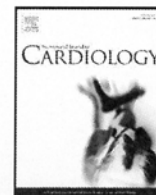高齢者の健康寿命延伸のためのシステムネットワーク構築と検証に関する研究分野

Letter to the Editor

Low-intensity exercise under ischemic conditions enhances metabolic stress in patients with heart failure

Masashige Takahashi [a], Shintaro Kinugawa [a,*], Shingo Takada [a], Kagami Hirabayashi [a], Takashi Yokota [a], Shouji Matsushima [a], Akimichi Saito [a], Koichi Okita [b], Hiroyuki Tsutsui [a]

[a] Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[b] Department of Sport Education, Hokusho University, Ebetsu, Japan

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It has been reported that maximum muscle strength (e.g., strength of the knee flexor muscles) and muscle mass are independent predictor for poor prognosis in patients with heart failure (HF) [1]. Therefore, resistance training could be effective therapy for HF. However, an appropriate exercise protocol of resistance training for HF patients has never been known. The increases in muscle strength and mass can be caused by high-intensity resistance training with a mechanical load greater than 60% of maximal muscle strength in healthy subjects [2]. High mechanical stress to the skeletal muscle has been reported to be a potent stimulus for muscle adaptations [2]. On the other hand, recent studies reported that metabolic stress followed by the accumulation of metabolic by-products, such as inorganic phosphate (Pi) (a decrease in phosphocreatine (PCr)) and H^+ (a decrease in muscle pH) in contracting muscle, played an important role on the effects by resistance training [2,3]. Our previous study using ^{31}P-magnetic resonance spectroscopy (MRS) clearly showed that low-intensity resistance exercise under ischemic conditions

enhanced metabolic stress [3]. Furthermore, metabolic stress was closely associated with the effects of resistance training [3]. It is often difficult for HF patients to perform high-intensity resistance training. The resistance exercises to enhance metabolic stress without increasing mechanical stress cause a sufficient training effect, and may be effective therapy for patients with HF. In the present study, we investigated metabolic stress in patients with HF during resistance exercises with 40–60% one-repetition maximum (1-RM), which has been recommended by a council statement from the American Heart Association [4]. Furthermore, we clarified an appropriate workload to obtain sufficient metabolic stress during resistance exercises under ischemic conditions in patients with HF.

Eight male patients with HF (52 ± 15 years, left ventricular ejection fraction (LVEF) 36 ± 9%, peak oxygen uptake 20.0 ± 6.5 ml/kg/min) and 8 age- and sex-matched healthy subjects as control (51 ± 4 years, LVEF 68 ± 6%, peak oxygen uptake 31.1 ± 3.5 ml/kg/min) were studied. Patients who had the implanted mechanical devices and New York Heart Association functional class IV were excluded. Patients with peripheral artery disease, angina pectoris, deep vein thrombosis, varix of lower extremity, and who could not perform exercise testing were also excluded. The protocol was approved by the medical ethics committee of Hokkaido University Hospital, and written informed consent was obtained from all participating subjects.

Muscle strength was measured by 1-RM method, and blood flow restriction (BFR) was carried out using 130% of the subject's resting systolic blood pressure with a pneumatic rapid inflator (E-20 rapid cuff inflator, Hokanson, Belleuve, WA), as previously described [3]. Patients with HF performed the exercises with 6 different protocols; 40%, 50%, and 60% 1-RM without BFR, and 20%, 30%, and 40% 1-RM with BFR. Control subjects performed the exercises with only a protocol; 60% 1-RM without BFR. Because it has been known that exercise training with this protocol leads to the significant increases in muscle strength and muscle mass in healthy subjects [2], 6 protocols in HF were compared with this protocol as a standard protocol. Measurements of ^{31}P-MRS and spectra analysis were performed, as described previously [3]. The spectra were obtained at rest and 2 min during plantar flexion exercises. The decrease in PCr or pH during the exercises was calculated by the difference between at rest and 2 min. The decreases in PCr and pH were presented as relative values to those obtained from the standards. Based on a previous study [5], sample size of study subjects were needed to be 8 for HF and 8 for control ($\alpha = 0.05$, $\beta = 0.90$). Data are expressed as means ± SD. To perform multiple pairwise
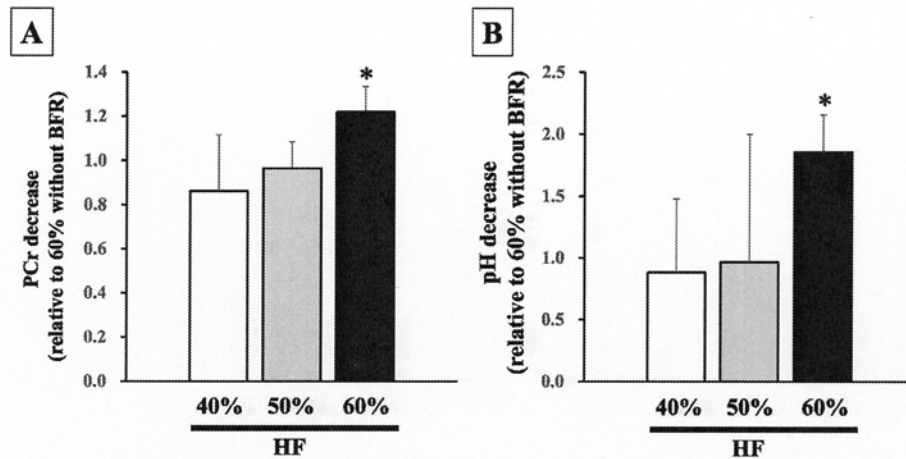
Fig. 1. PCr (A) and pH (B) decrease in 40% 1-RM, 50% 1-RM, and 60% 1-RM without BFR in HF patients (n = 8). All protocols were performed in a random order for 2 days with at least 30-min intervals between protocols. Before each subsequent protocol, we confirmed the recovery in altered energy metabolism to baseline levels. Data are expressed as means ± SD. *$P < 0.05$ vs. the reference. PCr, phosphocreatine; 1-RM, one-repetition maximum; BFR, blood flow restriction; and HF, heart failure.

comparisons to the reference, Dunnett's test was used. Statistical significance was defined as $P < 0.05$.

1-RM was significantly decreased in HF compared with control (35 ± 3 vs. 45 ± 3 kg, $P < 0.05$). PCr and pH at rest were comparable between control and HF. In HF patients, PCr and pH decreases were significantly higher in 60% 1-RM without BFR than the standards, but were comparable between the standards and other 2 protocols without BFR (Fig. 1). They were significantly lower in 20% 1-RM with BFR than the standards, but were comparable between the standards and other 2 BFR protocols (Fig. 2).

We found that metabolic stress during resistance exercise with 60% 1-RM was higher in HF patients than control subjects (Fig. 1). This suggests that energy supply from mitochondria is decreased and energy demand depends on PCr-ATP system in the skeletal muscle of HF patients [6,7]. Furthermore, this may be also associated with fiber type transition from slow (dominant oxidative capacity)-twitch fiber to fast (dominant glycolytic capacity)-twitch fiber in HF patients [8,9]. In a view of metabolic stress, exercise training with moderate-intensity (e.g., 40–50% 1-RM) under non-ischemic condition is sufficient for muscle adaptations in HF. We also clarified that resistance exercises with low- to moderate-

intensity (e.g., 30–40% 1-RM) under ischemic conditions enhanced metabolic stress in HF patients (Fig. 2). In the present study, we have never investigated the actual training effects including the increases in muscle strength and muscle mass by chronic resistance exercises. However, metabolic stress is a crucial factor for protein synthase of the skeletal muscle, which is possibly due to an increase in growth hormone secretion in blood and/or the activation of common signaling such as insulin-like growth factor-Akt-mammalian target of rapamycin signal in the skeletal muscle rather than inhibition of protein degradation [10]. Even if mechanical stress is small, resistance training under ischemic conditions could be effective in HF patients. Therefore, this therapeutic approach is very useful and safe in patients with HF. Our results propose that exercise trainings with 40–50% 1-RM under non-ischemic condition and with 30–40% 1-RM under ischemic condition are sufficient for muscle adaptation in patients with HF.
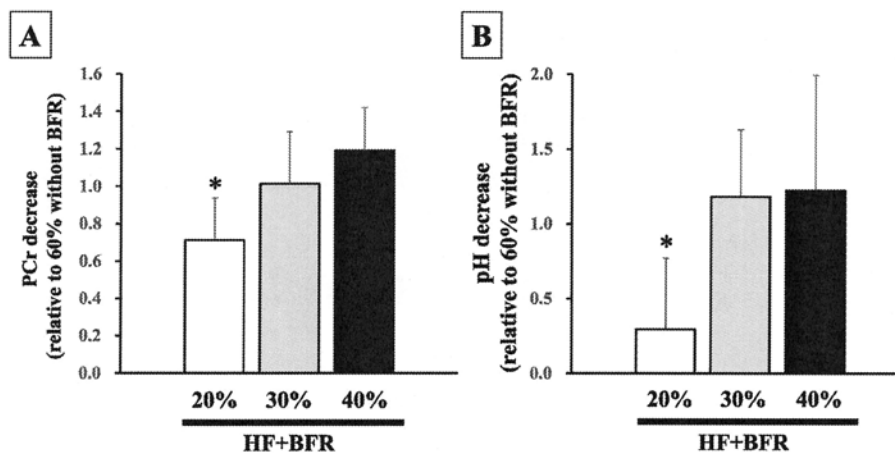
Fig. 2. PCr (A) and pH (B) decrease in 20% 1-RM, 30% 1-RM, and 40% 1-RM with BFR in HF patients (n = 8). Data are expressed as means ± SD. *$P < 0.05$ vs. the reference. PCr, phosphocreatine; BFR, blood flow restriction; and HF, heart failure.

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Skeletal Muscle Abnormalities in Heart Failure

Shintaro Kinugawa,[1] MD, Shingo Takada,[1] PhD, Shouji Matsushima,[1] MD,
Koichi Okita,[2] MD, and Hiroyuki Tsutsui,[1] MD

Summary

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

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

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

Skeletal Muscle Energy Metabolism

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

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

Skeletal Muscle Mass

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

Skeletal Muscle, Exercise Capacity and Life Expectancy

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

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

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

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

Molecular Mechanism of Skeletal Muscle Abnormalities

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

Regulation of mitochondrial function (Figure 1): The oxidative capacity of mitochondria is determined primarily by the amount of mitochondrial protein. Peroxisome proliferator-activated receptor γ (PPARγ) and PPARγ coactivator-1 (PGC-1) play key roles in mitochondrial biogenesis and functional regu-
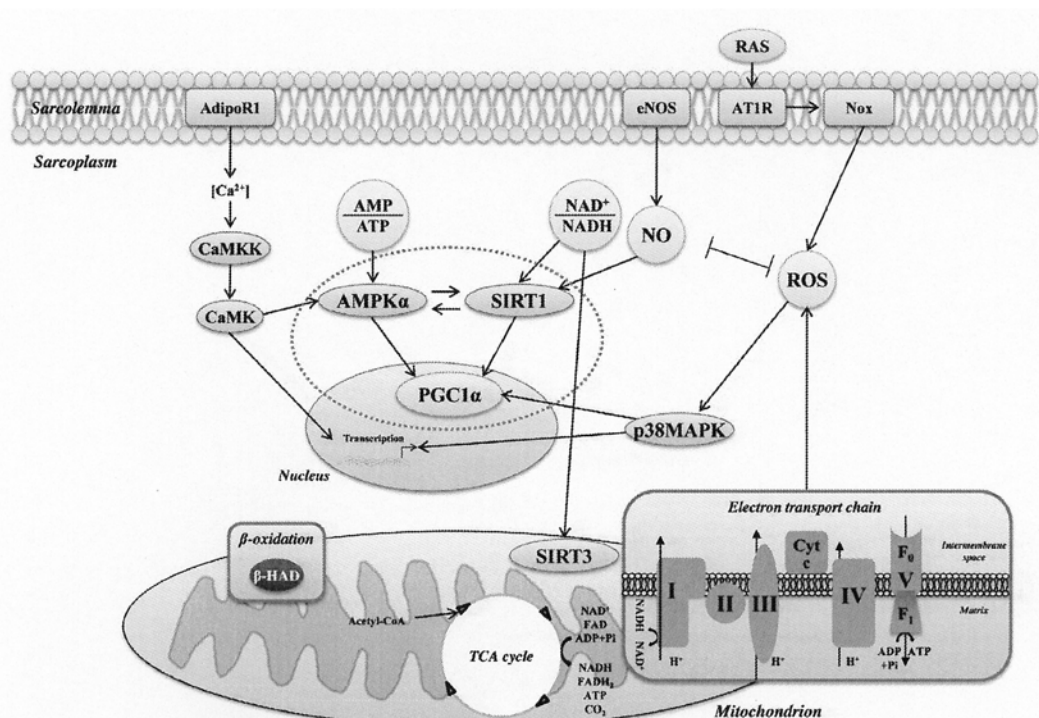
Figure 1. Regulation of mitochondrial function. AdipoR indicates adiponectin receptor; eNOS, endothelial nitric oxide synthase; RAS, renin-angiotensin system; AT1R, angiotensin II type 1 receptor; Nox, nicotinamide adenine dinucleotide phosphate-oxidase, CaMKK, calcium-calmodulin-dependent protein kinase kinase; CaMK, calcium-calmodulin-dependent protein kinase; NO, nitric oxide; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; AMPKα, AMP-activated protein kinase α; SIRT, sirtuin; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-α; β-HAD, β-hydroxy-acyl CoA dehydrogenase; TCA, tricarboxylic acid; and NAD, nicotinamide adenine dinucleotide. See text for details.
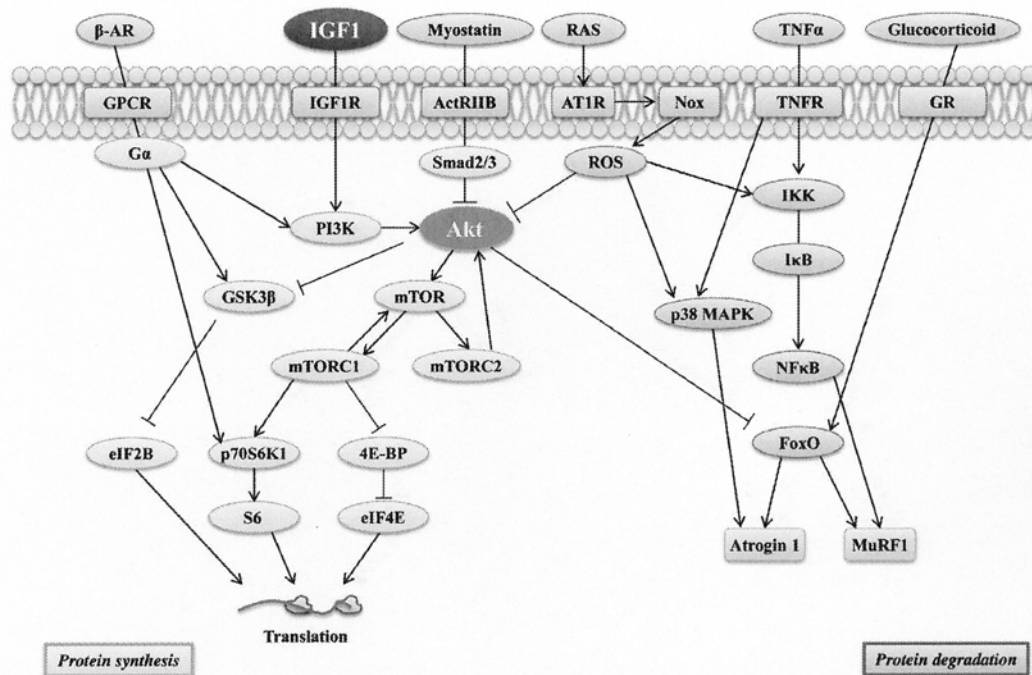
Figure 2. Skeletal muscle hypertrophy and atrophy. *β*-AR indicates *β*-adrenergic receptor; GPCR, G-protein-coupled receptor; IGF, insulin-like growth factor; IGFR, IGF receptor; ActRIIB, activin receptor IIb; RAS, renin-angiotensin system; AT1R, angiotensin II type 1 receptor; Nox, nicotinamide adenine dinucleotide phosphage-oxidase, TNF*α*; tumor necrosis factor *α*; TNFR, TNF receptor; GR, glucocorticoid receptor; GSK3*β*, glycogen synthase kinase-3*β*; PI3K, phosphoinositide-3-kinase; mTORC, mammalian target of rapamycin complex; p70S6K, p70 ribosomal S6 kinase; eIF2B, eukaryotic initiation factor-2B; 4E-BP, eIF4E-binding protein; I*κ*B, inhibitor of kappa B; IKK, I*κ*B kinase; NF*κ*B, nuclear factor kappa B; FoxO, forkhead box O; and MuRF1, muscle RING finger protein 1. See text for details.

lation.[16,17] PGC-1*α* increases the expression of transcription factors such as nuclear respiratory factor (NRF)-1 and -2.[16] NRFs not only increase the expression of nuclear gene-encoded mitochondrial proteins, but also increase the expression of mitochondrial transcription factor A (Tfam).[16] Tfam binds mitochondrial DNA (mtDNA) in the mitochondria and regulates the protein expression encoded in the mtDNA gene.[18,19] Overexpression of skeletal muscle-specific PGC-1*α* increased mitochondrial biogenesis and an oxidative muscle fiber-type, thereby increasing exercise capacity and creating a phenotypic change that equals aerobic exercise training.[17,20-22] Conversely, deletion of skeletal muscle-specific PGC-1*α* decreased mitochondrial biogenesis and increased the percentage of a glycolytic fiber-type, which caused a decline in exercise capacity.[22]

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

AMPK is a serine-threonine kinase that regulates intracellular metabolism. AMPK activation is regulated by the deficiency in intracellular energy such as the reduction of ATP and an increase in the AMP/ATP ratio.[16,23] AMPK is activated by aerobic exercise training and caloric restriction models and is inhibited in diabetes mellitus and aging models.[24-26] PGC-1*α* is phosphorylated by the activation of AMPK and mitochondrial biogenesis is regulated through this activation.[27] In fact, mitochondrial biogenesis is inhibited in skeletal muscle-specific AMPK-deficient mice[28] and mitochondrial biogenesis is increased with chronic activation of AMPK by AICAR and gene manipulation.[26,29]

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

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

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

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

Mitogen-activated protein kinases (MAPKs) are stress-response kinases that have an important role in mitochondrial biogenesis. MAPKs are activated by the mechanical stress of contraction in the skeletal muscle and stimulation of cytokines and oxidative stress.[46] This is related to MAPK activation during exercise. An MAPK family member, p38 MAPK, stimulates the upstream transcription factor of the PGC-1α gene and increases mitochondrial biogenesis in skeletal muscle by regulating the expression of PGC-1α.[24,47] On the other hand, in the absence of p38γ isoforms, an increase in mitochondrial biogenesis induced by exercise is inhibited.[48]

Muscle hypertrophy and muscle atrophy (Figure 2):

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

thesis not only through a protein synthesis pathway mediated by PI3K/Akt/mTOR, but also in an mTOR-independent manner.[55,62-66] Akt phosphorylates and inactivates glycogen synthase kinase 3β (GSK3β), leading to the activation of eIF2B, which is involved in protein synthesis. The PI3K/Akt/GSK3β-mediated pathway may also be involved in muscle hypertrophy.[55,62-66]

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

Another important signal for regulating skeletal muscle mass is the G protein-coupled receptor (GPCR)-mediated signal. The β2 adrenaline receptor stimulants, such as clenbuterol, are known to induce skeletal muscle hypertrophy.[72-74] The effect is mediated by GTP binding to a subunit, Gα$_s$, followed by Akt/mTOR signal cascade. Also, the activation of another GPCR, frizzled (Fzd)7-Wnt7a, is mediated through the Gα$_s$ pathway.[75] Skeletal muscle atrophy has been observed in Gα$_s$-deficient mice.[76] Conversely, in a recent study, skeletal muscle hypertrophy was observed through a PKC signal mediated by Gα$_i$2 activation.[76] Gα$_i$2 activation phosphorylated the downstream target of mTOR, particularly p70S6K and GSK3β, but not Akt.

