum triglycerides at least in part through improved skeletal muscle energy metabolism including fatty acid metabolism in MetS patients.

In the present study's MetS patients, the increase in AT, but not the increase in peak VO_2, correlated with the decrease in IMCL content after the pioglitazone treatment. As fatty acids are the primary substrate for ATP production in skeletal muscle during low- to moderate-intensity exercise[24], it seems reasonable that there was a relationship between the increase in submaximal aerobic capacity and the improvement in skeletal muscle fatty acid metabolism after pioglitazone treatment in our MetS patients. However, as the intensity of exercise is further increased, particularly near or at peak intensity, the main energy sources can be switched to glucose and lactate, which might be the reason why the increase in peak VO_2 did not significantly correlate with the improvement in skeletal muscle fatty acid metabolism after pioglitazone treatment.

In addition to improved fatty acid metabolism in the skeletal muscle, other mechanisms could also contribute to the increased aerobic capacity of MetS patients after pioglitazone treatment. It has been shown that insulin sensitivity *per se* is associated with aerobic capacity[25]. However, in the present study, the improvement in insulin sensitivity markers including fasting blood glucose, insulin and homeostasis model assessment of insulin resistance did not correlate with the increase in aerobic capacity produced by pioglitazone treatment. Pioglitazone has been reported to improve endothelial function[26], which can improve aerobic capacity through an increased O_2 supply into skeletal muscle. We cannot exclude the contribution of changes in peripheral blood flow by pioglitazone, because we did not assess endothelial function.

There are some limitations that should be acknowledged. First, the small sample size (n = 14) might limit our interpretation and discussion. Second, the study was not a double-blind study using a matching placebo. Further randomized clinical trials with larger sample sizes are required to confirm the data of the present study.

In conclusion, this is the first study to show that pioglitazone treatment improved MetS patients' whole-body aerobic capacity and skeletal muscle energy metabolism. The increased submaximal aerobic capacity might be at least in part attributable to the improved fatty acid metabolism in skeletal muscle after the pioglitazone treatment. Although there is no doubt that lifestyle interventions, such as exercise and diet therapy, are the most desirable treatment for MetS, the present findings raise the possibility that pioglitazone can be a potential drug treatment for obese and insulin-resistant patients whose exercise capacity is lowered.

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DISCLOSURE

The authors declare no conflict of interest.

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