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

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

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

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

Skeletal Muscle Abnormalities in HF

Exercise capacity in HF patients declines according to its severity. The skeletal muscle plays an important role in the reduced exercise capacity.[5,90,91] Many skeletal muscle abnormalities have been reported in association with HF. They include impaired skeletal muscle energy metabolism, mitochondrial dysfunction, fiber-type transition, and atrophy.[5,90-97] Skeletal muscle atrophy shows a decrease in muscle fiber size, and closely associates with the limited exercise capacity. Fiber-type transitions, the decreased ratio of type 1 (slow) fibers, and the relatively increased ratio of type 2 (fast) fibers were observed in skeletal muscle biopsy samples from patients with HF,[92,93,95-98] which was coincident with a shift from myosin heavy chain (MHC) 1 to fast fatigable MHC 2.[94,98] These fiber-type transitions are also known to be closely associated with exercise capacity.[94,98] We measured skeletal muscle energy metabolism during local exercise by lower limb using MRS in patients with HF.[5] They demonstrated a large PCr loss compared to healthy subjects (Figure 3A). A larger PCr loss indicated an impaired aerobic ATP production in skeletal muscle mitochondria. This PCr loss during localized exercise and the whole-body exercise tolerance (peak oxygen uptake; peak VO$_2$, and anaerobic threshold; AT) was closely linked (Figure 3B, C). Furthermore, we measured intramyocellular lipid (IMCL) levels by MRS (Figure 3D). IMCL was increased in HF patients compared to healthy subjects (Figure 3E) and its content was closely associated with skeletal muscle energy metabolism and whole-body exercise tolerance (Figure 3F, G).[91] This suggests that fatty acid oxidation is impaired in the mitochondria, and these results are coincident with a previous
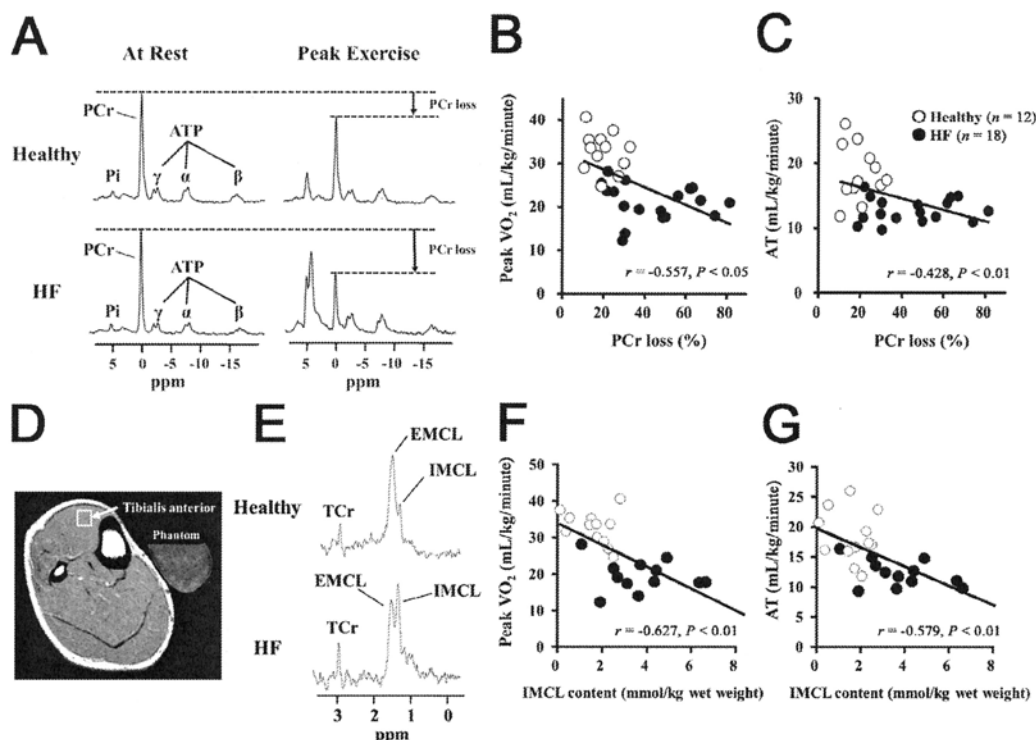
Figure 3. Skeletal muscle energy metabolism in patients with heart failure. Representative ^{31}P MR spectra recorded at rest and during exercise in triceps surae muscle from a healthy subject (upper) and a patient with HF (under) in the quantification of PCr content (**A**). Scatter plots between PCr loss and peak VO$_2$ (**B**) or AT (**C**) in healthy subjects (○; n = 12) and patients with HF (●; n = 18). Each plot represents the individual data of exercise capacity and ^{31}P-MRS obtained from the same subject. Representative ^{1}H MR spectra recorded at the resting tibialis anterior muscle (**D**) from a healthy subject (upper) and a patient with HF (under) in the quantification of IMCL content (**E**). Scatter plots between IMCL content and peak VO$_2$ (**F**) or AT (**G**) in healthy subjects (○; n = 12) and patients with HF (●; n = 18). Each plot represents the individual data of exercise capacity and ^{1}H-MRS obtained from the same subject. HF indicates heart failure; PCr, phosphocreatine; ATP, adenosine triphosphate; VO$_2$, oxygen uptake; AT, anaerobic threshold; IMCL, intramyocellular lipid; EMCL, extramyocellular lipid; and TCr, total creatine. Reproduced with permission from Hirabayashi, *et al.* Int J Cardiol 2014; 176: 1110-2.[91]

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

Skeletal muscle atrophy was observed in HF animal models 12 weeks after the induction of myocardial infarction (MI).[99,100] The atrophy was due to the enhanced protein degradation from activation of the UP system rather than a defect in protein synthesis, and the expressions of MAFbx and MuRF1 were also increased.[99,100] These were inhibited in mice with overexpression of skeletal muscle-specific IGF-1.[100] Furthermore, the activation of the UP system in skeletal muscle from post-infarct HF increased in mice lacking β2 adrenoreceptors, which was associated with a decline in Akt phosphorylation.[101] On the other hand, UP system activation occurred through NF-κB and p38 MAPK activation by NAD(P)H oxidase-derived oxidative stress in skeletal muscle from the same animal model.[99] We found that impairment of Akt phosphorylation in skeletal muscle from post-infarct HF mice occurred 4 weeks after surgery,[102,103] which was associated with NAD(P)H oxidase-derived oxidative stress and increased local angiotensin II in skeletal muscles.[102,103] Angiotensin II is thus considered to be important in skeletal muscle atrophy in HF. In this model, protein synthesis decreased due to impairment of Akt phosphorylation and protein degradation enhanced through protein ubiquitination.[104] A recent study reported that HF-induced skeletal muscle atrophy was inhibited in cardiac-specific myostatin-knockout mice.[105] This suggests that a cardiac-derived myostatin is involved in skeletal muscle atrophy in HF, thereby indicating a new mechanism of skeletal muscle abnormalities in HF.

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

We have shown that angiotensin II is possibly involved in skeletal muscle abnormalities in HF. Firstly, we administered a subpressor dose (50 ng/kg/minute) of angiotensin II into mice, and skeletal muscle mitochondrial dysfunction occurred and exercise capacity decreased without affecting skeletal muscle mass.[116] We also found that reactive oxygen species from NAD(P)H oxidase are involved in this model.[116] Furthermore, we investigated the effects of a pressor dose (1000 ng/kg/minute) of angiotensin II on the skeletal muscle abnormalities.[117] Angiotensin II directly induced mitochondrial dysfunction (Figure 4A) and fiber type transition to glycolytic (type IIb) from oxidative (type I) fibers (Figure 4B), followed by skeletal muscle atrophy (Figure 4C). Consequently, it reduced the exercise capacity (Figure 4D). Therefore, angiotensin II can be a key molecule that causes a series of skeletal muscle abnormalities observed in HF.
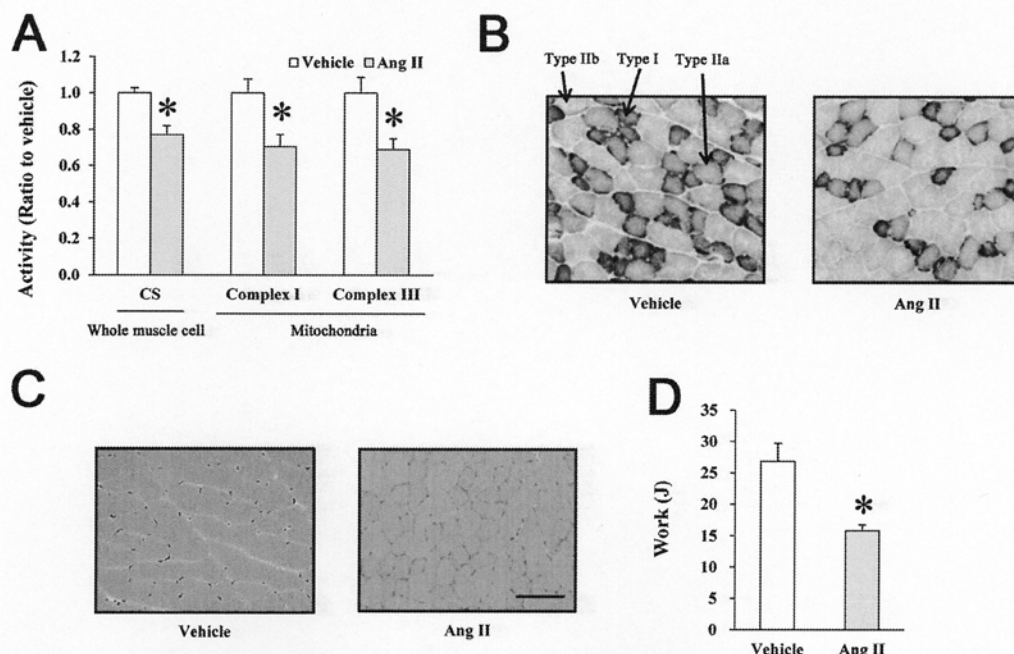
Figure 4. Skeletal muscle abnormalities in mice treated with angiotensin II. Citrate synthase activity (**A**, left), mitochondrial complex I (**A**, middle) and III (**A**, right) activities in the skeletal muscle tissue from vehicle and Ang II mice. Representative high-power photomicrographs of skeletal muscle tissue sections stained with nicotinamide adenine dinucleotide (NADH, **B**) and hematoxylin-eosin (HE) from vehicle and Ang II mice (**C**). Work during peak exercise in vehicle and Ang II mice (**D**). Scale bar, 100 μm. Ang II indicates angiotensin II; and CS, citrate synthase. Data are expressed the mean ± SE. *$P < 0.05$ versus vehicle. Reproduced with permission from Kadoguchi, *et al.* Exp Physiol 2015; 100: 312-22.[117]

HF and Diabetes Mellitus

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

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

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

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

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Research Paper

Sesamin prevents decline in exercise capacity and impairment of skeletal muscle mitochondrial function in mice with high-fat diet-induced diabetes

Shingo Takada[1], Shintaro Kinugawa[1], Shouji Matsushima[1], Daisuke Takemoto[2], Takaaki Furihata[1], Wataru Mizushima[1], Arata Fukushima[1], Takashi Yokota[1], Yoshiko Ono[2], Hiroshi Shibata[2], Koichi Okita[3] and Hiroyuki Tsutsui[1]

[1] *Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan*
[2] *Institute for Health Care Science, Suntory Wellness Ltd, Osaka, Japan*
[3] *Department of Sport Education, Hokusho University, Ebetsu, Japan*

Experimental Physiology

New Findings

- **What is the central question of this study?**
  Our aim was to examine whether sesamin can prevent a decline in exercise capacity in high-fat diet-induced diabetic mice. Our hypothesis was that maintenance of mitochondrial function and attenuation of oxidative stress in the skeletal muscle would contribute to this result.
- **What is the main finding and its importance?**
  The new findings are that sesamin prevents the diabetes-induced decrease in exercise capacity and impairment of mitochondrial function through the inhibition of NAD(P)H oxidase-dependent oxidative stress in the skeletal muscle. Sesamin may be useful as a novel agent for the treatment of diabetes mellitus.

We previously reported that exercise capacity and skeletal muscle mitochondrial function in diabetic mice were impaired, in association with the activation of NAD(P)H oxidase. It has been reported that sesamin inhibits NAD(P)H oxidase-induced superoxide production. Therefore, we examined whether the antioxidant sesamin could prevent a decline in exercise capacity in mice with high-fat diet (HFD)-induced diabetes. C57BL/6J mice were fed a normal diet (ND) or HFD, then treated or not with sesamin (0.2%) to yield the following four groups: ND, ND+Sesamin, HFD and HFD+Sesamin ($n = 10$ each). After 8 weeks, body weight, fat weight, blood glucose, insulin, triglyceride, total cholesterol and fatty acid were significantly increased in HFD compared with ND mice. Sesamin prevented the increases in blood insulin and lipid levels in HFD-fed mice, but did not affect the plasma glucose. Exercise capacity determined by treadmill tests was significantly reduced in HFD mice, but almost completely recovered in HFD+Sesamin mice. Citrate synthase activity was significantly decreased in the skeletal muscle of HFD mice, and these decreases were also inhibited by sesamin. Superoxide anion and NAD(P)H oxidase activity were significantly increased in HFD mice compared with the ND mice and were ameliorated by sesamin. Sesamin prevented the decline in exercise capacity in HFD-induced diabetic mice via maintenance of mitochondrial function, fat oxidation and attenuation of oxidative stress in the skeletal muscle. Our data suggest that sesamin may be useful as a novel agent for the treatment of diabetes mellitus.

Introduction

Patients with metabolic syndrome and type 2 diabetes show reduced exercise capacity and mitochondrial dysfunction in the skeletal muscle (Regensteiner *et al.* 2005; Mogensen *et al.* 2007; Yokota *et al.* 2011, 2013). Abnormalities in skeletal muscle energy metabolism are the key factor in reduced exercise capacity (Okita *et al.* 1998; Yokota *et al.* 2011, 2013). Moreover, this feature has been reported to be an independent predictor of mortality (Wei *et al.* 2000). We previously reported that the reduced exercise capacity and impaired skeletal muscle mitochondrial function in mice with high-fat diet (HFD)-induced type 2 diabetes were due to enhanced oxidative stress via the activation of NAD(P)H oxidase (Yokota *et al.* 2009; Takada *et al.* 2013, 2014; Kinugawa *et al.* 2015). Another study reported that NAD(P)H oxidase-induced superoxide anion ($O_2 \cdot^-$) production was increased in the skeletal muscle of mice with insulin resistance-induced diabetes (Bonnard *et al.* 2008). The NAD(P)H oxidase-induced enhancement of oxidative stress has also been demonstrated in skeletal muscle from patients with type 2 diabetes (Roberts *et al.* 2006). In our previous studies, angiotensin II type 1 receptor blocker or an insulin-sensitizing drug ameliorated the activation of NAD(P)H oxidase and partly improved the limited exercise capacity (Takada *et al.* 2013, 2014). NAD(P)H oxidase activity can be increased by high fatty acid levels and activation of the renin–angiotensin system, as well as by high glucose, insulin and insulin resistance levels (Yang & Kahn, 2006; Takada *et al.* 2013, 2014; Kadoguchi *et al.* 2015). Therefore, the activation of NAD(P)H oxidase by activation of the renin–angiotensin system and/or insulin resistance plays an important role in the limited exercise capacity of HFD-induced diabetic mice.

Sesamin, one of the lignans found in sesame seeds and oil, has multiple biological functions (Nakano *et al.* 2006, 2008; Hong *et al.* 2013). It has been reported that sesamin decreases blood glucose, insulin and lipid levels in type 2 diabetic mice (Hong *et al.* 2013). Sesamin also inhibits NAD(P)H oxidase-induced $O_2 \cdot^-$ production in the aorta in rats administered deoxycorticosterone acetate and salt (Nakano *et al.* 2006). Furthermore, a sesamin metabolite (SC-1; ($7\alpha,7'\alpha,8\alpha,8'\alpha$)-3,4-dihydroxy-3′,4′-methylenedioxy-7,9′: 7′,9-diepoxylignane) strongly inhibited xanthin/xanthine oxidase-induced $O_2 \cdot^-$ production (Nakai *et al.* 2003; Nakano *et al.* 2006, 2008). Given that sesamin has antioxidant effects, we hypothesized that it may have a favourable effect on mitochondrial function, preventing the decline in exercise capacity in HFD-induced diabetic mice by inhibiting NAD(P)H oxidase-induced production of reactive oxygen species. We therefore investigated whether sesamin could prevent the activation of NAD(P)H oxidase and decline in exercise capacity in HFD-induced diabetic mice.

Methods

Experimental animals

Male C57BL/6J mice were housed in an animal room in controlled conditions on a 12 h–12 h light–dark cycle. Mice were fed either a normal diet (ND) containing 4.2% fat and 54.6% carbohydrate or an HFD (HFD32) containing 32.0% fat and 29.4% carbohydrate for 8 weeks. Mice were further divided into groups with or without the addition of sesamin (0.2%) to their ND or HFD diet. Sesamin was prepared from refined sesame seed oil and purified as previously described (Fukuda *et al.* 1986). The quantities of food consumed by each mouse (2.4–2.5 g day^{-1} per mouse) and body weights were monitored every week (data not shown). The dose of sesamin in the present study was chosen on the basis of previous studies (Ashakumary *et al.* 1999; Ide *et al.* 2001*a*). The present study thus had the following four treatment groups: (i) ND; (ii) ND+Sesamin; (iii) HFD; and (iv) HFD+Sesamin ($n = 10$ for each group). These assignment procedures were performed using numerical codes to identity the animals. All procedures and animal care were approved by our institutional animal research committee and conformed to the Animal Care Guideline for the Care and Use of Laboratory Animals at Hokkaido University Graduate School of Medicine.

Blood samples were collected from the inferior vena cava before the mice were killed, under deep general anaesthesia induced with tribromoethanol-amylene hydrate [Avertin; 2.5% w/v, 250 mg (kg body weight)$^{-1}$, I.P.] (Sigma-Aldrich, St Louis, MO, USA). Epididymal fat and unilateral hindlimb skeletal muscles (quadriceps, gastrocnemius and soleus) were then excised and weighed. We used only the gastrocnemius muscle for mitochondrial function and biochemical analyses in all experiments ($n = 6$–10 for each assay).

In the *in vitro* study, we used mouse C2C12 myotubes and measured NAD(P)H oxidase activity ($n = 10$–11 for each group).

Blood pressure measurements

Systemic blood pressure and heart rate were measured using the tail-cuff method (BP-98A; Softron, Tokyo, Japan) without anaesthesia.

Biochemical measurements

Plasma insulin, total cholesterol, triglyceride and non-esterified fatty acid levels were measured as previously

described (Takada *et al.* 2013, 2014; Ono *et al.* 2015).

Plasma concentrations of sesamin and SC-1

Plasma samples were extracted after hydrolysis with β-glucuronidase/arylsulfatase. Sesamin and SC-1 were measured by ultraperformance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) as previously described (Tomimori *et al.* 2013).

Intraperitoneal glucose and insulin tolerance tests

For the glucose or insulin tolerance test, mice were fasted for 6 h and were given an I.P. injection of glucose (1 mg g^{-1}) or human insulin (0.25 mU g^{-1}) in purified water. Blood samples were repeatedly drawn from the tail vein of the same mice before and 15, 30, 60, 90 and 120 min after the injection. Blood glucose levels were determined using a glucometer (Glutest Ace R; Sanwa Kagaku Kenkyusho, Nagoya, Japan).

Treadmill testing with expired gas analysis and spontaneous physical activity

Mice were treadmill tested to measure indexes defining exercise capacity as previously described (Kinugawa *et al.* 2005; Yokota *et al.* 2009; Takada *et al.* 2013, 2014; Suga *et al.* 2014; Kadoguchi *et al.* 2015; Nishikawa *et al.* 2015). At the time of treadmill testing, each mouse was placed on a treadmill enclosed by a metabolic chamber, through which air flowing at a constant speed (1 l min^{-1}) was passed (Oxymax 2; Columbus Instruments, Columbus, OH, USA). Oxygen and carbon dioxide gas fractions were monitored at both the inlet and output ports of the metabolic chamber. Basal measurements were obtained over a period of 10 min. Mice were then provided with a 10 min warm-up period at 6 m min^{-1} with the ramp at 0 deg slope. After animals had warmed up, the angle was fixed at 10 deg, and the speed was increased incrementally by 2 m min^{-1} every 2 min until the mouse reached exhaustion. Exhaustion was defined as spending time (10 s) on the shocker plate (shock grid stimulus area, 51 mm × 51 mm; stimulus current range, 0.34–1.5 mA; stimulus voltage, 163 V) without attempting to re-engage the treadmill. Whole-body oxygen uptake and carbon dioxide production were automatically calculated every 10 s by taking the difference between the inlet and output gas flow. The respiratory exchange ratio (RER) was calculated as carbon dioxide production divided by oxygen uptake. Work was defined as the product of the vertical running distance and body weight. Spontaneous physical activity was measured using an animal movement analysis system (ACTIMO System; Shintechno, Fukuoka, Japan) as previously described (Takada *et al.* 2013).

Mitochondrial enzyme activities in the skeletal muscle

The enzymatic activity of citrate synthase (CS), a key enzyme of tricarboxylic acid cycle, was determined spectrophotometrically in the tissue homogenates from skeletal muscle samples, as described previously (Inoue *et al.* 2012; Suga *et al.* 2014; Takada *et al.* 2014; Kadoguchi *et al.* 2015; Nishikawa *et al.* 2015).

Immunoblotting in the skeletal muscle

Immunoblotting was performed using antibodies against the phosphorylated forms of AMPK and acetyl-CoA carboxylase-β (Cell Signaling, Danvers, MA, USA). Equal loading of protein was verified by immunoblotting with glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Cell Signaling), as previously described (Takada *et al.* 2013; Fukushima *et al.* 2014; Kadoguchi *et al.* 2015; Nishikawa *et al.* 2015; Ono *et al.* 2015).

Quantitative RT-PCR

Total RNA was extracted from the hindlimb skeletal muscle limb in the four groups of mice using QuickGene-810 (Fujifilm, Tokyo, Japan) according to the manufacturer's instructions. Complementary DNA was synthesized with a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) as previously described (Takada *et al.* 2013). TaqMan quantitative PCR was performed with the 7300 real-time PCR system (Applied Biosystems) to amplify samples for fatty acid binding protein 3 (*Fabp3*), fatty acid transport protein 1 (*Fatp1*), FAT/CD36 (*Cd36*), carnitine palmitoyltransferase-1b (*Cpt-1b*), superoxide dismutase (*Sod1*), *Sod2*, *Catalase*, glutathione peroxidase (*Gpx*), sirtuin 1 (*Sirt1*), peroxisome proliferator-activated receptor γ coactivator 1α (*Pgc-1*), nuclear respiratory factor-1 (*Nrf-1*) and mitochondrial transcription factor A (*Tfam*). Relative mRNA was analysed with the $\Delta\Delta$ threshold cycle ($\Delta\Delta C_T$) method and normalized to GAPDH as the internal control. Each C_T value was determined by subtracting the GAPDH RNA C_T value from the target gene C_T value. The C_T value was calculated by subtracting the C_T value of ND mice from the C_T value of ND+Sesamin, HFD and HFD+Sesamin mice. The value $2^{-\Delta\Delta C_T}$ represented the average relative amount of mRNA for each target gene as previously described (Takada *et al.* 2013).

Table 1. Animal characteristics

Parameter	ND group ($n = 10$)	ND+Sesamin group ($n = 10$)	HFD group ($n = 10$)	HFD+Sesamin group ($n = 10$)
Haemodynamic measurements				
Systolic blood pressure (mmHg)	101 ± 1	101 ± 2	99 ± 1	101 ± 2
Heart rate (beats min^{-1})	621 ± 40	610 ± 24	613 ± 42	603 ± 19
Body and organ weights				
Body weight (g)	30.3 ± 0.5	29.5 ± 0.2	43.1 ± 0.7*	37.5 ± 1.0*†
Fat weight (mg)	630 ± 64	460 ± 37	2241 ± 68*	2006 ± 109*
Quadriceps muscle weight (mg)	221 ± 5	234 ± 4	225 ± 3	222 ± 4
Gastrocnemius muscle weight (mg)	171 ± 3	172 ± 3	184 ± 2	170 ± 2
Soleus weight (mg)	11.0 ± 0.2	11.8 ± 0.2	14.4 ± 0.2	12.7 ± 0.3

Data are expressed as means ± SEM. *$P < 0.05$ *versus* ND; †$P < 0.05$ *versus* HFD. Abbreviations: HFD, high-fat diet; and ND, normal diet.

Superoxide anion and NAD(P)H oxidase activity in the skeletal muscle *in vivo*

The chemiluminescence elicited by $O_2 \cdot^-$ in the presence of lucigenin (5 μmol l^{-1}) was measured in hindlimb skeletal muscle using a luminometer (AccuFLEX Lumi 400; Aloka, Tokyo, Japan) as previously described (Yokota *et al.* 2009; Takada *et al.* 2013, 2014; Fukushima *et al.* 2014; Suga *et al.* 2014; Kadoguchi *et al.* 2015; Nishikawa *et al.* 2015; Ono *et al.* 2015). NAD(P)H oxidase activity was measured in the homogenates isolated from hindlimb skeletal muscle by the lucigenin assay after the addition of NAD(P)H (300 μmol l^{-1}) as previously described (Yokota *et al.* 2009; Takada *et al.* 2013, 2014; Fukushima *et al.* 2014; Suga *et al.* 2014; Kadoguchi *et al.* 2015; Nishikawa *et al.* 2015; Ono *et al.* 2015).

NAD(P)H oxidase activity in C2C12 myotubes *in vitro*

The mouse C2C12 myoblast cell line (American Type Culture Collection, Manassas, VA, USA) was seeded on culture plates with medium containing 2% horse serum. Differentiation of C2C12 myoblasts into myotubes occurred in 6–7 days, as confirmed by light microscopy showing morphological alignment, elongation and fusion, as previously described (Fukushima *et al.* 2014; Nishikawa *et al.* 2015). After pre-incubation at 37°C in serum-free conditions, C2C12 myotubes were incubated at 37°C with with 1 μmol l^{-1} angiotensin II (Sigma-Aldrich) for 24 h in the absence or presence of 1 or 10 μmol l^{-1} SC-1, which was prepared as previously described (Urata *et al.* 2008). After 24 h of incubation, cells were harvested and stored at −80°C for measurement of NAD(P)H oxidase activity. The NAD(P)H oxidase activity was measured in the homogenates of the C2C12 myotubes by a lucigenin (5 μmol l^{-1}) assay after the addition of NAD(P)H (100 μmol l^{-1}) as previously described (Fukushima *et al.* 2014; Nishikawa *et al.* 2015).

Statistical analysis

Data are expressed as means ± SEM. For multiple-group comparisons, two-way ANOVA followed by Tukey's test was performed. In I.P. glucose and insulin tolerance tests, differences between groups were determined with repeated-measures ANOVA. A value of $P < 0.05$ was considered statistically significant.

Results

Animal characteristics

Table 1 shows the characteristics of the animals in the four groups. Body weight was significantly higher in HFD compared with ND mice, and this was accompanied by a significant increase in the epididymal fat weight (Table 1). There were no differences in the quadriceps, gastrocnemius and soleus muscle weights, systolic blood pressure or heart rate between ND and HFD mice (Table 1). Fasting blood glucose, insulin, triglyceride, total cholesterol and non-esterified fatty acid levels were significantly higher in HFD mice (Table 2). Moreover, blood glucose levels during the I.P. glucose and insulin tolerance tests were significantly higher in HFD than in ND mice (Fig. 1).

Plasma sesamin and SC-1 were detected in ND+Sesamin and HFD+Sesamin mice (sesamin, 380 ± 354 and 323 ± 186 nmol l^{-1}; and SC-1, 1.00 ± 0.43 and 1.85 ± 0.43 μmol l^{-1}). Sesamin significantly suppressed an increase of body weight, but did not affect haemodynamic measurements (Table 1). There were also no significant differences in epididymal fat weight, total skeletal muscle weight, fasting blood glucose or blood glucose levels between HFD and HFD+Sesamin mice during the I.P. glucose tolerance test (Tables 1 and 2 and Fig. 1). In contrast, the increases in plasma insulin, triglyceride, total cholesterol and non-esterified fatty acid

Table 2. Blood measurements

Parameter	ND group ($n = 8$)	ND+Sesamin group ($n = 8$)	HFD group ($n = 8$)	HFD+Sesamin group ($n = 8$)
Fasting glucose (mg dl^{-1})	106 ± 6	106 ± 5	$244 \pm 7*$	$220 \pm 10*$
Insulin (ng ml^{-1})	0.47 ± 0.09	0.81 ± 0.10	$1.96 \pm 0.32*$	$0.76 \pm 0.16^{\dagger}$
Triglyceride (mg ml^{-1})	40 ± 2	45 ± 7	$82 \pm 4*$	$54 \pm 6^{\dagger}$
Total cholesterol (mg ml^{-1})	74 ± 6	55 ± 4	$198 \pm 6*$	$81 \pm 15^{\dagger}$
Non-esterified fatty acid (mequiv l^{-1})	0.25 ± 0.02	0.20 ± 0.03	$0.87 \pm 0.02*$	$0.37 \pm 0.05^{\dagger}$

Data are expressed as means $\pm$ SEM. *$P < 0.05$ versus ND; $^{\dagger}P < 0.05$ versus HFD.

levels were completely attenuated in HFD+Sesamin mice. These results showed that HFD feeding for 8 weeks induced type 2 diabetes with the characteristics of obesity and glucose intolerance, and that sesamin prevented the increases in blood insulin and serum lipid levels.

Exercise capacity and spontaneous physical activity

Figure 2 shows the indices of exercise capacity. The work, run distance and run time to exhaustion were significantly decreased in HFD compared with ND mice. The reduced
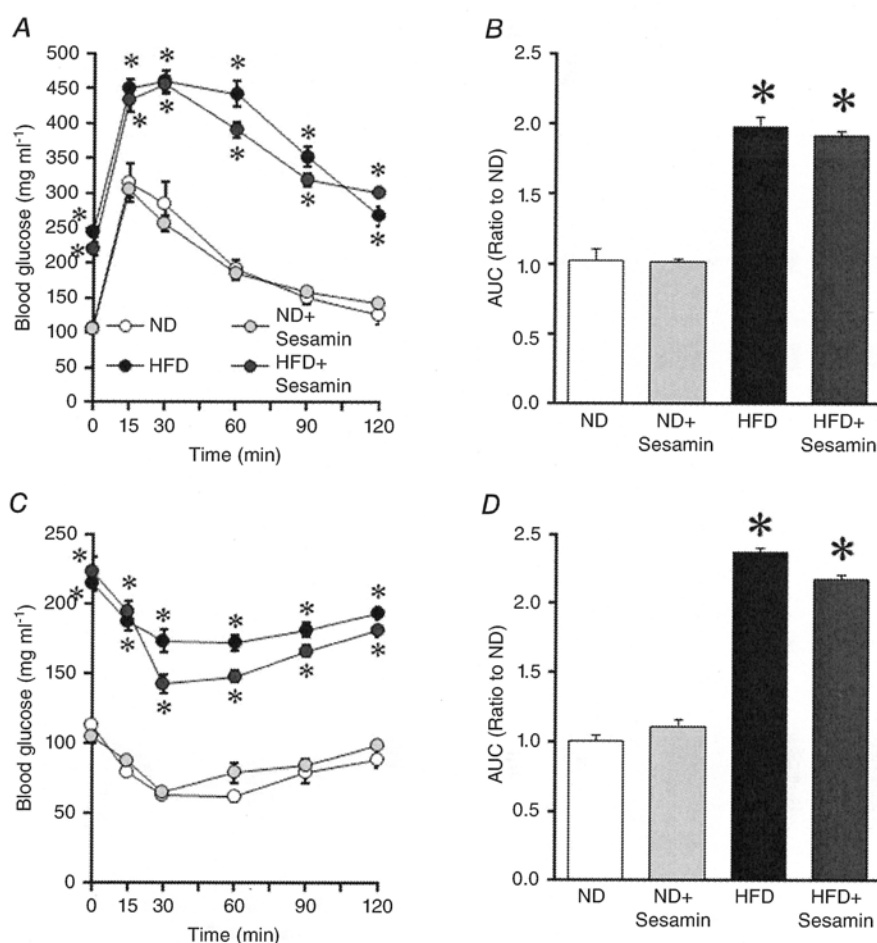
Figure 1. Glucose tolerance test and insulin tolerance test
Blood glucose levels during I.P. glucose tolerance test (*A*) and insulin tolerance test (*C*) in the normal diet (ND), ND+Sesamin, high-fat diet (HFD) and HFD+Sesamin mice ($n = 9$–10 for each group). Area under the curve (AUC) of blood glucose levels during I.P. glucose tolerance test (*B*) and insulin tolerance test (*D*) in the ND, ND+Sesamin, HFD and HFD+Sesamin mice. Data are shown as means $\pm$ SEM. Experiments were performed after 8 weeks of feeding in all groups. *$P < 0.01$ versus ND.

exercise capacity was partly ameliorated in HFD+sesamin mice (Fig. 2A–D). In contrast, spontaneous physical activity was significantly decreased in HFD compared with ND mice, and this effect was not altered by sesamin (Fig. 2F).

Mitochondrial function and biogenesis in the skeletal muscle

Exercise capacity is largely dependent on mitochondrial function in the skeletal muscle. It has been reported that the activity of CS, a key enzyme of the tricarboxylic acid cycle, in the skeletal muscle plays a critical role in exercise capacity (MacDougall et al. 1998; Kanatous et al. 1999; Park et al. 2014). Moreover, CS activity closely is related to mitochondrial quantity, complex activities and respiration in the permeabilized muscle fibre (Kanatous et al. 1999; Park et al. 2014). Therefore, the activity of

this mitochondrial enzyme was measured (Fig. 3). Skeletal muscle CS activity was significantly decreased in HFD compared with ND mice, and this decrease was reversed by sesamin. In contrast, there were no differences between groups in the phosphorylation of the mitochondrial biogenesis-related protein AMPK or in the gene expression of Pgc-1 mRNA (Fig. 4). Gene expressions of Sirt1, Nrf-1 and Tfam mRNA were significantly decreased in HFD compared with ND mice, and these differences were not affected by sesamin (Fig. 4).

Fatty acid oxidation and glucose metabolism in the skeletal muscle

Acetyl-CoA carboxylase-β phosphorylation and β-hydroxyacyl-CoA dehydrogenase activity in the skeletal muscle were significantly decreased in HFD compared with ND mice, and these decreases were

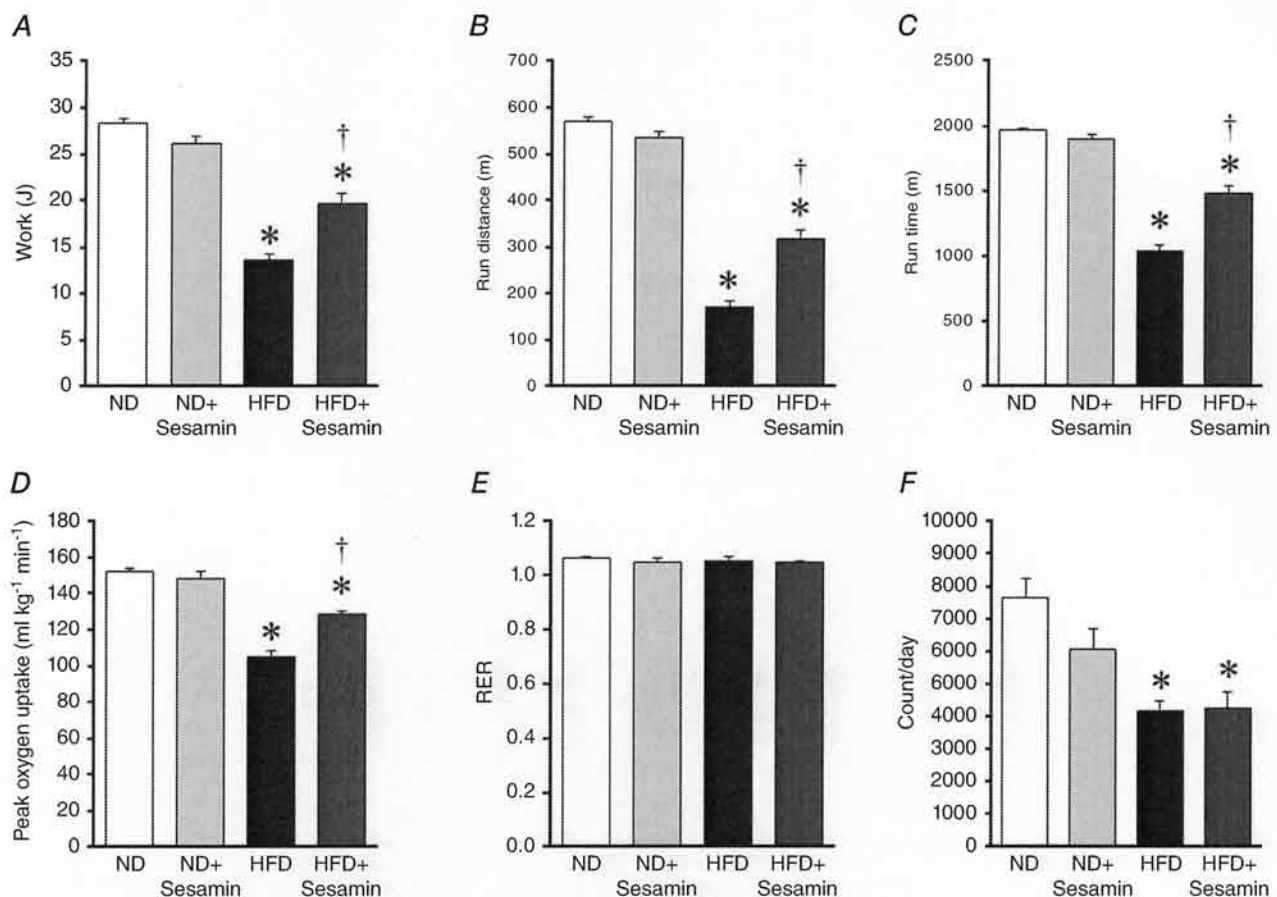**Figure 2. Exercise capacity and spontaneous physical activity**
The summarized data of the work (A), distance run (B), run time (C), peak oxygen uptake (D), respiratory exhange ratio (RER) to exhaustion (E) and spontaneous physical activity (F) in the ND, ND+Sesamin, HFD and HFD+Sesamin mice (n = 10 for each group). Data are shown as means + SEM. *$P < 0.05$ versus ND; †$P < 0.05$ versus HFD.

inhibited by sesamin (Fig. 5A and C). *Cpt-1b* mRNA was significantly decreased in the skeletal muscle from HFD compared with ND mice, but this difference was not affected by sesamin (Fig. 5B). *Fabp3*, *Fatp1* and *Cd36* mRNA also tended to be decreased in HFD mice and

were not affected by sesamin. In contrast, there were no differences in glucose transporter 4, hexokinase 2 and pyruvate kinase m2 among the four groups (Fig. 5D–G).

Oxidative stress in the skeletal muscle

Superoxide anion production and NAD(P)H oxidase activity were significantly increased in the skeletal muscle from HFD compared with ND mice, and this change was completely inhibited by sesamin (Fig. 6A and B). Moreover, SC-1 at a dose of 1 μmol l^{-1} significantly suppressed an increase in NAD(P)H oxidase activity by angiotensin II stimulation in C2C12 myotubes (Fig. 7). *Sod1* and *Catalase* mRNA in the skeletal muscle were significantly decreased in HFD compared with ND mice, but were not affected by sesamin (Fig. 6C).

Discussion

In the present study, mice with HFD-induced diabetes exhibited lowered exercise capacity, decreased activity of the enzyme CS and activation of NAD(P)H oxidase in the skeletal muscle; all these effects were significantly ameliorated by chronic oral administration of sesamin to HFD mice. Therefore, dietary sesamin was shown to prevent the decline in exercise capacity and the impairment of mitochondrial function in mice with HFD-induced diabetes.
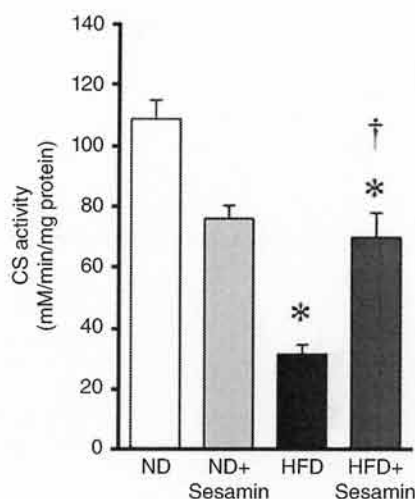
Figure 3. CS activity
The summarized data for citrate synthase (CS) in the skeletal muscle from the ND, ND+Sesamin, HFD and HFD+Sesamin mice ($n = 6$ for each group). Data are shown as means + SEM. *$P < 0.05$ *versus* ND; †$P < 0.05$ *versus* HFD.
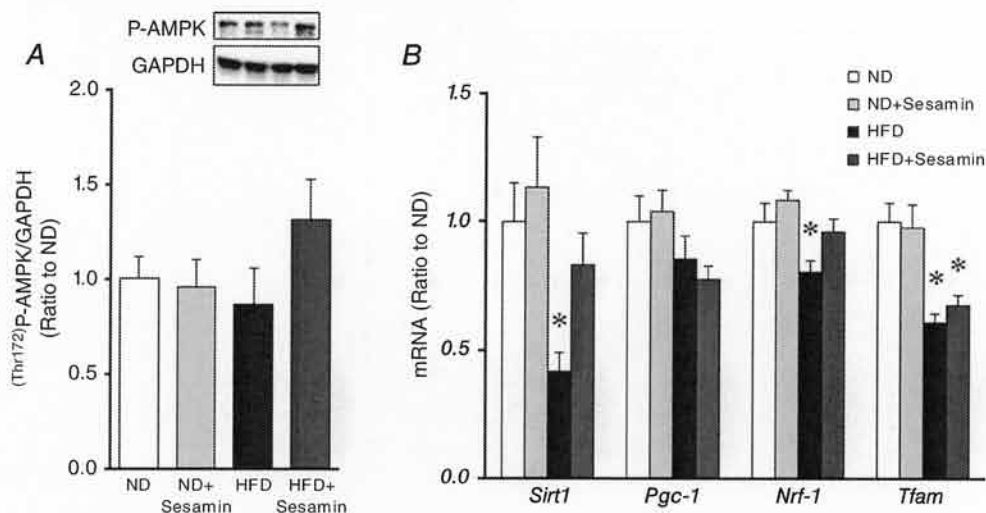
Figure 4. Mitochondrial biogenesis related-protein and gene expressions
Quantitative analysis of the phosphorylated form (Thr172) of AMP kinase (AMPK) protein (A) and gene expressions of sirtuin1 (*Sirt1*), peroxisome proliferator-activated receptor γ coactivator 1 (*Pgc-1*), nuclear respiratory factor 1 (*Nrf-1*) and mitochondrial transcription factor A (*Tfam*) mRNA (B) in skeletal muscle obtained from the ND, ND+Sesamin, HFD and HFD+Sesamin mice ($n = 6$–8 for each group). Protein expression was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Gene expressions were normalized to GAPDH and depicted as the ratio to ND. Data are shown as means + SEM. *$P < 0.05$ *versus* ND.

Effects of sesamin on substrate metabolism

Sesamin has hypoglycaemic and hypolipidaemic effects in diabetic mice (Nakano *et al.* 2006, 2008; Hong *et al.* 2013). Hong *et al.* (2013) reported that sesamin decreased blood glucose, insulin and lipid levels in mice with type 2 diabetes. Sesamin also increased hepatic CPT activity in mice or fat oxidation in rats (Shimoda *et al.* 2006; Ide *et al.* 2009). Coinciding with these results, in the present study sesamin prevented the impairment in lipid metabolism in the skeletal muscle (Fig. 5) and, consequently, decreased blood lipids in HFD mice (Table 2). In contrast, it did not affect the fasting glucose level, glucose and insulin

tolerance (Table 2 and Fig. 1) or glucose metabolism in the skeletal muscle (Fig. 4). Therefore, these results suggest that sesamin specifically maintains the lipid substrate metabolism and may have beneficial effects on energy production in the skeletal muscle.

Effects of sesamin on exercise capacity and NAD(P)H oxidase activity

The most significant finding of the present study was that chronic administration of sesamin to HFD mice prevented the decrease in exercise capacity (Fig. 2) and
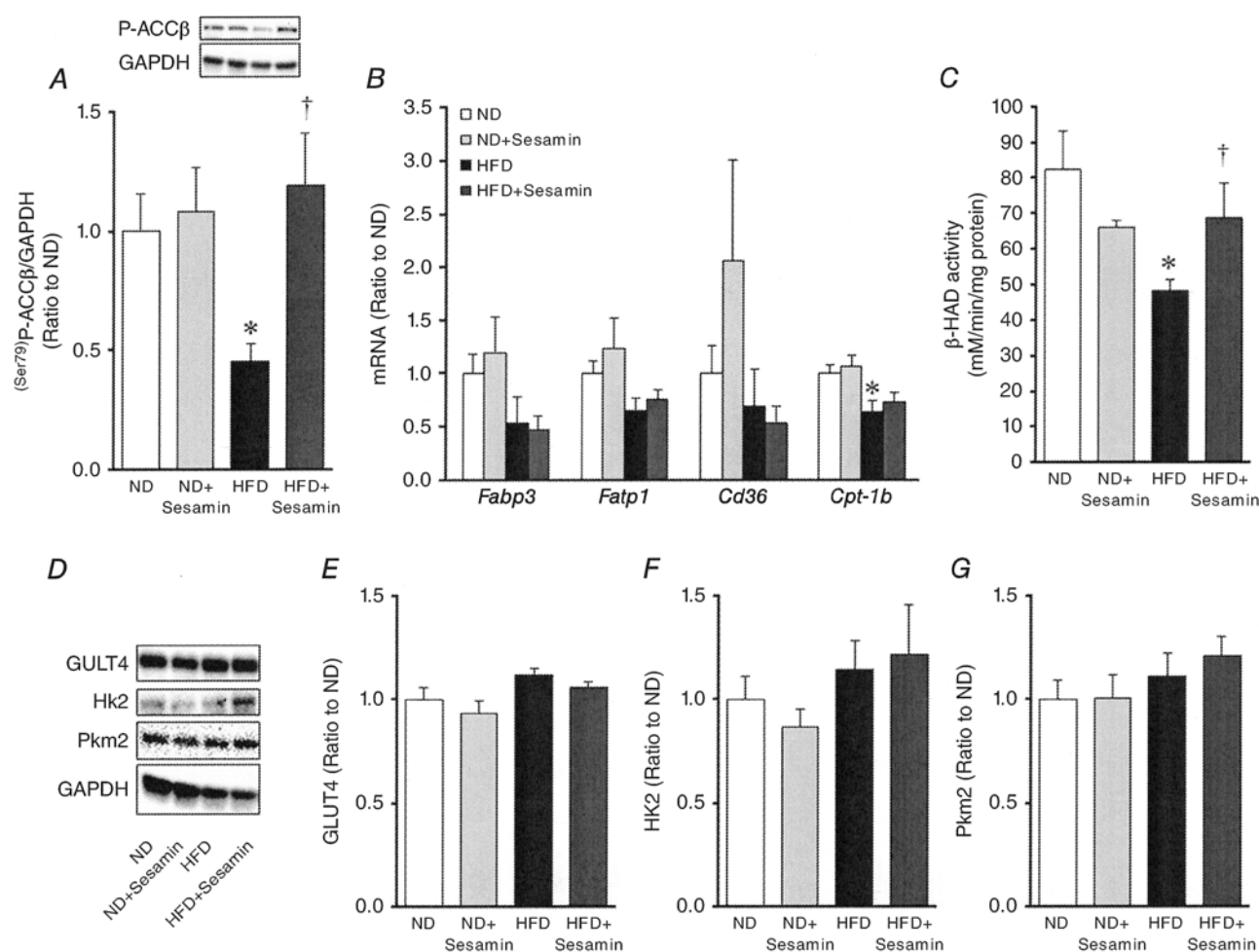
Figure 5. Substrate metabolism related-protein and gene expressions
Summarized data of quantitative analysis of the phosphorylated form (Ser79) of acetyl-CoA carboxylase-β (ACCβ) protein (*A*), gene expressions of fatty acid binding protein 3 (*Fabp3*), fatty acid transport protein 1 (*Fatp1*), *Cd36* (FAT/CD36) and carnitine palmitoyltransferase-1b (*Cpt-1b*) mRNA (*B*) and β-hydroxyacyl-CoA dehydrogenase (β-HAD) activity (*C*) in skeletal muscle obtained from the ND, ND+Sesamin, HFD and HFD+Sesamin mice ($n = 6$–8 for each group). Representative bands (*D*) and quantitative analysis of protein expressions of glucose transporter 4 (GLUT4; *E*), hexokinase 2 (Hk2; *F*) and pyruvate kinase m2 (Pkm2; *G*) in the skeletal muscle obtained from ND, ND+Sesamin, HFD and HFD+Sesamin mice ($n = 6$ for each group). Data are shown as means + SEM. *$P < 0.05$ *versus* ND; †$P < 0.05$ *versus* HFD.

the impairment of mitochondrial function, including activity of the tricarboxylic acid cycle (Fig. 3). In contrast, sesamin did not affect the signalling associated with mitochondrial biogenesis (Fig. 4). These results show that sesamin maintains mitochondrial function without changing the number of mitochondria in the skeletal muscle.

Our previous papers showed that NAD(P)H oxidase-induced $O_2 \cdot^-$ production impaired mitochondrial function in the skeletal muscle of HFD mice (Yokota et al. 2009; Takada et al. 2013; Suga et al. 2014). However, the mechanisms for mitochondrial dysfunction induced by NAD(P)H oxidase-dependent $O_2 \cdot^-$ in skeletal muscle are not fully understood. The decrease of mitochondrial electron transport chain complex I and III activities can potentially be explained by direct oxidative damage to mitochondrial complexes (Doughan et al. 2008; Yokota et al. 2009). Mitochondria can be the primary target for oxidative damage when production of reactive oxygen species exceeds the capacity of the endogenous reactive oxygen species scavenging system. Superoxide anion easily impairs these electron transport chain complexes because they include an iron–sulfur centre. Multiple iron–sulfur centres exist in complexes in complex I and II. In mice lacking superoxide dismutase, destruction of the iron–sulfur centres in the mitochondria has been described (Li et al. 1995; Morten et al. 2006). In addition, oxidative damage to mitochondrial DNA can also result in a decrease of electron transport chain complex activities (Ide et al. 2001b). Furthermore, impaired mitochondrial

DNA may adversely affect mitochondrial biogenesis. In contrast, given that sesamin has an antioxidant effect, we hypothesized that it may have a favourable effect on mitochondrial function in HFD-induced diabetic mice.

Several studies have reported that sesamin increases superoxide dismutase, catalase and glutathione peroxidase activities in the liver or aortic tissue (Roghani et al. 2011; Hong et al. 2013). In the present study, skeletal muscle expressions of antioxidant genes, in particular Sod1 and Catalase mRNA, were significantly decreased in HFD mice, and sesamin did not affect them (Fig. 6C). Therefore, sesamin attenuated oxidative stress without affecting the antioxidant enzymes in the skeletal muscle of HFD mice.

Effects of SC-1 on inhibition of NAD(P)H oxidase activity

Direct inhibition of the activation of NAD(P)H oxidase by sesamin or SC-1 may be associated with the present results as another possible mechanism. It has been reported that oral sesamin (1% w/w) feeding attenuated deoxycorticosterone acetate- and salt-induced increases in NAD(P)H-dependent $O_2 \cdot^-$ production in the rat aorta (Nakano et al. 2008). In a previous study investigating the metabolic pathway of sesamin, it has been shown that the methylenedioxyphenyl moiety in the structure of sesamin is changed into a dihydroxyphenyl (catechol) moiety in
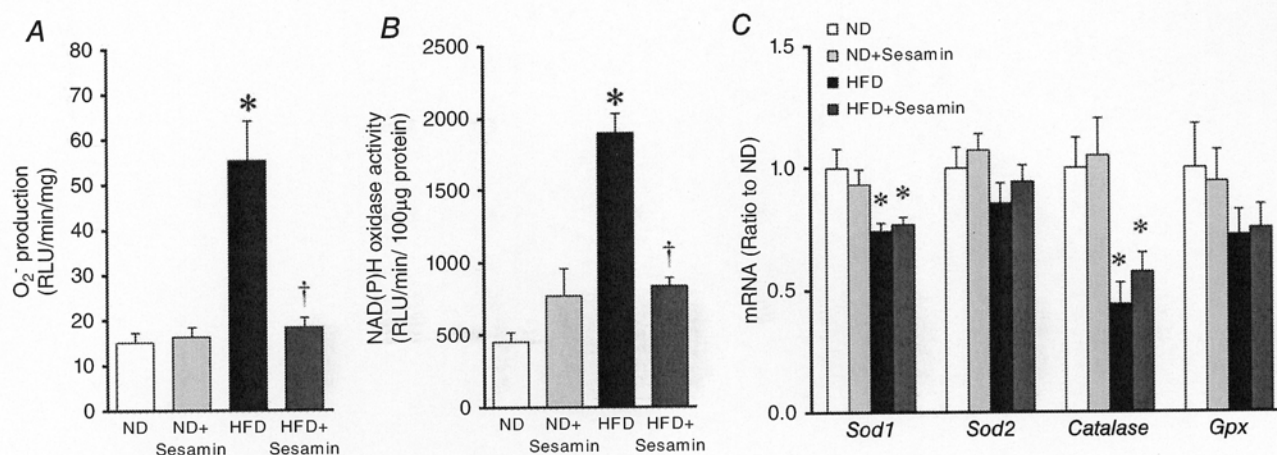
Figure 6. Oxidative stress and antioxidant capacity
Superoxide ($O_2 \cdot^-$; A) and NAD(P)H oxidase activity (B) measured by lucigenin chemiluminescence in the skeletal muscle obtained from ND, ND+Sesamin, HFD and HFD+Sesamin mice ($n = 6$–10 for each group). Quantitative gene expressions of superoxide dismutase 1 (Sod1), Sod2, Catalase and glutathione peroxidase (Gpx) mRNA (C) in skeletal muscle obtained from the ND, ND+Sesamin, HFD and HFD+Sesamin mice ($n = 8$ for each group). Data are shown as means + SEM. Abbreviation: RLU, relative light units. *$P < 0.05$ versus ND; †$P < 0.05$ versus HFD.

the liver (Nakai *et al.* 2003). SC-1, one of the metabolites of sesamin, was shown to inhibit $O_2 \cdot^-$ production in rat aorta (Nakano *et al.* 2006). In the present study, after 8 weeks of feeding sesamin, plasma concentrations of SC-1 were 1.00 ± 0.43 and 1.85 ± 0.43 μmol l^{-1} in the ND+Sesamin and HFD+Sesamin mice, respectively. SC-1 at a dose of 1.0 μmol l^{-1} significantly inhibited NAD(P)H oxidase activity induced by angiotensin II stimulation in C2C12 myotubes (Fig. 7). Therefore, SC-1 may have a direct inhibitory effect on NAD(P)H oxidase activity in HFD-induced diabetic mice.

Clinical implications

The incidence of type 2 diabetes has been increasing markedly, creating both medical and social challenges in industrialized countries. Our present data showed that supplemental treatment with sesamin prevented the increases in insulin and lipid levels and the decline in exercise capacity in type 2 diabetic mice. Given the close association between exercise capacity and prognosis, sesamin could be useful for treatment of type 2 diabetes in humans.

Figure 7. Effect of SC-1 on NAD(P)H oxidase activity
NAD(P)H oxidase activity measured by lucigenin chemiluminescence in the C2C12 myotubes obtained from the control, angiotensin II (Ang II) 1 μM and Ang II+SC-1 (1 μM) groups ($n = 10$–11 for each group). Data are shown as means + SEM. Abbreviation: RLU, relative light units. *$P < 0.05$ *versus* ND; †$P < 0.05$ *versus* HFD.
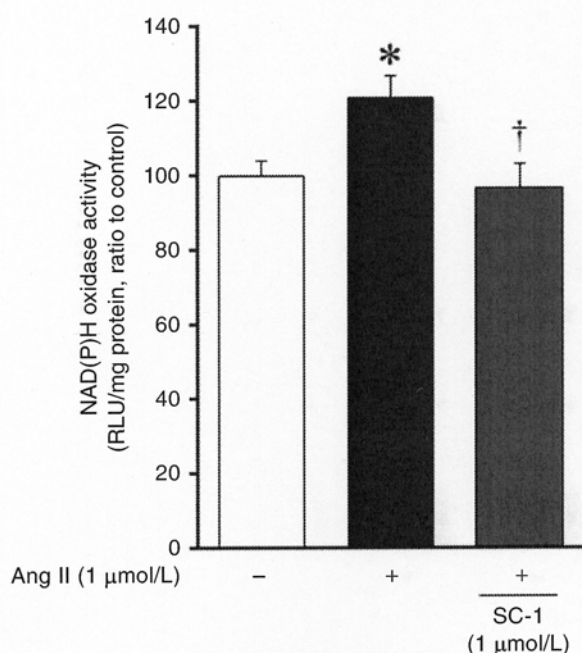
Conclusion

Sesamin prevented the decline in exercise capacity in mice with HFD-induced diabetes by maintenance of mitochondrial function, fat oxidation and attenuation of oxidative stress in the skeletal muscle. Our data suggest that sesamin would contribute novel ameliorating activities to the treatment of diabetes mellitus, especially by improving patients' lowered exercise capacity.

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Additional information

Competing interests

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Author contributions

S.T. designed experiments, performed experiments, analysed data and wrote the manuscript. S.K. conceived and designed experiments and wrote the manuscript. T.F., W.M. and A.F. performed experiments, analysed data and wrote the manuscript. D.T., Y.O. and H.S. performed experiments, analysed data and contributed to discussions. S.M., T.Y., K.O. and H.T. designed experiments, contributed to discussions and reviewed and edited the manuscript. All authors have read and approved the manuscript.

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Clinical Investigations

Serum Brain-Derived Neurotropic Factor Level Predicts Adverse Clinical Outcomes in Patients With Heart Failure

ARATA FUKUSHIMA, MD, PhD,[1] SHINTARO KINUGAWA, MD, PhD,[1] TSUNEAKI HOMMA, MD, PhD,[1]
YOSHIHIRO MASAKI, MD, PhD,[1] TAKAAKI FURIHATA, MD,[1] TAKASHI YOKOTA, MD, PhD,[1]
SHOUJI MATSUSHIMA, MD, PhD,[1] SHINGO TAKADA, PhD,[1] TOMOYASU KADOGUCHI, BS,[1] KOJI OBA, PhD,[2]
KOICHI OKITA, MD, PhD,[3] AND HIROYUKI TSUTSUI, MD, PhD[1]

Sapporo and Ebetsu, Japan

ABSTRACT

Background: Brain-derived neurotropic factor (BDNF) is involved in cardiovascular diseases as well as skeletal muscle energy metabolism and depression. We investigated whether serum BDNF level was associated with prognosis in patients with heart failure (HF).
Methods and Results: We measured the serum BDNF level in 58 patients with HF (59.2 ± 13.7 years old, New York Heart Association functional class I–III) at baseline, and adverse events, including all cardiac deaths and HF rehospitalizations, were recorded during the median follow-up of 20.3 months. In a univariate analysis, serum BDNF levels were significantly associated with peak oxygen capacity (β = 0.547; P = .003), anaerobic threshold (β = 0.929; P = .004), and log minute ventilation/carbon dioxide production slope (β = -10.15; P = .005), but not Patient Health Questionnaire scores (β = -0.099; P = .586). A multivariate analysis demonstrated that serum BDNF level was an independent prognostic factor of adverse events (hazard ratio 0.41, 95% confidence interval 0.20–0.84; P = .003). The receiver operating characteristic curve demonstrated that low levels of BDNF (<17.4 ng/mL) were associated with higher rates of adverse events compared with high levels of BDNF (≥ 17.4 ng/mL; log rank test: $P < .001$).
Conclusions: Decreased serum BDNF levels were significantly associated with adverse outcomes in HF patients, suggesting that these levels can be a useful prognostic biomarker. (*J Cardiac Fail* 2015;21:300–306)
Key Words: Heart failure, brain-derived neurotrophic factor, exercise capacity, prognosis.

Heart failure (HF) remains highly prevalent and is characterized by repeated hospitalizations with a heavy health burden, and it is associated with excess morbidity and

mortality.[1] Lowered exercise capacity is well known to be closely related to the poor prognosis in patients with HF.[2,3] Various skeletal muscle abnormalities, including a transition of myofibers from type I to type II, a reduction in muscular strength, muscle atrophy, and impaired energy metabolism, each play an important role as a determinant factor of lowered exercise capacity in individuals with HF.[4] Cytokines and growth factors were recently reported to be secreted by skeletal muscle and to regulate skeletal muscle mass or function.[5] Such factors may be associated with skeletal muscle abnormalities and exercise capacity in HF patients, and they may be potential biomarkers predicting the severity and prognosis of HF.

Brain-derived neurotropic factor (BDNF) is a member of the neurotropin family, which regulates various neurotropic functions, including neuroregeneration, neuroprotection, and synaptic plasticity.[6] BDNF was discovered in the brain

and has been shown to be linked to the pathophysiology of psychiatric disorders, including major depression and dementia.[7-9] In addition, BDNF expression is reported to be up-regulated in the ischemic heart and to protect the heart against ischemic injury.[10] Indeed, a low plasma BDNF level was shown to predict poor prognosis in patients with angina pectoris.[11]

Interestingly, BDNF can also be produced in skeletal muscle,[12] and exercise training can increase its serum levels.[13] We recently reported that the serum BDNF level was decreased in HF patients compared with normal subjects, and that it was positively correlated with their peak oxygen capacity (peak VO_2).[14] Because peak VO_2 is a strong prognostic marker of HF,[2] these findings raise the possibility that BDNF may be involved in lowered exercise capacity and may help predict the prognosis of HF patients. However, it has not been determined whether the serum BDNF level can provide prognostic information in HF patients. In the present study, we determined whether the serum BDNF level could predict the prognosis, including all cardiac death and HF readmission, during a median follow-up of 20.3 months in HF patients. We also investigated whether BDNF levels were associated with depressive symptoms, because depression is highly prevalent and associated with adverse outcomes in HF patients.[15]

Methods

Patients

The present study enrolled a total of 88 consecutive patients with HF who were admitted to Hokkaido University Hospital from April 2012 to June 2013. Inclusion criteria were: (1) age 20–75 years; (2) HF symptoms as defined by the Framingham criteria; (3) New York Heart Association (NYHA) functional class I–III on optimized pharmacotherapy; (4) absence of the following HF etiologies: severe valve disease, congenital disease, pericardial disease, and pulmonary artery embolism; and (5) absence of the following comorbidities: active infectious disease, cancer, and renal failure requiring dialysis. HF was diagnosed on the basis of standard criteria and the presence of systolic or diastolic functional impairment by means of echocardiography according to the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines by ≥2 cardiologists.[16]

Patients were excluded when they had any of the following within 2 weeks before the study: changes in NYHA functional class, changes in HF medications, or administration of any intravenous medication for HF. In addition, patients were excluded if they had signs of or a history of a psychiatric disorder, such as major depressive disorder, schizophrenic disorder, and organic brain disorders; were taking antidepressant medications; had a stroke within the past 3 months; or were unable to perform a maximal exercise test because of a neurologic deficit.

According to these inclusion and exclusion criteria, we excluded patients with NYHA functional class IV (n = 10), severe valvular heart disease (n = 6), major depression (n = 4), active infection (n = 3), stroke with significant neurologic deficit (n = 1), acute pulmonary embolism (n = 1), cancer (n = 1), and renal failure (n = 4). A final total of 58 patients were enrolled in the present study. The protocol was approved by the Medical Ethics Committee of

Hokkaido University Hospital, and written informed consents were obtained from all participating subjects.

Baseline Patient Characteristics

Each patient's body weight and height were measured, and his or her body mass index (BMI; body weight/height^2, kg/m^2) was calculated because circulating BDNF in patients with newly diagnosed type 2 diabetes showed a positive correlation with BMI.[17] Ischemic heart disease was diagnosed based on coronary artery disease according to angiography, hypertensive heart disease based on left ventricular hypertrophy with hypertension, valvular heart disease based on moderate valve disease according to echocardiography, and dilated cardiomyopathy based on both left ventricular dilation and dysfunction without any cause.

Hypertension, diabetes mellitus (DM), history of stroke, chronic obstructive pulmonary disease (COPD), sleep apnea syndrome, and paroxysmal or chronic atrial fibrillation were determined based on the patient's medical records. Depressive symptoms were assessed with the use of the Patient Health Questionnaire (PHQ-9). The PHQ-9 is a 9-item self-report measure of depressive symptoms using 0–3 scales, established for the screening of depression in various populations.[18]

The left ventricular end-diastolic dimension (LVEDD) was measured in the parasternal long axis view by means of transthoracic echocardiography. The LV ejection fraction (LVEF) was calculated from the apical 4- and 2-chamber views according to the biplane Simpson method.[19] Cardiopulmonary exercise testing was performed with the use of an upright electromechanical bicycle ergometer (Aerobike 75XLII; Combi Wellness, Tokyo, Japan) with a ramp protocol as previously described.[20] Briefly, after 3 minutes of unloaded cycling, the exercise load was increased in 10–15 W/min increments to symptom-limited maximal work. Minute ventilation (VE), oxygen capacity (VO_2), and carbon dioxide production (VCO_2) were measured continuously throughout the exercise period with the use of a 280E Aeromonitor (Aeromonitor AE-300S; Minato Medical Science, Osaka, Japan). Peak VO_2 was defined as the maximal VO_2 attained during exercise. The anaerobic threshold (AT) was determined by the V-slope method as described.[21] VE and VCO_2 responses throughout the exercise were used to calculate the VE/VCO_2 slope by means of a least squares linear regression ($y = mx + b$; m = slope).[22]

Serum BDNF Levels and Biochemistry

Peripheral venous blood samples were collected in serum tubes from all subjects between 6:00 and 9:00 am before cardiopulmonary exercise testing. All samples were allowed to clot before being centrifuged at 1,000g for 15 minutes and were stored at −80°C until analysis. Serum BDNF levels were determined by a commercially available enzyme immunoassay kit (R&D Systems, Minneapolis, Minnesota) according to the manufacturer's protocol as previously described[23]; its detection limit was 20 pg/mL. To ensure accurate measurements, all of the samples were analyzed in duplicate by investigators blinded to clinical information. The inter- and intra-assay coefficients of variation (CVs) were 6.1% and 2.3%, respectively.

Plasma B-type natriuretic peptide (BNP) was measured by means of a chemiluminescence immunoassay kit (Architect BNP-JP; normal reference values: 18.4 pg/mL) in an automated analyzer (Architect; Abbott Japan, Tokyo). The Architect BNP assay was validated as previously described.[24] The limit of detection of the assay was 5.8 pg/mL. The within-run and total CVs

were 2.4 and 3.4%, respectively. Linearity was acceptable with recoveries within 5.0% of the target values over a concentration range of 429—2,894 pg/mL. Our comparison of the Architect BNP-JP assay results with those of another chemiluminescent enzyme immunoassay (MI02 Shionogi BNP kit; Shionogi, Japan) by the Passing-Bablok method resulted in slopes ranging from 1.00 to 1.03 and correlation coefficients of 0.98. The estimated glomerular filtration rate (eGFR) was calculated from the serum creatinine values and age with the use of the Japanese equation[25]:

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

Procedures and Clinical Follow-Up

At enrollment, the patients underwent a complete clinical and physical examination, including the collection of blood samples for the measurement of eGFR, plasma BNP, and serum BDNF levels. The examination included chest X-ray, transthoracic echocardiography, PHQ-9 score, NYHA functional class determination, and cardiopulmonary exercise test. The entire protocol was performed at baseline (at the time of BDNF measurement). All patients were then followed prospectively with regular outpatient visits up to March 2014. The median follow-up period was 20.3 months (interquartile range [IQR] 11.5—22.8 months) after the measurement of serum BDNF.

Clinical information about major adverse events, including all cardiac deaths and rehospitalizations due to worsening HF, during the follow-up period was provided by the patients' cardiologists without knowledge of the serum BDNF levels. Cardiac death was defined as death from worsening HF or sudden cardiac death, and HF rehospitalization was defined as an unplanned hospital admission requiring intravenous diuretics, vasodilators, or inotropic agent infusion. Cardiac death during the rehospitalization was counted as a single event. Additionally, 2nd or further rehospitalizations were not counted as additional events but rather as a single event at the time of the first rehospitalization.

Statistical Analysis

Continuous variables are expressed as mean ± SD for normally distributed variables, and median and IQR for nonnormally distributed variables. Categoric variables are expressed as number and percentage. Serum BDNF levels, peak VO₂, and AT were normally distributed as proven by the Shapiro-Wilk test. In contrast, age, BMI, LVEDD, LVEF, VE/VCO₂ slope, eGFR, plasma BNP, and PHQ-9 score were not normally distributed. The VE/VCO₂ slope and plasma BNP values were transformed to log VE/VCO₂ slope and log BNP, respectively. There were 3 missing data in peak VO₂, AT, and log VE/VCO₂ slope, respectively, because of 1 patient's inability to participate in the exercise test.

We used a univariate linear model to determine the correlations between serum BDNF levels and other variables. Serum BDNF and plasma BNP levels were compared for their ability to predict adverse outcomes of HF by means of a receiver operating characteristic (ROC) curve analysis. The optimal ROC curve cutoff value for the prediction of adverse outcomes was chosen as the value maximizing sensitivity plus specificity. To determine whether serum BDNF levels were incremental prognostic markers in addition to plasma BNP, we compared the areas under the ROC curve (AUCs) of these variables with the use of the DeLong algorithm.[26] The DeLong method is a different approach from that of Hanley[27] to estimate the AUC (c-index) regarding the estimation of variance. Because the variance was derived from the assumption of

2 underlying negative exponential distributions in the Hanley approach, it is known that it can underestimate the variance when the AUC is close to 0.5 and can overestimate it when the AUC is close to 1.0.[27] We therefore used the DeLong approach in the present study.

We calculated Kaplan-Meier survival plots from baseline to the time of all cardiac death or rehospitalization due to worsening HF, and we used the log-rank test to compare the results. Univariate and multivariate analyses with the Cox proportional hazard regression model were used to determine significant predictors of all cardiac death and HF rehospitalization. Variables that were significant with a P value of $<.01$ in the univariate analyses, including NYHA functional class, log BNP, and BDNF values, were entered into the multivariate Cox proportional hazard analysis, which was adjusted for age and sex. Peak VO₂, AT, and log VE/VCO₂ slope data were excluded from the multivariate analysis to avoid problems related to multicollinearity, because these variables were significantly associated with serum BDNF levels (Table 1), as consistent with our previous study.[14] We also selected variables in the multivariate Cox model based on the stepwise forward selection method owing to the small number of events in this study. All analyses were performed with the use of JMP 10.0.2 (SAS Institute, Cary, North Carolina). The differences were considered significant when P values were $<.05$.

Results

Patients' Characteristics

The clinical characteristics of the study patients are summarized in Table 2. The etiologies of HF were dilated cardiomyopathy in 18 patients (31%), ischemic heart disease in 17 (29%), hypertensive heart disease in 10 (17%), valvular heart disease in 5 (8%), and others in the remaining 8 (13%). Five patients were in NYHA functional class I, 39 in class II, and 14 in class III. Hypertension was identified in 18 patients (31%), DM in 19 (32%), history of stroke in 7 (12%), COPD in 8 (13%), sleep apnea syndrome in 8

Table 1. Univariate Linear Model of Serum BDNF in Patients With HF

Variable	HF Patients (n = 58)	
	β	P Value
Age, y	0.043	.425
Sex (male = 0)	−0.714	.412
Body mass index, kg/m²	0.208	.172
LVEDD, mm	−0.002	.967
LVEF, %	0.007	.902
Peak VO₂, mL/kg/min	0.547	.003
AT, mL/kg/min	0.929	.004
Log VE/VCO₂ slope	−10.15	.005
eGFR (mL min⁻¹ 1.73 m⁻²)	0.013	.523
Log BNP, pg/mL	−1.215	.047
PHQ-9 score	−0.099	.586
Change in peak VO₂ from baseline	0.200	.233
Exercise session per week	−0.548	.047

BDNF, brain-derived neurotropic factor; HF, heart failure; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; VO₂, oxygen uptake; AT, anaerobic threshold; VE, minute ventilation; VCO₂, carbon dioxide production; eGFR, estimated glomerular filtration rate; BNP, B-type natriuretic peptide; PHQ-9, Patient Health Questionnaire.

Table 2. Patients' Characteristics

	All Patients (n = 58)
Demographic factors	
Age, yrs (mean ± SD)	59.2 ± 13.7
Male, n (%)	44 (75)
Body mass index, kg/m^2	23.4 ± 4.9
Causes of heart failure, n (%)	
Dilated cardiomyopathy	18 (31)
Ischemic heart disease	17 (29)
Hypertensive heart disease	10 (17)
Valvular heart disease	5 (8)
NYHA functional class (I/II/III)	5/39/14
Medical history, n (%)	
Hypertension	18 (31)
Diabetes mellitus	19 (32)
Stroke	7 (12)
Paroxysmal or chronic atrial fibrillation	27 (46)
COPD	8 (13)
Sleep apnea syndrome	8 (13)
PHQ-9 score	3.9 ± 4.2
Echocardiographic parameters	
LVEDD, mm	62.9 ± 11.5
LVEF, %	34.7 ± 12.7
Cardiopulmonary exercise test	
Peak VO$_2$, mL kg^{-1} min^{-1}	14.0 ± 3.9
AT, mL kg^{-1} min^{-1}	9.1 ± 2.2
VE/VCO$_2$ slope	38.5 ± 8.2
Peak RER	1.23 ± 0.1
Medications, n (%)	
ACE-I or ARB	52 (89)
β-Blockers	56 (96)
Spironolactone	35 (60)
Laboratory tests	
eGFR, mL min^{-1} 1.73 m^{-2}	55.5 ± 20.3
Plasma BNP, pg/mL	410 ± 646
Serum BDNF, ng/mL	19.0 ± 5.6

NYHA, New York Heart Association; COPD, chronic obstructive pulmonary disease; RER, respiratory exchange ratio; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II type I receptor blocker; other abbreviations as in Table 1.

(13%), and paroxysmal or chronic atrial fibrillation in 27 (46%). According to the PHQ-9 scores, 4 patients (6%) were identified to be in a clinically significant depressive state (PHQ-9 ≥10). The mean LVEF was 34.7 ± 12.7%, and the mean peak VO$_2$ was 14.0 ± 3.9 mL kg^{-1} min^{-1}. Angiotensin-converting enzyme inhibitors (ACE-Is) or angiotensin II type I receptor antagonists (ARBs) were used in 89% of the patients, β-blockers in 96%, and spironolactone in 60%. The mean serum BDNF level in the HF patients was 19.0 ± 5.6 ng/mL.

Serum BDNF Levels Correlate With Exercise Capacity

In the univariate linear model, the patients' serum BDNF levels were significantly associated with their peak VO$_2$ (β = 0.547; P = .003), AT (β = 0.929; P = .004), and log VE/VCO$_2$ slope (β = −10.15; P = .005), but not age, sex, BMI, LVEDD, LVEF, eGFR, and plasma BNP levels (Table 1). There was no significant association between serum BDNF levels and depressive status according to PHQ-9 scores (β = −0.099; P = .586). Moreover, there was no significant association between serum BDNF levels and the change in peak VO$_2$ from baseline until the end of their follow-ups (β = 0.410; P = .233) among the 19

patients who performed the exercise pulmonary test repeatedly after their enrollment.

To assess the role of serum BDNF in adherence to exercise training, we obtained data regarding each patient's participation in cardiac rehabilitation from their exercise diaries. One session of cardiac rehabilitation included 30 minutes of aerobic exercise with a cycle ergometer. Patients who participated in >1 session per week throughout the follow-up period were defined as adherent in cardiac rehabilitation. As a result, 25 patients (43%) were considered to be adherent. There was no significant difference in serum BDNF levels between the adherent and nonadherent patients (19.0 ± 5.5 ng/mL vs 19.1 ± 5.7 ng/mL; P = .945). In contrast, the patients' serum BDNF levels were negatively associated with the number of sessions completed weekly (β = −0.548; P = .047).

Serum BDNF Levels Predict Adverse Outcomes

During the median follow-up of 20.3 months (IQR 11.5–22.8 months), there were 19 (32%) adverse events, including 8 cardiac deaths and 11 rehospitalizations due to worsening HF. Of these, the death of 5 patients was due to HF and that of 3 patients sudden death. In addition, 3 patients (5%) had a new onset of stroke. None of the studied patients were lost within the follow-up period. The variables to predict adverse outcomes were identified by the univariate (Table 3) and multivariate (Table 4) Cox proportional hazard analyses.

In the univariate analysis, serum BDNF was significantly associated with all cardiac death and HF rehospitalization (per SD increase: hazard ratio [HR] 0.47, 95% confidence interval [CI] 0.29–0.75; P = .001). NYHA functional class, LVEDD, LVEF, peak VO$_2$, AT, log VE/VCO$_2$ slope, and log BNP were also related to adverse outcomes. The

Table 3. Univariate Analysis of Predictors of All Cardiac Death and HF Rehospitalization

Variable	HR	95% CI	P Value
Age*	1.00	0.65–1.53	.982
Sex (male vs female)	3.35	0.96–21.1	.057
BMI*	0.91	0.58–1.42	.685
NYHA functional class (III vs I/II)	4.36	1.68–13.4	<.001
COPD	1.41	0.32–4.26	.594
Sleep apnea syndrome	0.68	0.10–2.36	.590
PHQ-9	1.04	0.94–1.13	.324
LVEDD*	1.56	1.00–2.43	.046
LVEF*	0.53	0.29–0.95	.021
Peak VO$_2$, mL kg^{-1} min^{-1}*	0.32	0.16–0.64	<.001
AT, mL kg^{-1} min^{-1}*	0.27	0.13–0.59	<.001
Log VE/VCO$_2$ slope*	71.6	5.82–1017	<.001
ACE-I or ARB	0.11	0.01–2.22	.121
β-Blockers	0.22	0.06–1.43	.100
Spironolactone	1.49	0.59–4.21	.402
Estimated GFR*	0.76	0.41–1.43	.357
Log BNP*	2.63	1.64–4.21	<.001
BDNF*	0.47	0.29–0.75	.001

HR, hazard ratio; CI, confidence interval; BMI, body mass index; other abbreviations as in Tables 1 and 2.
*Per 1 SD increase.

Table 4. Multivariate Analysis of Predictors of All Cardiac Death and HF Rehospitalization

Variable	HR	95% CI	P Value
Age*	1.35	0.83–2.20	.127
Sex (male vs female)	3.11	0.82–20.2	.098
NYHA functional class (III vs I/II)	2.02	0.70–6.03	.186
Log BNP*	2.54	1.15–5.60	<.001
BDNF*	0.41	0.20–0.84	.003

Abbreviations as in Tables 1–3.
*Per 1 SD increase.

multivariate analysis revealed that among these variables, the serum BDNF level and log BNP were independent predictors of adverse outcomes. The results based on the stepwise forward selection method showed that NYHA functional class, log BNP, and BDNF were significant prognostic factors, and these results were consistent with the 1st model which included all potential candidates as covariates (Table 4).

Comparison of Serum BDNF and Plasma BNP as Prognostic Indicators

The ROC curves of serum BDNF values and plasma BNP concentrations for the prediction of all cardiac death or HF rehospitalization are shown in Figure 1. The AUC of the serum BDNF levels for the prediction of adverse events was 0.798 (95% CI 0.641–0.897; $P < .001$), whereas that of the plasma BNP concentration was 0.827 (95% CI 0.694–0.910; $P < .001$). There was no significant difference in AUC between serum BDNF and plasma BNP (difference of AUC −0.03, 95% CI −0.192 to 0.133; $P = .721$). A serum BDNF level of 17.4 ng/mL and a BNP level of 246.7 pg/mL were defined as the optimal cutoff points for discriminating adverse outcomes. The best performing value of serum BDNF (17.4 ng/mL) to predict adverse events was associated with 75% sensitivity, 79% specificity, 60% positive predictive value, and 85%
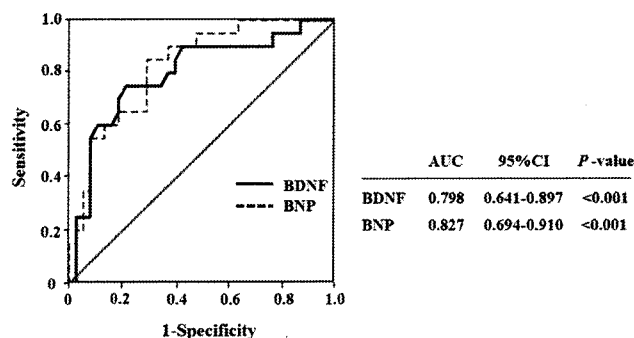
Fig. 1. Predictive ability of serum brain-derived neurotropic factor (BDNF) and plasma B-type natriuretic peptide (BNP) levels for adverse outcomes. The receiver operating characteristic curve was created to predict all cardiac death and rehospitalization due to worsening of HF based on serum BDNF and plasma BNP levels. AUC, area under the receiver operating characteristic curve.

negative predictive value. This tended to be a slightly weaker performance than that observed for plasma BNP, with 85% sensitivity, 71% specificity, 60% positive predictive value, and 90% negative predictive value. Adverse events occurred significantly more frequently in the low (<17.4 ng/mL) BDNF group compared with the high (≥17.4 ng/mL) BDNF group (66% vs 16%; $P < .001$; Fig. 2).

Discussion

The major finding of the present study was that lower serum BDNF levels were associated with a higher incidence of adverse outcomes including all cardiac death and HF rehospitalization among HF patients. Importantly, this is the 1st report to demonstrate that the serum BDNF level was an independent prognostic factor for adverse events by means of a multivariate Cox proportional hazard analysis.

Role of BDNF in Lowered Exercise Capacity in HF Patients

Our results showed a significant positive association between serum BDNF level and exercise capacity measured as the peak VO_2, AT, and VE/VCO_2 slope. These findings were consistent with those of our previous study.[14] Qualitative abnormalities in skeletal muscles' energy metabolism are well known to determine the exercise capacity in HF patients.[2,4] BDNF was shown to be produced in skeletal muscle, and it is increased by muscle contractions to enhance fat oxidation in an 5′-adenosine monophosphate—activated protein kinase—dependent fashion, which can regulate glucose and fatty acid metabolism.[12,28] In addition, we recently demonstrated that intramyocellular lipid is
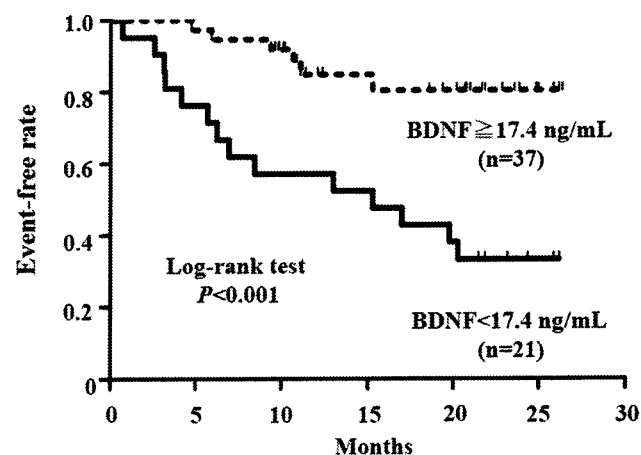
Fig. 2. Kaplan-Meier event-free curves for serum brain-derived neurotropic factor (BDNF) levels. The event-free rate from cardiac death and rehospitalization caused by the worsening of heart failure was compared between patients with high and low serum BDNF levels. The cutoff level of the serum BDNF concentration (17.4 ng/mL) was determined by the receiver operating characteristic curve. The significance of separation between the 2 groups was examined with the use of a log rank test at 20.4 months.

increased in the skeletal muscle of patients with dilated cardiomyopathy with lowered exercise capacity.[29]

These findings suggest that serum BDNF can be a useful marker for exercise capacity in HF patients, reflecting impaired fatty acid metabolism in the skeletal muscle. In the present study, the change in peak VO_2 from baseline did not correlate with the serum BDNF level in the exercise capacity—limited patients, but further study is needed to determine whether serum BDNF predicts the future exercise capacity in HF patients. In contrast, there was a negative association between serum BDNF levels and the number of completed exercise sessions, suggesting that patients with low BDNF levels may need more intensive exercise training than those with high BDNF levels.

Predictive Value of Serum BDNF for Prognosis in HF Patients

Our results demonstrated the role of serum BDNF as a prognostic marker in HF. Jiang et al reported that multiple cardiovascular risk factors were associated with plasma BDNF level in patients with angina pectoris, and that low plasma BDNF was related to future coronary events and mortality in those patients.[11] To our knowledge, the present study is the first to demonstrate the predictive role of BDNF in HF. Because we found that serum BDNF levels were associated with cardiopulmonary exercise variables, these prognostic values of BDNF may be partly explained by its being a surrogate marker for exercise capacity. Novel biomarkers reflective of ventricular remodeling and fibrosis have been reported as having incremental prognostic value[30]; however, potential biomarkers that reflect skeletal muscle abnormalities and exercise capacity have not, to our knowledge, been investigated.

Even after adjustments for powerful prognostic variables, including the NYHA functional class and plasma BNP, we observed that the serum BDNF level was an independent predictor of adverse cardiac events. These findings suggest that measurements of serum BDNF could provide further information about the prognosis in HF. In addition, the optimal cutoff value of BDNF determined by ROC analysis had a prognostic ability similar to that of BNP, suggesting that serum BDNF could be a novel marker in addition to plasma BNP, which is the first-line biomarker for risk stratification in HF.[16]

Role of BDNF in Depressive Symptoms and Adherence to Exercise Training in HF

Circulating and hippocampal BDNF levels in patients with major depression have been reported to be lower and associated with the severity of this disease.[31,32] In the present study, however, there was no association between serum BDNF and depressive symptoms according to PHQ-9 scores. This discrepant result may be due to the relatively small number of patients who were in a clinically significant depressive state (6%). On the other hand, higher serum BDNF levels were reported to protect against the future occurrence of dementia in the offspring of

participants in the Framingham study.[9] Cognitive impairment is prevalent among HF patients and is associated with increased mortality risk,[33] and it was also reported to affect poor treatment adherence in HF.[34] In the present study, we investigated the role of BDNF in adherence to exercise training, and we found that there was no difference in serum BDNF levels between the adherent and nonadherent patients, suggesting that serum BDNF levels may have a minor role in the adherence to exercise training in HF.

Study Limitations

There are several potential limitations which should be acknowledged in the present study. First, this study had the possibility of creating an overfitted model, with only 19 events, and we could not create a prognostic model with proper validation and calibration. However, the present study was categorized as prognostic factor research, not prognostic model research, as shown by a recent review.[35] Therefore, we used a Cox model for adjusting the confounding bias to evaluate the size of association between the outcome and BDNF, not for prediction. Furthermore, the relatively small number of studied subjects limits the statistical power for detecting the prognostic value of serum BDNF levels. Therefore, a study on a larger scale is warranted to confirm the relationship between worse prognosis and decreased serum BDNF levels in HF patients. Second, an accurate investigation for a new biomarker has never been performed with the use of specific statistical tests including discrimination, calibration, and reclassification analyses as recommended.[36,37] Moreover, there was no control group in the present study. Third, there were no data about cognitive function, daily physical activity, or medication adherence. Finally, the sources of serum BDNF are skeletal muscle, heart, and brain, which could not be identified here. Despite these limitations, our observation that serum BDNF can predict the prognosis in HF patients is unique and had not been reported previously.

Conclusions

Decreased serum BDNF levels were related to all cardiac death and HF readmission in HF patients. Serum BDNF may be a promising biomarker to predict the adverse outcomes in HF. Further studies are needed to demonstrate the prognostic incremental value of BDNF compared with the standard cardiovascular biomarkers and to determine the cost-effectiveness of its measurement in patients with HF.

Disclosures

None.

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● 基　礎 ●

寒冷刺激に対する生理的応答

森田憲輝[*1]，沖田孝一[*2]

はじめに

　人は暑さや寒さなどさまざまな外的環境に身体を適応させ，生命活動の維持そして日々の生活を営んでいる．人は知恵を活用することでさまざまな手段で寒さへの対処を行っているが，寒さへの生理的な反応や適応も進化，そして生命を繋いでくるなかで重要であったことはいうまでもない．では，「寒冷」という刺激に対して具体的にどのような生理的反応が生じているのか．本稿では，寒冷刺激への急性応答，および慢性応答，そしてそれらを巧妙に制御する生理学的メカニズムについて概説する．

寒冷刺激に対する急性応答

　人が寒冷刺激（低温，浸水，強風）に曝露された場合には，その個体は身体機能を正常に維持するために諸々の生理的反応を示す．体温（特に深部体温）は，「熱産生」と「熱放散」という2つの相反する生理機能のバランスによって制御され，常に約37℃で維持されている．暑熱環境においては，熱放散量が低下することで「熱産生＞熱放散」となりやすく，結果として深部体温の過度な上昇，つまり熱中症の危険性が生じる．一方，寒冷環境では，多くの熱が外部に奪われるため「熱放散＞熱産生」という状態になり，特に厳しい寒冷環境では，深部体温低下の危険性が生じる．そこで，このような異常状態にならないようホメオスタシスとして生じる反応に「① 熱放散の抑制」，「② 熱産生の亢進」があり，体温調節機能も適宜

応答することで，体内の熱が過剰に外部環境に奪われないようにしている．これらが具体的にどのような反応かを以下に解説する．

1. 熱放散の抑制

　体内の熱は皮膚にある末梢血管から外部に放出されており，寒冷刺激を受けた場合，速やかに皮膚末梢血管の収縮が生じる．これによって皮膚血流が低下し，身体内部（深部）と体表面間の熱の対流性移動が減少し，結果として体表面からの熱放熱量が減少する[1]．この反応により皮膚温および皮下組織温度が低下するが，身体内部の温度を一定に保つことがより重要なため，不可避な応答といえる．

　この熱放散抑制反応の調節機序を図1に示す[2,3]．この反応は主に2つの機序によって制御されている．1つは局所因子である血管内皮一酸化窒素（NO）系の産生阻害による血管拡張反応の抑制であり，もう1つは中枢性因子でもあるノルエピネフリンによる血管収縮反応である．

　血管内皮NO系は血管拡張反応に作用しており，皮膚組織の寒冷刺激によってNOの産生機序が阻害され，局所NO濃度の低下が起き，血管拡張反応の低下，つまり血管収縮反応が促進される．また，ノルエピネフリンは寒冷刺激により，全身性にも局所的にも分泌が促進されるため，熱放散部位である皮膚表面において血管収縮反応が起こる．

2. 熱産生の亢進

　体熱産生の亢進には，2つの要素がある．1つは不随意的筋活動，つまりふるえ（shivering）によるもので，もう1つは非ふるえ熱産生（non-shivering thermogenesis）である．この2つの機序によって必要な熱産生に見合った，つまり寒冷刺激の程度に見合った代謝量の上昇が生じる（図2）[4]．

*1　北海道教育大学岩見沢校スポーツ文化専攻
*2　北翔大学生涯スポーツ学部スポーツ教育学科

図1 ◦ 急性寒冷刺激による皮膚血流の
低下メカニズム
NEPI：ノルエピネフリン，NO：一酸化窒
素
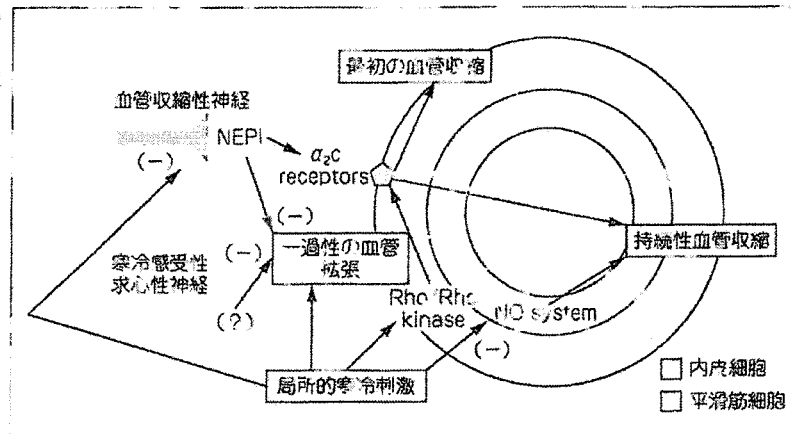
図2の左側は寒冷刺激によって生じる熱産生のた
めの代謝応答を示す．至適温度域（thermoneutral
zone）の範囲内では代謝量の増減がみられないも
のの，至適温度域の下限値（下臨界温）を下回ると
外部環境に奪われた熱量分に応じた代謝量の上昇
が生じる．

図3は寒冷刺激による代謝および熱産生応答の
急性期から慢性期への移行を示している[5]．図3
より急性期での熱産生亢進の大部分はふるえによ
る筋活動の増加が大きく関与していることがわか
る．その後，寒冷刺激が持続することで，徐々に
ふるえが減少していく．このふるえの減少は，も
う一方の熱産生機序である非ふるえ熱産生の亢進
によって必要な熱産生が賄われることで減少して
いくとされている．したがって，非ふるえ熱産生
は急性期の熱産生上昇分としての貢献は少ないと
理解されている．しかし，寒冷環境に適応した後
には，一過性の寒冷刺激に対してもふるえによる
熱産生反応が減少し，非ふるえ熱産生反応によっ
て大部分の熱産生が賄われるようになる．

ふるえ熱産生の制御メカニズムも徐々に明らか
になってきている[6,7]．そのメカニズムとして，
まず皮膚の冷覚受容器が寒冷刺激を感知し，その
刺激が脊髄を経て視床下部内に伝わる．そのシグ
ナルが視床下部で各種の温感受性ニューロン
（warm-sensitive neuron）の抑制に働き，骨格筋に
連絡している交感神経系を介してα運動神経
ニューロンを活性化させ不随意的な活動，つまり
ふるえを生じ，体熱産生を促進するとされてい
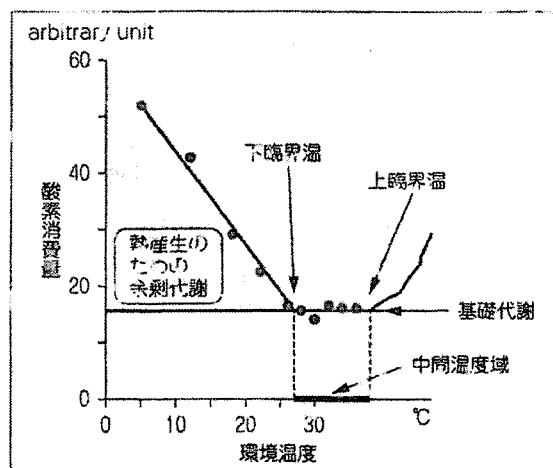
図2 ◦ 外部環境による代謝応答（酸素消費）の違い
マウスを用いた実験．
（文献4より引用）

る[7]．さらに，このふるえをもたらす神経経路は
非ふるえ熱産生経路とも近接・並行して存在し，
多量な熱産生が必要な条件下においては随意運動
を阻害して，非随意的活動であるふるえによって
熱産生を行うとの知見が得られている[6]．

ふるえによる代謝上昇量の最大量は，Eyolfson
らによると最大酸素摂取量の40％程度もしくは
安静時代謝の5倍程度にも相当すると報告されて
おり[8]，寒冷刺激によるふるえは快適な温度環境
での低強度運動を凌ぐほどのエネルギー代謝上昇
効果を有していることになる．また，ラットを用
いた研究では，この「ふるえ熱産生」のために利用
しているエネルギー基質は，蛋白質約15％，糖
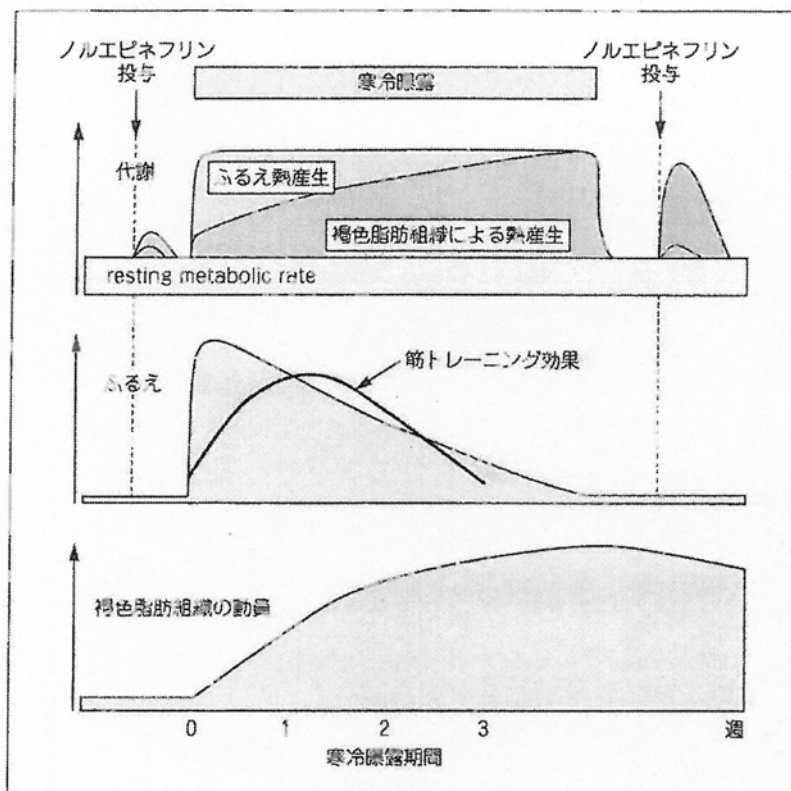
図3 ● 急性および慢性寒冷刺激に対する代謝応答および体熱産生の変化
マウス，ラットを用いた実験．
（文献5より引用改変）

質約35％，そして脂質が約50％であったとの報告がある[9,10]．

一方，非ふるえ熱産生の主要な機序として，実験動物においては褐色脂肪細胞による熱産生亢進が報告されていたが，人では褐色脂肪細胞は関与しないと考えられてきた．しかし，寒冷刺激で健常人の褐色脂肪細胞が活性化することが2009年に報告された（本稿では褐色脂肪"様"細胞の「ベージュ」もしくは「ブライト」脂肪細胞も褐色脂肪細胞と表記する）[11]．そのため，現在では人でも褐色脂肪細胞が非ふるえ熱産生の重要な因子と考えられている．しかしながら，必ずしもすべての人で褐色脂肪細胞の存在が認められているわけでない[11]．Saitoらによると褐色脂肪細胞は寒冷刺激応答性であり，寒冷刺激を行った場合には20歳代では半数以上で検出でき，50歳代以降では10％未満の対象者でしか検出できなかったことを報告している[12,13]．

褐色脂肪細胞による熱産生における重要な因子として，ノルエピネフリンおよび熱産生蛋白質と

しての脱共役蛋白質（uncoupling protein 1：UCP1）があげられる．上述したようにノルエピネフリンは皮膚血管収縮，つまり熱放散抑制に作用しつつ，非ふるえ熱産生でも重要な役割を果たしている．寒冷刺激によってノルエピネフリンが中枢性および局所性に分泌され褐色脂肪細胞の細胞膜上にあるβ_3受容体に結合することでサイクリックAMP（3'-5'-cyclic adenosine monophosphate：cAMP）およびprotein kinase A（PKA）の活性化，そして脂質分解の促進，ミトコンドリア内での酸化的リン酸化を経て産生されたエネルギーを「熱」に変換する[5,14]．この熱を血液が受け取ることで，全身に熱が供給される．その他，褐色脂肪細胞および交感神経系以外の非ふるえ熱産生の寄与因子として甲状腺ホルモン，副腎髄質ホルモン，副腎皮質ホルモンがある．

寒冷刺激への慢性期応答

寒冷刺激への慢性適応，つまり寒冷馴化には3
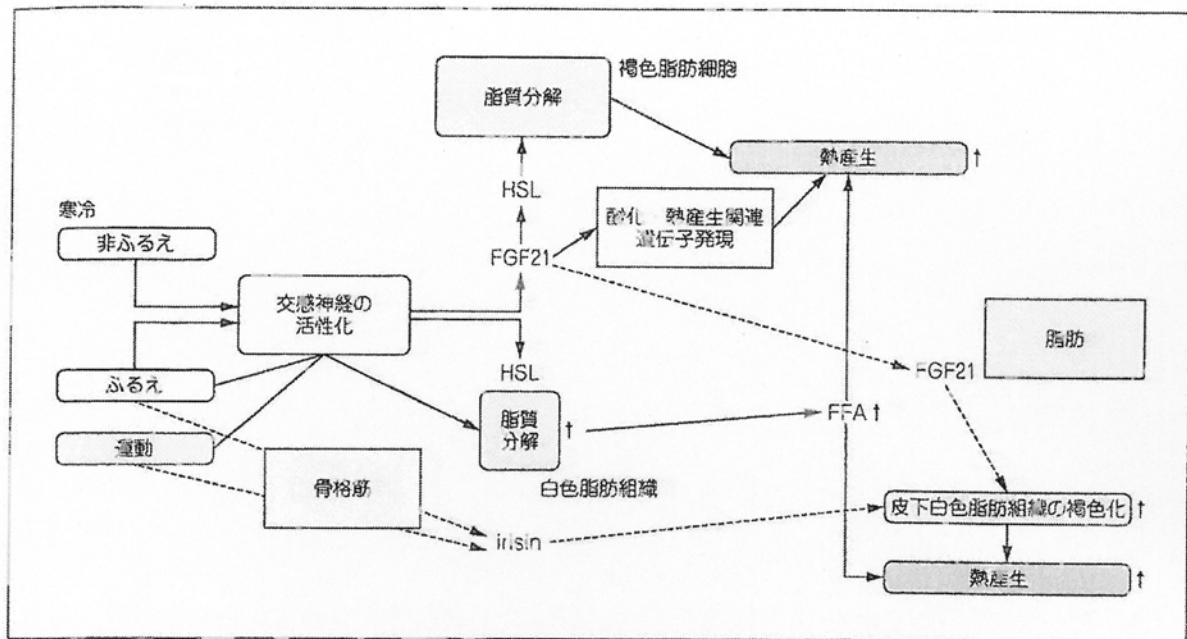
図4 寒冷および運動によるFGF21，irisin発現と脂肪組織における熱産生亢進
（文献14より引用）

つのパターンがあるとされている[1,15]．1つは慣れ（habituation）であり，寒冷馴化が起こる以前の状態より寒冷刺激に対する応答が鈍感になる適応である．これにより，ふるえの減少および皮膚血管収縮の鈍化が生じるとされている．

2つ目の適応としては，上述したように非ふるえ熱産生の亢進である（図3）．これは熱産生に寄与する褐色脂肪細胞前駆細胞の分化による褐色脂肪細胞数の増加および褐色脂肪細胞内のミトコンドリアの増加による熱産生量の増大によるとされている[5]．また，人への6週間の寒冷刺激（17℃）によって褐色脂肪細胞活性の増加，それに加えて研究開始当初では8名中3名のみに検出された褐色脂肪細胞が，介入後には8名中6名で検出できたと報告されている[16]．このことは動員の増加のみならず白色脂肪細胞の褐色化（browning）が生じることによる熱産生増加を示唆する．この白色脂肪細胞のbrowningの機序，そして熱産生能力の向上については図4のようにまとめられている[14]．熱産生能力の向上には，骨格筋由来因子であるIrisinの関与も示唆されており，運動が熱産生応答に影響することも興味深い．

慢性期適応としては断熱性の向上があげられる．これは急性応答で述べた皮膚血管収縮応答の迅速化により，寒冷馴化以前の状態よりも速やかに深部体温と体表面との熱移動を最小化する応答である．これに加え，皮下脂肪が増加すると深部組織と体表組織間の断熱性はより高まる．

食欲制御ホルモンであるグレリンに着目した研究において，肥満者では45分間の寒冷刺激（8℃）後にビュッフェスタイルで食事をしたところ，通常温度時よりも血中グレリンの増加，そして摂取エネルギー量の増加が報告されている[17]．ただ，寒冷刺激の食欲および摂取エネルギー量への影響については方法論などに議論の余地がある．

運動能力への寒冷刺激の影響

寒冷刺激によって発揮筋力，筋収縮速度，最大酸素摂取量のいずれの能力も低下するとされている[15,18]．さらに，寒冷刺激の影響には量反応性，つまり低温であればあるほど身体能力の低下量も大きくなるとされている[18]．いずれの身体能力の発揮にも骨格筋収縮が必要なわけであるが，筋

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収縮には生化学的反応がその基盤にあり，それらの生化学的反応は低温になるほど反応速度が低下することが影響していると考えられる．また，短期記憶や集中のような認知機能も低下するとされていることから[15]，身体能力だけでなく競技スポーツなどでも強い寒冷ストレスが競技能力の低下を招く可能性は高い[15,18,19]．

まとめ

寒冷刺激への生理的な応答をまとめると，急性期および慢性期応答とも，深部体温の維持を目的として1) 熱放散の抑制（血管収縮），2) 熱産生の亢進（ふるえおよび褐色脂肪細胞活性化）にあり，それらでホメオスタシスを維持するように反応する．注目すべきは，ふるえ熱産生による代謝量増加が軽運動と同程度の代謝活性化になっていること，そして寒冷刺激による褐色脂肪細胞活性化メカニズムが肥満および糖尿病治療を進歩させうる可能性をもっていることである．重大な健康被害をもたらす可能性がある寒冷刺激であるが，その生理的機序を活用することで代謝性の生活習慣病予防そして改善の一助となっていくことが期待される．

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巨大な内分泌器官としての骨格筋，そして中枢神経系との連関

Skeletal muscle tissue as a great endocrine organ and its interrelation with brain

おきたこういち
沖田孝一

Key words：運動療法，ミオカイン，骨格筋，脳，動脈硬化，BDNF

抄録

心臓リハビリテーションにおける運動療法の目的は，運動耐容能，QOL および疾患・生命予後の改善である．運動療法により，骨格筋機能が向上する一方で，虚血性心疾患，慢性心不全の予後および糖・脂質代謝異常を含む様々な病態が改善する．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．単純に考えると，運動は「筋の収縮」であり，生理学的には，ある程度の強さの筋収縮により機械的ストレスおよびエネルギー代謝による化学的（代謝的）ストレスが発生する．この筋活動により発生した代謝的ストレスは，活性酸素種(ROS)を介して細胞シグナル伝達のトリガーとなり，抗酸化防御物質・酵素を，また一方で成長因子やミオカインの産生を誘導する．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，動物種の寿命に関連し，一方，成長因子やミオカインも，オートクリンあるいはパラクリンに作用し，糖・脂質代謝の改善，筋肥大，血管増生，抗炎症，抗酸化，抗動脈硬化，抗腫瘍性など多様な治療効果を発揮する．そしてもう一つ，「運動は脳の活動」でもあり，運動による精神神経系機能の改善や脳保護作用が注目されている．

はじめに

心臓リハビリテーションにおける運動療法の目的は，疾患の改善，QOL および生命予後の改善・向上である．運動トレーニングにより虚血性心疾患，慢性心不全のみならず多数の疾患の病態や生命予後の改善が得られることが，多くの研究において証明されている（**表1**）[1~6]．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[7~10]．ゆえに，運動トレーニングは，心臓リハビリテーションの最も重要な構成要素となっている．

それでは，運動とは何か？運動に伴うどのような刺激あるいは変化が，**表1**に示すような良好な効果をもたらすのか？…意外なことに，この答えは明らかにされていない．

運動とは？どういう刺激なのか？最も簡単に考えると，運動は，「筋の収縮」であることは間違いない．生理学的には，ある程度の強さの筋収縮により機械的ストレスおよびエネルギー代謝による化学的（代謝的）ストレスが発生することになる．近年の研究において，この筋活動により発生した代謝的ストレスは，酸化ストレスを介して細胞シグナル伝達のトリガーとなり，抗酸化防御物質・酵素を，また一方で成長因子およびミオカインの産生を誘導することが示されている（**図1**）[11]．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，動物種の寿命の長さに関連すると考えられている[12]．一方，ミオカインは，アディポカインとは逆に様々な善玉作用（後述）を発揮する[7~9]．これが，科学的事実であれば，運動の様々な効果の説明となり，骨格筋の性質が良好なほどあるいは筋量が多いほど予後がよいとのエビデンス[7~10]にも合致する．そしてもう一つ，「運動は脳の活動」でもあり，また筋の疲労は，脳・中枢神経系への求心性刺激となる．最近，運動が脳機能に与える影響や脳保護作用についての研究報告がさかんに行われているが[13~15]，これらを統合すると，運動の多面的効果を矛盾なく説明できるように思える．

北翔大学生涯スポーツ学部スポーツ教育学科

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表1　科学的に証明された身体活動の効果（文献1-6）．

死亡率の減少	疾病の予防	神経機能改善	その他
全死因	高血圧	うつ状態改善	体力増進
虚血性心疾患	脂質異常症	痴呆改善	QOL向上
慢性心不全	肥満	認知機能改善	酸化ストレス軽減
脳血管障害	糖尿病	海馬容積の増加	抗酸化酵素増加
がん	メタボリック症候群	アルツハイマー病のリスク軽減	免疫力向上
（大腸，乳腺，前立腺等）	骨粗鬆症	パーキンソン病の予後改善	寿命延長？
HIV？		脳損傷回復促進	老化予防？

図1　運動に伴うROS発生によるシグナル活性化と抗酸化システムおよびミオカインの誘導．運動に伴うROS発生によるシグナル活性化．(a) ROS（Reactive oxygen species）は，ミトコンドリア電子伝達系からの電子漏出や前述のHypoxanthineの代謝により発生する．(b) ROSは，レドックス感受性転写因子（x, y, z：未知の因子）に作用し，細胞間シグナル伝達を制御する．それにより，PGC1α，酸化ストレス防御因子やミオカインが誘導される．(c) PGC1αは，ミトコンドリア機能を増大する遺伝子を活性化し，一方でSOD（superoxide dismutase），GPX1（glutathione peroxidase-1）およびcatalaseなどの内因性抗酸化物質は増加したROSを緩衝する．(e) ミオカインは，オートクリンあるいはパラクリンに働き，筋肥大（IL-6, LIF, IL-15），血管増生（IL-8, Fstl-1），代謝改善作用（IL-6）を発揮する．LIF：leukemia inhibitory factor, Fstl-1：follistatin-like1．（文献11より改変引用）

運動疫学

運動が，生活習慣病の予防に役立ち，健康を維持するために重要であることを否定する人はいないであろう．科学的研究結果に基づく運動の効能は，まさに多様であり，"運動療法"に匹敵する治療法はほかにない．その結果が，Paffenbarger[3,4]やWannamethee[5,6]の研究によって明らかにされた習慣的な身体活動は死亡率を低下させるというエビデンスに集約される．さらに体力と生存率は深く関係しており，健常者でも心疾患でも持久力あるいは筋力があるほど生存率が高いことが報告されている[7~10]．注目すべきことは，運動習慣により心血管死亡が減るだけではなく，がんなどを含めた全死因による死亡率が低下することである[4]．この

ことは，慢性心不全における運動療法のメタ解析でも示されている[16]．

心血管疾患・動脈硬化と運動療法

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異なってくる．心筋梗塞後に運動療法を実施すれば，再発や心不全の発症率が著明に低下し，予後が改善する[17~19]．狭心症では，Hambrechtら[20,21]が，安定した1枝病変患者において，カテーテル治療群と運動療法群に無作為に割り付けて追跡調査を行い，運動療法群の方が有意に再発や悪化が少なかったと報告している．また慢性心不全でさえも適切な運動療法を施行した
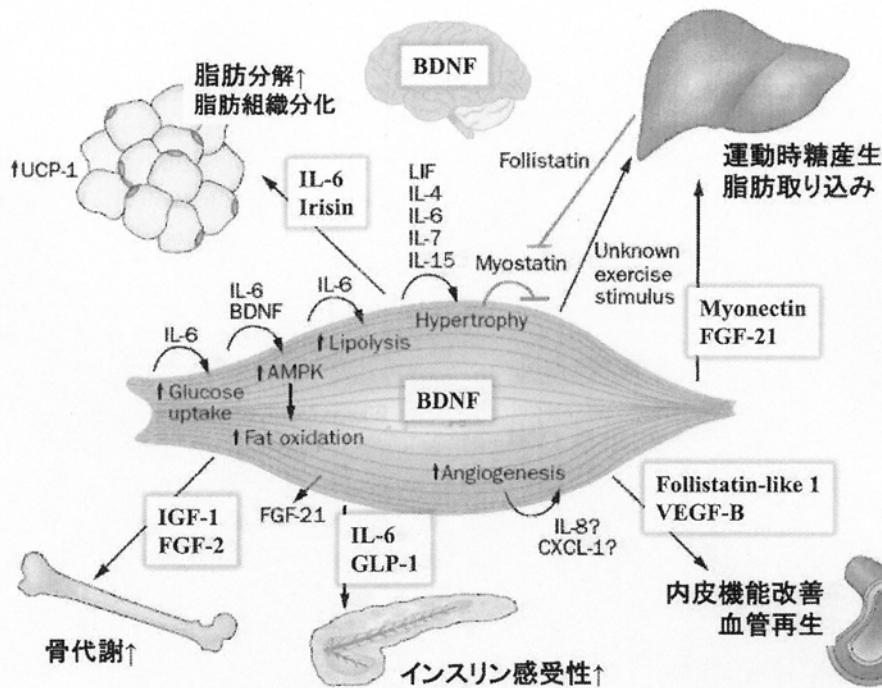
図2　巨大な内分泌器官としての骨格筋（文献29より引用改変）.

群の生存率が高くなることが無作為化比較試験で証明されている[16, 22].

心血管疾患の多くは，動脈硬化に起因するが，粥状動脈硬化進展過程における危険因子やバイオマーカーを指標とした研究により，運動療法・心臓リハビリテーションは，脂質の接着から粥腫破裂までのほぼすべての過程・因子の改善に有効であることが示されている[23, 24]. 我々も中年女性を対象に2ヶ月間の有酸素運動トレーニングを施行し，冠危険因子と炎症マーカーの変化の調べ，運動療法後にCRP（C-reactive protein），SAA（serum amyloid A protein）およびTNFα（tumor necrosis factor-alpha）が有意に減少することを証明した[25].

骨格筋は巨大な内分泌器官（図2）

近年の研究により，骨格筋は脂肪組織と並び，サイトカインを産生・分泌する内分泌器官であることが示されている．脂肪組織から分泌されるサイトカインは，アディポカインと呼ばれ，骨格筋由来のものは，ミオカインと呼ばれる．2000年に，運動中の骨格筋からIL-6が遊離することが明らかになり[26]，現在ではIL-4，IL-7，IL-8，IL-15，BDNF（brain-derived neurotrophic factor），LIF（leukemia inhibitory factor），IGF-1，GLP-1，FGF（fibroblast growth factor)-2，FGF-21，follistatin-like-1，myostatin，myonectin，irisinなどがミオカインとして同定されている[27~30]. かつて炎症惹起性サイトカインと考えられていたIL-6は，糖取り込み促進，脂肪酸化亢進，インスリン感受性改善，また筋細胞の修復・増生など善玉としての働きが明らかになって

きている[26, 27]. 筋の収縮時のエネルギー代謝による化学的（代謝的）ストレスがトリガーとなり，抗酸化防御物質が誘導され，一方で筋内に種々の成長因子およびミオカインが産生されてオートクリンにあるいはパラクリンに作用すると推定されている（図1）[11]. 抗酸化防御物質は，老化，動脈硬化，腫瘍発生を抑制し，動物種の寿命の長さに関連すると考えられており[12]，一方，ミオカインも，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，抗腫瘍性など様々な生体防御作用を発揮する[26~30]. これは，多様な運動効果の説明となり，骨格筋機能や筋量が予後に関連するというエビデンスにも当てはまる[7~10].

運動効果における骨格筋と脳・神経系の関連

大脳が活性化して指令を出さなければ骨格筋は運動しない．「運動は脳の活動」でもある．また筋の疲労は，脳・神経系への求心性刺激となる．運動療法による海馬の増大など，運動が脳機能に与える影響や脳保護作用（図3）についての近年の研究報告には特筆すべきものがあり[14, 15]，これらを加えると，運動の多面的効果を矛盾なく説明できるように思える．

骨格筋と脳に共通する液性因子：BDNF

BDNFは，神経調節因子の一つであり，神経細胞の寿命，成長および維持に中心的な役割をしており，学習，記憶，認知機能に深く関連している[31]. それを裏付けるようにアルツハイマー氏病患者の海馬においてBDNFの発現が低下していること（ドナー献体の調査），アルツハイマー氏病，パー
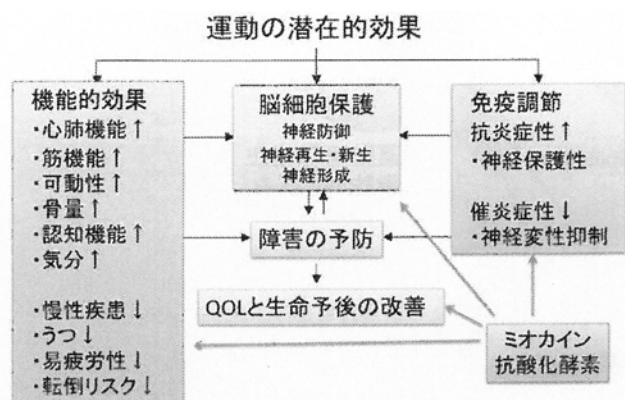
図3　運動による脳保護（neuroprotection）作用（文献14引用改変）．

キンソン氏病，うつ病患者の血中BDNF濃度が低下していることが報告されている[32~34]．最近，このBDNFが，ミオカインの一つとして注目されるようになった[27~29]．BDNFは，運動中，脳-大脳皮質・海馬において産生され，循環血液中に放出されるが，骨格筋内でも生成され，運動療法の効果発現において重要な役割を果たしている可能性が次々に明らかにされてきている[27~30]．我々は，慢性心不全患者の血中BDNFレベルが運動耐容能と正相関し[35]，さらに予後に関連することを明らかにした（JCS 2014 meeting）．このことは，慢性心不全における骨格筋萎縮（悪液質）が生命予後を悪化させる理由の一つではないかと考えている．

おわりに―Exercise is a multi-organ harmony against diseases―

運動は，骨格筋と大脳・神経系を中心とした全心体における限りなく調和した生命現象である．また筋活動に伴い抗酸化防御機構が誘導され，一方でミオカインが産生される．抗酸化防御機能の強化は，さらなる強度の運動を安全に施行することを可能にする．それらを統括する脳は発達し，神経調節因子を産生しつつ，さらなる調和を実現する．そして，神経性因子とミオカインは，骨格筋，脳神経系，血管などすべての組織に“治療効果”を発現していく．これが，Medicineとしての運動療法であり，「運動」が心身の「健康」に寄与できる理由ではないかと考えている．

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精神及び身体機能高度低下例における複合的音楽療法の可能性

Potential usefulness of combined music therapy in neurologically and physically frail elderly

沖田孝一[*1]，森田憲輝[*2]，高田真吾[*3]，舛谷夕貴[*4]，絹川真太郎[*3]，佐々木浩子[*5]，澤田悦子[*6]

抄 録

"Exercise is medicine" に象徴されるように，運動療法を主軸とした心臓リハビリテーションは，疾患治療ツールとして発展してきた．しかしながら，超高齢化が進む臨床現場では，歩行すら困難な患者が増加しており，運動療法の限界が懸念される．

身体負荷が少ない音楽療法などの芸術療法は，特にうつ状態や認知機能の改善を目的として民間療法的に施行されているが，ターゲットとする精神・神経機能への効果を客観的に評価することが難しく，科学エビデンスに乏しいため，医療には組み込まれていない．一方，精神・神経機能は，心疾患者の予後に影響することはよく知られている．脳由来神経栄養因子 (BDNF) は，神経細胞の寿命，成長，維持に中心的な役割をしており，その血中レベルが，学習，記憶，認知機能やうつ，さらには狭心症患者の予後に関連することが報告されている．そこで我々は，身体機能が著しく低下した高齢者において，血中 BDNF を精神・神経機能のバイオマーカーとして用い，歌唱，簡易楽器の演奏からなる複合的な音楽療法の効果を検討し，良好な結果を得たので，心リハ領域における音楽療法の潜在的有用性について報告する．

背景・目的

我が国は空前の高齢化社会を迎え，高齢者の健康を維持・増進することが極めて重大な課題になっている．高齢者は心血管疾患有病率が高く，また運動機能障害およびうつや認知機能低下などの精神・神経機能障害を来しやすい．そのために自立性が著しく損なわれる．このような虚弱高齢者における弊害を予防・改善するために運動療法が試みられているが，現実的には施行が困難である例が多い．一方，民間および福祉・介護分野を中心に，精神・神経機能改善・維持を目的にした音楽療法を含む芸術療法が試みられている．

各種介入が身体に与える影響は，運動能力や心血管危険因子の血液生化学的測定などにより評価できる[1,2]．一方，精神・神経機能への影響も極めて重要な要素であるが，その評価はアンケート調査が主体であり，客観的に評価することが難しい．ゆえに，芸術療法はサイエンスとして発展して来なかった．

脳由来神経栄養因子 (brain-derived neurotrophic factor, BDNF) は，神経調節因子の一つとして発見され，脳組織，特に海馬に高い濃度で存在し，神経細胞の発生，成長，維持，修復に働き[2~4]，学習，記憶および情動などの脳機能に重要な役割を担うことが明らかにされている[5~9]．BDNF は血液脳関門を通過し，血中 BDNF は大脳皮質 BDNF レベルと密接な相関関係を示すことが報告されている[3~9]．近年の研究報告において，血中 BDNF 濃度が大うつ病性障害やアルツハイマー氏病患者において減少していること，また認知機能障害やうつ状態の重症度と相関関係を示すことが

*1北翔大学生涯スポーツ学部スポーツ教育学科，*2北海道教育大学岩見沢校スポーツ教育課程，*3北海道大学大学院医学研究科循環病態内科学，*4北海道札幌平岸高等学校，*5北翔大学人間福祉学部介護福祉学科，*6北翔大学短期大学部こども学科

- 簡易楽器を用いての歌唱
- 珍しい楽器を用いての演奏
- よく知られた歌の歌唱
- 新しい歌を覚えて歌唱
- 全体でハーモニーを奏でる

報告され[10~12]，精神・神経機能を反映するバイオマーカーとしての可能性が示唆されている[3,10~12]．本研究では，身体機能が著しく低下しているために運動療法が施行困難であった軽症認知症高齢者において，精神・神経機能改善を目的とした音楽療法の効果を従来の血液生化学的指標と血清BDNFを用いて評価することを試みた．

方　法

対　象

軽症認知症を有し身体機能が高度に低下した71～103才（平均90±8才）の高齢者13名（男性6/女性7名）を対象とした．被験者および家族（あるいは身元引き受け者）に研究の主旨，安全性，個人情報の管理等について説明し，署名による同意書を得た．本研究は，北翔大学研究倫理委員会において承認されている（HOKUSHO2011-01）．

検査測定

早朝空腹時採血と精神機能の評価としてSTAIスコア（State-Trait Anxiety Inventory）の調査を複合的音楽療法前後で施行した．

測定項目

・一般血液検査：末梢血液，血液像，脂質，糖代謝，尿酸
・特殊血液検査：高感度CRP，テストステロン，DHEAS（長寿ホルモン），BDNF
・アンケート調査(STAI：State-Trait Anxiety Inventoryスコア)

複合的音楽療法

音楽療法は，歌唱，簡易楽器の演奏からなる6つのメニューで行い，課題達成を目標に1回60分，週2回，4週間施行した．具体的なプログラムは以下の通りである．

音楽プログラム（所要時間60分）

1) 浜千鳥
使用楽器：レインスティック，鈴，トライアングル，バードコール
ねらい：不安や緊張感の減少．自然音の再現．

2) りんごの歌
使用楽器：カスタネット，鈴，ウッドブロック，タンブリン
ねらい：簡易楽器演奏の役割と順番の記憶，上肢の運動．

3) 赤い花白い花
使用楽器：ハンドベル（カラー）
ねらい：知らない曲を覚える．簡易楽器演奏の役割と順番の記憶．

4) ジャズ（フランクシナトラ）
ねらい：音楽に合わせてスティックでリズムを叩き，協調，共有と一体感，上肢の運動．

5) 青い山脈
ねらい：歌唱を楽しむ．気道，胸郭組織の活性化．

6) 見上げてごらん
使用楽器：トーンチャイム，ツリーチャイム，シンバル
ねらい：ハーモニーの役割と合奏による運動，協調と一体感．

表1　複合的音楽療法前後の各指標の変化

	介入前	介入後	検定
血液生化学的指標			
総コレステロール，mg/dL	160±32	169±37	ns
中性脂肪，mg/dL	109±36	118±43	ns
HDL，mg/dL	46±11	48±11	ns
LDL，mg/dL	94±27	96±30	ns
HbA1c，%	5.8±1.0	5.8±0.9	ns
AST，U/L	21±6	20±5	ns
ALT，U/L	14±5	16±3	ns
γ-GTP，U/L	30±25	31±24	ns
尿酸，mg/dL	5.6±2.0	5.5±1.8	ns
赤血球，×10^6/μL	363±61	368±54	ns
白血球，/μL	5715±1839	5508±1701	ns
高感度CRP，mg/L	1.28±1.45	2.78±3.64	ns
テストステロン，ng/dL	1.59±1.88	1.56±1.90	ns
DHEAS，μg/dL	55.7±46.9	59.5±48.3	ns
BNP，pg/mL	143.8±193.6	91.4±104.9	ns
BDNF，ng/mL	16.8±0.5	19.4±0.5	$p < 0.05$
STAI			
Score S	40±8	40±9	ns
Score T	40±8	44±9	ns
Total score	80±15	84±17	ns

平均±標準偏差．HDL, high-density lipoprotein；LDL, low-density lipoprotein；AST, aspartate transaminase；ALT, alanine aminotransferase；γ-GTP, gamma-glutamyl transpeptidase；CRP, C-reactive protein；DHEAS, dehydroepiandrosterone sulfate；BNP, brain natriuretic peptide；BDNF, brain-derived neurotrophic factor；STAI, State-trait qnxiety inventory.

音楽療法の使用楽器

　ヤマハ社製鈴（ヤマハベル YBR-318, 321），トライアングル（TR-106），カスタネット（YHC-G2），タンバリン（TMB-221），シンバル，スズキ社製トーンチャイム（HB250），タンバリン（STR-21），ツリーチャイム（MM-35），バードコール，ZEN-ON 社製カラーハンドベル（MB-C），ドラムスティックおよび歌唱とした．伴奏にキーボード（ヤマハ社製 EZ-J210）を用いた．音楽療法のスタッフ：統括者，統括補助，音楽療法士1名，補助者（伴奏）1名，学生補助（5人/回）にて毎回の音楽療法を組織した（写真）．

統計解析

　データは，平均（標準偏差）で表示した．音楽療法前後の各指標の比較には，対応のある t 検定を用いた．統計学的有意水準は，$p < 0.05$ とした．すべての検定は，WINDOWS 用統計解析ソフト Statview 5.0（SAS Institute）を用いて行った．

結　果

　体調不良により，途中欠席した参加者もみられたが，最終的には，脱落者なく，介入を終えることができた．参加者の年齢が高く，慣れない動作にストレスを感じた方，また難しいとの苦情を訴える参加者もいたが，細かいサポートを行い継続することができた．最終的には，顕著な上達がみられ，本人および施設のスタッフも満足感と充実感が得られる結果となった．

　各指標の変化は，表1の通りである．BNP 高値例が多く，潜在的心不全の合併が疑われた．介入後，ほとんどの指標において有意な変化はみられなかった．一方，血清 BDNF には介入後に有意な上昇が認められた．

考　察

　本研究では，身体機能が高度に低下した軽症認知症高齢者において精神・神経機能改善を目的とした複合的音楽療法を施行し，その効果を STAI スコア，従来の血液生化学的指標および血清 BDNF を用いて評価することを試みた．その結果，介入後に BDNF が上昇するという画期的な知見を得ることができた．BDNF が上昇した理由を明確にするのは難しいが，対象者が最後に音楽プログラムを覚え，間違えることなく遂行することができるようになったこと，すなわち，脳機能の改善を反映しているのではないかと推測している．本研究では，STAI スコアを評価することができたが，そのスコアに有意な変化は認められなかった．STAI スコアは精神的満足度を反映するものであり，入所施設のケアのレベルが高く，介入前より十分高いスコアだったためと推測される．当初の研究計画では，認知機能を測定す

る予定であったが，施設スタッフの同意が得られず，実行できなかった．BDNF の増加を考えると，認知機能スコアは改善していた可能性がある．

従来の血液指標については有意な改善が得られなかったが，この理由は身体活動としては極めて低いレベルであったことなどが考えられる．本研究では，対象者の身体機能が高度に低下していたため，常に座位で介入を行った．立位や歩行を取り入れたプログラムを用いることが出来れば，身体活動による効果も得られたかも知れない．

おわりに

身体機能高度低下のため運動療法施行困難であった軽症認知症高齢者において，精神・神経機能改善を目的とした複合的音楽療法を施行し，血清 BDNF という客観的指標を用いて有効性を検証できた．この成果は，高齢化社会が進む我が国において，脳機能，高次運動機能維持・改善のための新しい視点からの介入方法を提言するとともにこれまで困難であった芸術療法の客観的評価方法の可能性を示唆するものである．将来的に心臓リハビリテーション領域において芸術療法が活用・応用されていくことを期待したい．

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糖質制限は心臓リハビリテーションにおいて有効かあるいは支障になるのか？

Does low-carbohydrate diet provide merits or demerits in cardiac rehabilitation?

沖田孝一[*1]，高田真吾[*2]，門口智泰[*2]，森田憲輝[*3]，井出幸二郎[*1]，川初清典[*1]

Key words：食事療法，糖質制限，体力，肥満，心臓リハビリテーション

抄　録

今日，減量あるいは糖尿病治療のための，糖質制限を主体にした食事療法が，米国糖尿病学会で認められ，欧米を中心に普及してきている．一方，我が国では，依然として否定的見解の学識者が多いが，ふすまパン，大豆麺，豆腐米などがすでに市場に出回り，むしろ民間を基盤に浸透しつつある．心臓リハビリテーションでは，体力増進のみならず，心疾患や糖・脂質代謝異常などの原因となった肥満を改善するために運動療法が施行され，さらに食事療法が組み合わされる．糖質制限食の考え方は，血糖値を上昇させる唯一の栄養素である糖質の摂取を抑えて，「食後高血糖→インスリン分泌亢進→体脂肪蓄積」を防ぐことであるが，糖をエネルギーとする脳あるいは体力への悪影響の可能性など問題点も指摘されている．しかしながら，糖質制限が体力面に与える影響を検討した研究は少なく，特に日本では見られない．今回我々は，糖質制限あるいは総カロリー制限が，体組成，体力，気分および知力に与える影響について調べたので，その結果と先行研究を踏まえ，肥満や糖尿病を有する心臓リハビリテーション患者における糖質制限食の有用性と問題点について提言する．

背景・目的

生活習慣病の予防には，主に運動療法と食事療法およびそれらの混合療法が用いられており，各々の有用性が証明されている[1,2]．食事療法は，バランスよく摂取カロリーを減少させる総カロリー制限食が標準的な方法として実施されている[3]．しかしながら，特に糖尿病，肥満に関しては，その有効性が疑問視されており，一方で糖質の摂取のみを極端に制限する糖質制限食の有用性が注目されている．糖質制限とは，Richerd K. Bernstein 博士や Robert C. Atkins 博士らが考案した食事法であり[4~6]，糖質を制限することで，血糖値を一定に保ち，インスリン分泌亢進による体脂肪蓄積を防ぐという考え方に基づいている．この概念は，未治療の 1 型糖尿病患者が，インスリンの分泌がないため食事量に関わらず痩せてゆく病態に重なる．糖質制限の有効性は，近年，多数の研究で検証されている[7~20]．我が国においても，NIPPON DATA80 に基づく 29 年間のコホート研究から，低糖質食の程度が高い群ほど死亡率が低かったという衝撃的な結果が報告された[9]．しかしながら，我が国では，未だ積極的には推奨されていない．

糖質制限の問題点として，身体活動時において主要なスタミナ源となる糖質が不足することによって，体力が低下しないか，あるいはインスリン分泌亢進を抑えることにより骨格筋量の減少，筋力低下が起こらないか，また脳代謝の主要なエネルギー源であるブドウ糖不足により知力低下が起こらないかという懸念がある．そこで我々は，糖質制限あるいは総カロリー制限が，体組成，体力，気分および知力に与える影響について調べたので，その結果と先行研究を踏まえ，肥満や糖尿病を有する心臓リハビリテーション患者における糖質制限食の有用性と問題点について提言する．

[*1]北翔大学生涯スポーツ学部スポーツ教育学科，[*2]北海道大学大学院医学研究科循環病態内科学，[*3]北海道教育大学岩見沢校スポーツ教育課程
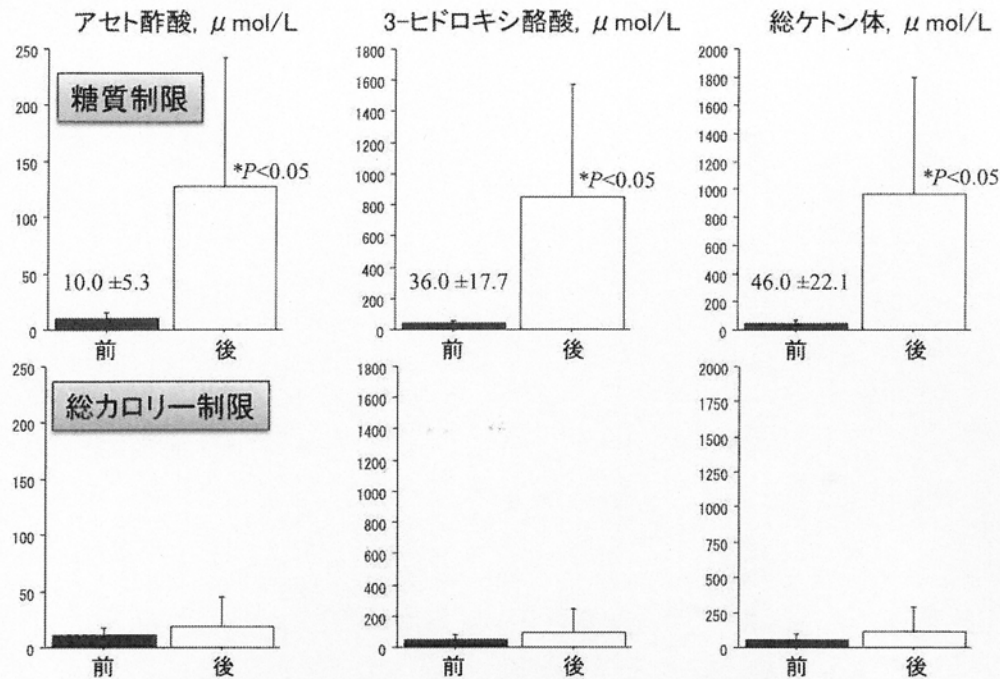
図1　食事療法前後のケトン体の変化．糖質制限群においてケトン体の著明な増加が認められた．

方　法

対　象

　肥満，耐糖能異常あるいは糖尿病の家族歴を持ち，運動習慣を持たない健常者12名（男5，女7）を対象とした．すべての被験者には研究の主旨，安全性，個人情報の管理等について説明し，署名による同意書を得た．本研究は，北翔大学研究倫理委員会において承認されている（HOKUSHO2013-05）．

　被験者を総カロリー制限群あるいは糖質制限食群に無作為に割り分け，1ヶ月間の介入を実施した．総カロリー制限は，1日（身長−100）×25 kcal，糖質制限は，糖質摂取量を1日40 g以下とし，カロリー制限は行わなかった．介入前後に，以下の項目を評価した．

測定項目

①一般血液検査

　血清脂質，耐糖能，肝機能，腎機能，尿酸，血球数を測定した．

②特殊血液検査

　インスリン，ケトン体（アセト酢酸，3-ヒドロキシ酪酸，総ケトン体），高感度C反応性蛋白（CRP）および血清BDNF（brain-derived neurotrophic factor）を測定した．

③身体・体力測定

　身長，体重，血圧，体脂肪率，腹囲，大腿四頭筋厚，膝伸展筋力，全身持久力（自転車エルゴメーターによる最大酸素摂取量）を測定した．

④うつ・不安・認知機能の評価

　BDI（Beck Depression Inventory：ベックうつ病調査表），STAI（State-Trait Anxiety Inventory：特性不安検査），及びカナ拾いテスト（認知機能）にて評価した．

統計処理

　本文，表および図は平均値±標準偏差で表した．独立した2群間の比較は，unpaired t-test により検定した．各群における介入前後の指標の比較は，paired t-test および二元配置反復測定分散分析（2-way repeated-measures ANOVA）を用いて行った．食事条件間の交互作用を認めた場合は，Bonferroni の多重比較（post-hoc）により検定した．統計学的有意水準は，$p < 0.05$ とした．

結　果

　糖質制限群において，ケトン体が著明に増加していた（図1）．介入前後の理学所見，体力指標および精神機能の変化を表1に示した．体格指数と腹囲は，糖質制限群においてのみ有意に減少していた．一方，糖質制限群，総カロリー制限群とも，体力指標，気分，知力に有意な変化は認められなかった．

　血液生化学指標の変化は表2の通りであり，糖質制限群のみで耐糖能指標の改善がみられた．有意差はなかったが，糖質制限群で総コレステロールおよびLDLの増加傾向が認められた．

表1　介入前後の理学所見，体力指標および精神機能の変化

測定項目	糖質制限群		総カロリー制限群	
	介入前	介入後	介入前	介入後
BMI, kg/m²	24.8±4.7	23.4±4.4*	25.3±4.4	25.0±4.5
腹囲, cm	84.0±11.9	79.9±9.9*	87.1±12.5	86.4±12.5
体脂肪率, %	31.9±8.6	31.0±8.4	31.3±7.9	31.4±8.6
大腿筋厚, cm	33.7±6.7	32.5±6.3	36.5±5.5	34.7±4.8
膝伸展筋力, W/kg	40.6±12.6	39.2±12.1	41.9±12.9	37.5±9.9
握力, kg	31.9±8.2	30.0±10.4	33.9±8.5	31.4±10.2
持久力, ml/min/kg	31.7±8.0	29.6±6.1	27.7±7.0	28.0±6.0
収縮期血圧, mmHg	115±10	109±14	111±12	110±14
拡張期血圧, mmHg	70±8	64±5	71±6	69±7
心拍数, beat/min	76±7	75±9	76±13	72±6
BDI	6±4	7±2	8±4	6±4
STAI	86±7	87±11	93±8	90±7
かな拾いテスト	48±14	50±12	43±13	48±10

BMI, body mass index；BDI, Beck Depression Inventory；STAI, State-Trait Anxiety Inventory.
*p<0.05，介入前 vs 介入後.

表2　介入前後の血液生化学的指標の変化

項目	糖質制限群		総カロリー制限群	
	介入前	介入後	介入前	介入後
AST, IU/L	21±5	20±5	30±23	23±8
ALT, IU/L	22±9	22±9	41±51	31±30
GGT, IU/L	21±13	20±17	23±13	21±9
TC, mg/dL	185±31	208±47	168±15	165±15
LDL, mg/dL	109±28	131±40	98±4	96±3
中性脂肪, mg/dL	77±21	64±32	84±62	69±34
HDL, mg/dL	61±13	64±18	53±16	55±17
空腹時血糖, mg/dL	98±17	89±7*	88±11	84±10
HbA1c, %	5.4±0.5	5.1±0.3*	5.5±0.7	5.4±0.6
インスリン, μU/mL	7.5±5.5	6.2±4.7	12.4±10.0	14.3±10.1
HOMA-R	1.79±1.30	1.40±1.19	2.56±1.74	2.95±2.21
尿酸	6.2±2.2	6.7±2.0	4.9±1.7	3.6±0.9
クレアチニン	0.67±0.09	0.66±0.09	0.69±0.14	0.60±0.08
白血球	5.55±1.63	5.03±1.63	5.50±1.05	5.90±1.16
高感度CRP	0.46±0.41	0.31±0.35	0.59±0.87	0.40±0.29
BDNF	30.1±4.9	29.6±7.5	32.1±4.3	32.6±4.2

AST, aspartate aminotransferase；ALT, alanine aminotransferase；GGT, gamma-glutamyl transpeptidase；TC, total cholesterol；LDL, low-density lipoprotein；HDL, high-density lipoprotein；CRP, C-reactive protein；BDNF, brain-derived neurotrophic factor. *p<0.05，介入前 vs 介入後.

結　論

　心臓リハビリテーションの主要な目的には，運動トレーニングによる運動耐容能の向上と冠危険因子の改善が含まれる．運動トレーニング法については，ガイドラインがほぼ確立されている．一方，食事療法の特異的なガイドラインはない．糖質制限は，冠危険因子の改善には極めて有効であるが，運動耐容能向上については，負の影響を及ぼす可能性があり，心臓リハビリテーションに応用するには，十分な検討が必要であると思われた．しかしながら，本研究においては，糖質制限による明らかな体力面への悪影響は認められず，また精神面への影響も同様であった．したがって，糖質制限は，肥満や糖尿病を有する心臓リハビリテーション患者には，考慮すべき食事療法の一つであると考えられた．

おわりに

　肥満や食後高血糖が，冠動脈疾患，がん，精神神経疾患

など様々な疾患の危険因子として認識されてきているにも関わらず，糖質を基盤とした食事療法が，スタンダードとして漫然と推奨されている．極端な糖質制限による弊害の可能性や代替食品の問題等は残されているが，多数の論文が示す通り，糖質制限の応用は，肥満・糖尿病の改善に非常に有用であることは間違いない．心臓リハビリテーションにおける運動療法と食事療法は，疾患の予防・改善を共通の目的とするものであり，特に肥満や糖尿病を有する心リハ患者では，運動療法と食事療法の併用が必要である．我々は，高脂肪食が糖尿病の原因であるという概念を刷り込まれてきた感がある．実験マウスでは，高脂肪食により糖尿病モデルを作成する．しかしながら，マウスは固形物しか食べないため，餌は糖質で固めた高糖質・高脂肪食なのである．工夫して高脂肪のみ摂取させても糖尿病マウスを作成できないようである．古い概念に固執せず研究データが積み重ねられ，運動療法のみならず，食事療法も含めたオーダーメイド療法が考案されていくことに期待している．

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平成26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

井　出　幸二郎[1]		上　田　知　行[1]	
Kojiro	IDE	Tomoyuki	UEDA
小　坂　井　留　美[2]		小　田　史　郎[2]	
Rumi	KOZAKAI	Shiro	ODA
本　多　理　沙[3]		竹　田　唯　史[1]	
Risa	HONDA	Tadashi	TAKEDA
増　山　尚　美[1]		竹　内　　　晶[4]	
Naomi	MASHIYAMA	Akira	TAKEUCHI

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

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5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	9月7日（土）10時〜12時	多目的研修センター	正しい姿勢でウォーキング講座【担当】本多理沙
第3回	10月13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】小坂井留美，他
第4回	11月16日（日）10時〜12時	総合体育館	がたリンピック【担当】本多理沙
第5回	1月17日（土）10時〜13時	月形小学校グラウンド	ゴルポッカ【担当】小田史郎，他
第6回	3月16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）北方圏生涯スポーツ研究センター

4）月形町教育委員会

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26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26年5月18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3に示した。参加者数は

表2. 参加者の身体的特徴

		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=10）	52 ± 16	135 ± 16	78 ± 13	167 ± 10	69 ± 10	25 ± 2
	65歳以下（n=8）	45 ± 10	136 ± 18	77 ± 14	171 ± 7	73 ± 6	25 ± 2
	65歳以上（n=2）	78 ±	133 ±	80 ±	154 ±	54 ±	23 ±
女性	全体（n=22）	57 ± 18	125 ± 19	74 ± 13	156 ± 8	55 ± 8	23 ± 3
	65歳以下（n=11）	41 ± 12	118 ± 16	72 ± 14	162 ± 5	58 ± 10	22 ± 4
	65歳以上（n=11）	73 ± 5	133 ± 20	75 ± 12	151 ± 7	52 ± 6	23 ± 2

平均±標準偏差

表3. 参加者の身体機能

		握力（kg）	ファンクショナルリーチ (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=10）	41.7 ± 8.6	42.0 ± 6.4	38.6 ± 12.3	90.5 ± 48.7	5.8 ± 1.6	4.0 ± 1.0	25.2 ± 8.0
	65歳以下（n=8）	43.9 ± 7.8	43.8 ± 6.0	39.9 ± 13.5	110.9 ± 25.7	5.7 ± 1.8	4.0 ± 1.2	26.9 ± 7.9
	65歳以上（n=2）	33.1	36.0	33.3	8.7	6.1	4.1	18.0
女性	全体（n=22）	24.3 ± 4.3	37.8 ± 6.8	36.4 ± 8.6	67.2 ± 51.2	7.1 ± 1.5	4.7 ± 1.0	21.5 ± 7.8
	65歳以下（n=11）	25.8 ± 5.0	42.1 ± 5.8	37.2 ± 11.0	82.6 ± 51.9	6.4 ± 1.1	4.0 ± 0.6	21.6 ± 8.5
	65歳以上（n=11）	22.9 ± 3.0	33.4 ± 4.6	35.6 ± 5.7	51.7 ± 47.8	7.8 ± 1.6	5.4 ± 1.0	21.3 ± 7.4

平均±標準偏差

32名であった。今年度の参加者は，こ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名が平成24年の第1回の測定から連続で参加しており，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反復測定分散分析を用いた。この7名の内訳は男性3名，女性4名で，平成26年の測定時の年齢は72±7歳であった。肥満度及び血圧に経年変化は認められなかった。また，握力，柔軟性，平衡機能，動
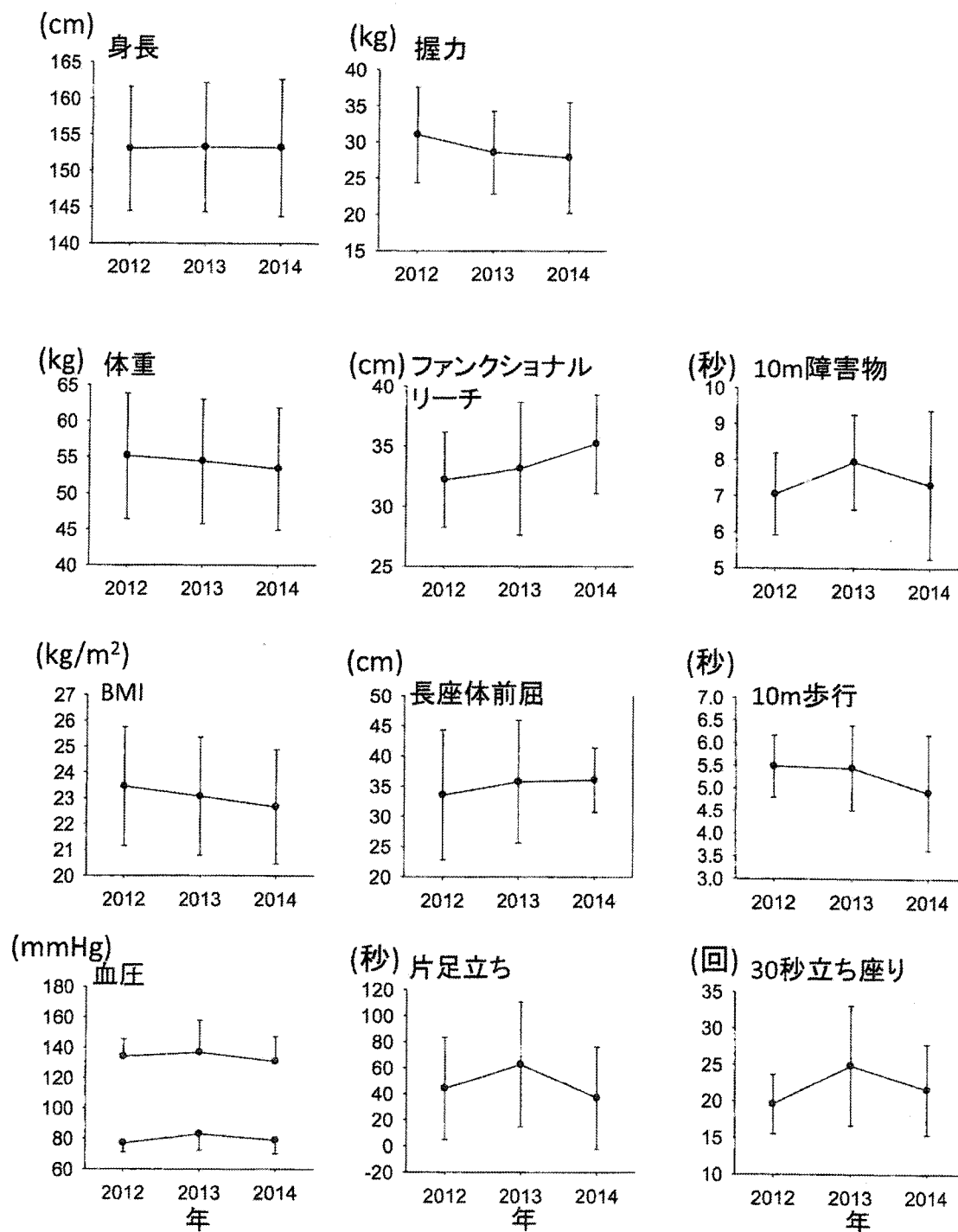
図1　月形町町民体力測定会に継続して参加している高齢者の身体的な変化
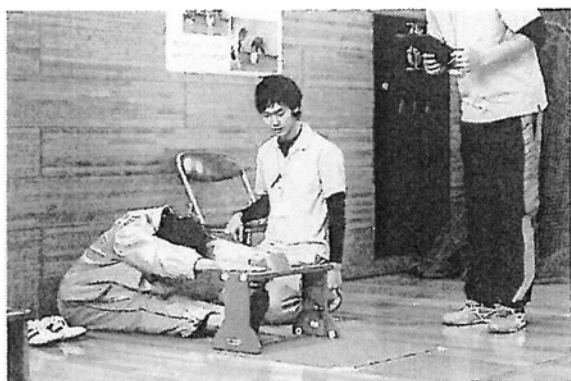
写真1　長座体前屈

的平衡機能，歩行機能，下肢パワーの低下は認められなかった。僅か7名のデータが月形町民の体力変化を反映しているとは考えにくいが，少数でもこのように体力全般的に経年による低下が認められていないことは，高齢者の転倒予防という観点からも良い傾向である。これまで我々は地域住民の主に体力に注視してきた。身体機能の低下は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]，認知症の予防のためにも注意を向ける必要がある。体力だけではなく認知機能や心の健康状態も評価することは，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が必要である。

Ⅲ．第二ステップ　ヘルシーミーティング

　6月22日（日）月形町多目的研修センターにて，20歳以上の月形町民を対象に「健康づくりに役立つ食生活」と題し，北翔大学生涯スポーツ学部小田嶋名誉教授が講話を行った。また，前回の体力測定の参加者に対しては体力測定結果と歩行時の連続画像をフィードバックした。講話後，参加者に対して体力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教員及び学生スタッフが指導した。具体的には，参加者は，前の週の体力測定に参加した者とそれに不参加であった者も含め，計23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「正しい姿勢でウォーキング講座」，第3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4回「がたリンピック」，第5回「ゴルポッカ」，第6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。「正しい姿勢でウォーキング講座」では，①姿勢の改善，②歩き方の確認，③筋力トレーニング，④ラダートレーニング，⑤ノルデックウォーキングの指導を行った。引き続いて，「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，前回の「正しい姿勢でウォーキング講座」を活かし，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，6kmと9km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

写真2　町民歩け歩け大会

写真3　ゴルポッカ

歩け歩け大会とも参加した本学学生が月形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。「ゴルポッカ」は，月形小学校グラウンドにて行った。月形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行える運動と，高齢者でも冬期に雪上でも行えるゴルポッカ，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。

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H26年度も引き続き実施した。平成26年1月10日，月形町と北翔大学は包括的連携協定を締結した。これを機に，月形町の発展と月形町民の健康体力づくり事業が活発となり，さらに連携を通じた教育研究活動が益々盛んになることが望まれる。

謝　辞

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平成26年度地域まるごと元気アッププログラム体力測定会実施報告

Report on Physical Fitness Measurement Meeting in Participants of " Chiiki Marugoto Genki Up Program "

上　田　知　行[1]　　井　出　幸二郎[1]　　小坂井　留　美[2]　　小　田　史　郎[2]
小田嶋　政　子[4]　　本　間　美　幸[2]　　佐　藤　郁　子[2]　　佐々木　浩　子[3]
本　多　理　紗[4]　　相　内　俊　一[4,5]

Tomoyuki UEDA[1]　　Kojiro IDE[1]　　Rumi KOZAKAI[2]　　Shiro ODA[2]
Masako ODAJIMA[4]　　Miyuki HONMA[2]　　Ikuko SATO[2]　　Hiroko SASAKI[3]
Risa HONDA[4]　　Toshikazu AIUCHI[4,5]

キーワード：介護予防，自治体，高齢者，体力測定

Ⅰ．はじめに

　産学官で協働した地域におけるソーシャルビジネスの研究として実施している，「地域まるごと元気アッププログラム」は行政・民間企業・非営利団体・大学の協働によって行なわれている。60歳以上の高齢者を中心とした一般町民に向けた体力測定会の結果による市民の生活体力の把握をはじめ，比較的低体力者から中程度の体力者までの3クラスの高齢者対象の運動教室，中学生と高齢者の世代間交流，高齢者向け音楽会などを平成22年から実施しているものである[1][2][3][4]。

　このうち体力測定会については，平成26年度にこれまでの3市町に加え，新たに3町が加わった。また運動教室については，8市町村で実施した。本研究では，平成26年度の体力測定実施地域におけるその報告を行う。

Ⅱ．体力測定会実施の概要

　平成26年度の体力測定会を実施した地域と会場数，測定実施人数は表1のとおりである。

　体力測定項目については，握力・長座体前屈・開眼片足立ち・ファンクショナルリーチ（以下F/R）・10m全力歩行・10m障害物歩行・30秒椅子立ち座りテストを行った。実施前には，血圧測定および体調の確認を行い，測定の危険性や情報の取り扱う範囲について，十分説明し同意を得たうえで，十分な準備体操を実施したうえで行った。また，既往症や現病歴，運動実施の程度についても聞き取りによる調査を行った。

Ⅲ．結　果

　測定結果を性別ごとに表2および表3に示す。
　また，性別ごとに5歳区分ごとの測定結果を表4およ

表1：平成26年度体力測定会の概要

実施地域	期間（期日）	会場数	実施人数
Ak市	8月20日～22日	10会場	235名
As市	8月21日	1会場	59名
Y町	8月25日，8月29日	5会場	207名
Nu町	7月9日	3か所	29名
Su町	9月8日～9月9日	1か所	100名
Ku町	9月17日～9月18日	1か所	40名
合計		21か所	670名

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）北翔大学教育文化学部教育学科

4）北翔大学北方圏生涯スポーツ研究センター

5）NPO）ソーシャルビジネス推進センター

表2：平成26年度体力測定会結果（男性）

男性 (184名)	握力 (kg)	F／R (cm)	長座体 前屈 (cm)	開眼片 足立ち (秒)	10m全力 歩行（秒）	10m障害 物歩行 (秒)	30秒椅子 立ち座り (回)
平均値	36.17	36.01	32.38	65.12	5.09	7.53	21.23
標準偏差	8.24	6.96	10.23	48.55	1.43	2.13	7.16

表3：平成26年度体力測定会結果（女性）

女性 (479名)	握力 (kg)	F／R (cm)	長座体 前屈 (cm)	開眼片 足立ち (秒)	10m全力 歩行（秒）	10m障害 物歩行 (秒)	30秒椅子 立ち座り (回)
平均値	22.53	33.24	36.88	59.19	5.73	8.99	20.15
標準偏差	4.54	6.61	8.44	46.97	1.47	46.97	6.99

表4：平成26年度体力測定会結果（男性）

	握力 (kg)	F／ R(cm)	長座体 前屈 (cm)	開眼片 足立ち (秒)	10m全力 歩行（秒）	10m障害 物歩行 (秒)	30秒椅子 立ち座り (回)
60歳未満（20名）							
平均値	44.37	41.73	34.78	114.00	3.85	6.21	26.82
標準偏差	6.84	4.12	9.19	22.05	0.60	1.24	6.29
60〜64歳未満（19名）							
平均値	42.59	40.24	36.29	93.15	4.19	6.07	25.75
標準偏差	7.79	5.85	10.62	35.97	0.57	0.99	5.55
65〜69歳未満（21名）							
平均値	39.22	37.28	33.07	92.24	4.83	7.09	21.95
標準偏差	5.78	7.41	9.37	41.44	1.43	2.73	7.93
70〜74歳未満（37名）							
平均値	34.76	36.76	33.28	58.08	4.96	7.43	20.72
標準偏差	5.52	5.52	9.90	44.61	1.06	1.89	6.28
75〜79歳未満（46名）							
平均値	34.44	35.78	30.96	58.47	5.23	7.68	20.68
標準偏差	5.51	5.35	9.43	47.63	1.13	1.64	20.68
80〜84歳未満（29名）							
平均値	32.31	30.90	30.98	27.18	5.75	8.49	18.18
標準偏差	6.06	5.54	10.86	31.04	1.22	1.42	4.84
85歳以上（13名）							
平均値	27.56	29.08	26.96	24.26	7.14	10.49	13.80
標準偏差	7.10	7.25	11.17	33.61	2.19	2.84	6.06

び表5に示す。

表5：平成26年度体力測定会結果（女性）

	握力 (kg)	F／ R(cm)	長座体 前屈 (cm)	開眼片 足立ち (秒)	10m全力 歩行（秒）	10m障害 物歩行 (秒)	30秒椅子 立ち座り (回)
60歳未満（13名）							
平均値	27.48	42.23	40.65	106.82	4.48	6.92	20.65
標準偏差	2.96	4.32	7.35	31.43	0.43	0.92	5.03
60〜64歳未満（14名）							
平均値	27.69	41.50	39.50	107.76	4.47	6.89	21.11
標準偏差	2.96	4.92	8.22	3.48	0.41	0.89	5.12
65〜69歳未満（93名）							
平均値	24.06	35.86	37.98	80.93	5.15	7.93	21.32
標準偏差	3.62	5.22	8.85	44.92	0.77	16.11	7.16
70〜74歳未満（103名）							
平均値	22.70	33.68	37.30	55.38	5.51	8.59	21.61
標準偏差	4.32	5.76	8.58	44.72	1.24	2.78	6.19
75〜79歳未満（129名）							
平均値	21.90	32.23	36.92	45.61	5.79	9.25	19.83
標準偏差	4.06	5.84	8.56	40.65	1.23	2.42	6.46
80〜84歳未満（67名）							
平均値	19.72	29.93	35.66	40.11	6.53	10.41	16.00
標準偏差	5.01	6.78	7.93	39.22	1.69	2.95	5.85
85歳以上（31名）							
平均値	19.19	25.73	33.52	9.77	12.60	12.60	12.69
標準偏差	3.31	5.73	7.20	9.54	3.62	3.52	4.34

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介護予防運動プログラム指導者への研修プログラムの作成

Study on Development of a Training Program for Care Prevention Exercise Instructors

上 田 知 行[1]　　井 出 幸二郎[1]　　小坂井 留 美[2]　　小 田 史 郎[2]
小田嶋 政 子[3]　　本 間 美 幸[2]　　本 多 理 紗[3]　　相 内 俊 一[3,4]

Tomoyuki UEDA[1]　　Kojiro IDE[1]　　Rumi KOZAKAI[2]　　Shiro ODA[2]
Masako ODAJIMA[3]　　Miyuki HONMA[2]　　Risa HONDA[3]　　Toshikazu AIUCHI[3,4]

キーワード：介護予防，指導者，養成，運動プログラム

Ⅰ．はじめに

これまで産学官で協働した地域におけるソーシャルビジネスの研究として，「地域まるごと元気アッププログラム」を実施してきた。「地域まるごと元気アッププログラム」は行政・民間企業・非営利団体・大学の協働によって行なわれている。60歳以上の高齢者を中心とした一般町民に向けた体力測定会の結果による市民の生活体力の把握をはじめ，比較的低体力者から中程度の体力者までの3クラスの高齢者対象の運動教室，中学生と高齢者の世代間交流，高齢者向け音楽会などを平成22年から実施している[1][2][3][4]。

これまで行なってきた「地域まるごと元気アッププログラム」（以下「まる元」）では，体力測定会や健康講演会，運動教室を実施してきた。そのうち運動教室については，自治体の介護予防事業のうち，地域支援事業にも位置づけられ一定の成果を果たしている[2][3][4]。高齢化が進む北海道の地域市町村では，介護予防のための運動教室が実施されている。しかし，「まる元」運動教室のように，健康づくりのための運動指導の専門家である健康運動指導士に運動指導を任せるには，人的資源が不足しており，保健師や管理栄養士などが運動指導を担うケースも少なくない。さらに，継続した運動により，介護予防のための体力が維持されることは明らかになっているが，多くの事業では3か月間や半年間など，運動習慣を身に着けることまでは至っておらず，通年型の「まる元」運

動教室を導入しようとする自治体が増加することが予測される。こういったニーズに対応するには，体力の維持向上を図り，楽しく運動指導を行える運動指導者の輩出が不可欠であるが，この人的資源を輩出するための研修プログラムが整備されているとは言い難い。そこで本研究では，より質の高い運動指導者を輩出するために，健康運動指導士を取得して，地域における介護予防運動プログラムを指導しようとする運動指導者を育成するため研修プログラムを策定し実施した。

Ⅱ．概　要

1．目的

高齢者の健康づくりや介護予防の運動指導者として，教室の指導能力，市町村の課題に合ったプログラムを展開する能力，教室運営上の他業種との連携等が必要となる。この研修では，健康運動指導士としての指導上の知識，ホスピタリティ，運動の動機づけ，リスク管理等を身につけ，地方市町村での運動指導ができることを目的とする。

2．到達目標

・健康運動指導士として，安心・安全・楽しく効果的で継続できる運動指導ができる。

・参加者のQOLを向上できるような動機づけができる。

・多様な体力の方々に対する運動プログラムの構成ができる。

・リスクを予測し，リスク管理ができる。

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）NPO) ソーシャルビジネス推進センター

・ホスピタリティを理解し，コミュニケーションがとれる。
・評価についての知識と方法を習得する。

3．参加条件

　健康運動指導士または健康運動指導士と同等の知識や技能を有する者

4．開催時期および講義時間

　研修は1モジュールを3時間とし16モジュールに加えて，特別講義8回からなる60時間の計画とした。実施した期間は，平成26年4月7日（月）から平成26年5月26日（月）の毎週月曜日に，各モジュール180分および特別講座90分を実施した。各モジュールと特別講義のテーマ

表1：各モジュールの概要と特別講義のテーマ

モジュール	内容
1	「テーマ」オリエンテーション 健康運動指導士としての基本的知識の確認。まる元各クラスの目的や内容の確認。指導者・支援者としての目標設定。
2	「テーマ」コミュニケーションワーク ホスピタリティについての理解とホスピタリティトレーニング。 指導者としてのホスピタリティについて。コミュニケーション技術について。コミュニケーショントレーニング。
3	「テーマ」アイスブレーキング アイスブレーキングの意義と基本技術　様々なアイスブレーキングの体験とアレンジ方法。
4	「テーマ」アイスブレーキング アイスブレーキングの基本プログラミングと実践。（アセスメントの理解）
5	「テーマ」トレーニング トレーニング論を踏まえた運動プログラムの構成 ストレッチング・整理体操の確認（注意点なども含めて）と実践
6	「テーマ」トレーニング まる元で行っている筋力トレーニングの確認と実践　ボールを使用しての軽体操の実践
7	「テーマ」レクリエーションの理論 レクリエーションとは？　4つの分類からのレクリエーションの理解　福祉レクリエーションの考え方
8	「テーマ」レクリエーションの実際① 虚弱な高齢者へのレクリエーションの実際（Aクラスの想定） レクリエーション実施中に予測されるリスクと回避方法
9	「テーマ」レクリエーションの実際②③ 元気な高齢者へのレクリエーションの実際（B・Cクラスの想定） 身近な素材や道具を使用した様々なレクリエーションやリズム体操
10	「テーマ」レクリエーションの計画・実践 レクリエーションの指導案作成の説明と指導案作成をする。実際に実践をし，お互いに評価を行う。
11	「テーマ」ケーススタディ① 様々な事例からプログラムを構成できるようにする。指導案作成の説明
12	「テーマ」ケーススタディ② 様々な事例からプログラムを構成できるようにする。指導案作成の説明。
13	「テーマ」プログラム実践発表 様々なケースを想定し，プログラムの説明を行い発表する。（時間管理も含む。）自己の評価・反省と他者からの評価を行う。（Aクラス想定）
14	「テーマ」プログラム実践発表 様々なケースを想定し，プログラムの説明を行い発表する。（時間管理も含む。）自己の評価・反省と他者からの評価を行う。（Aクラス想定）
15	「テーマ」プログラム実践発表 様々なケースを想定し，プログラムの説明を行い発表する。（時間管理も含む）自己の評価・反省と他者からの評価を行う。（混合クラス想定）
16	「テーマ」プログラム実践発表 様々なケースを想定し，プログラムの説明を行い発表する。（時間管理も含む）自己の評価・反省と他者からの評価を行う。（混合クラス想定）
特別講座	内容
1	「バランスト・スコアカードと顧客志向」
2	「健食プログラム」
3	「行動変容を促す基礎要因」
4	「体力行動面から捉えた事業評価」
5	「睡眠力」
6	「老年学」
7	「運動と認知機能」
8	「地域の介護認定者の現状　介助の基本など」

を表1に掲載する。

Ⅲ．指導プログラムの内容

　各モジュールのうち，モジュール2およびモジュール7について次のようにまとめた。

1．モジュール2―コミュニケーションワーク
・ホスピタリティについての理解とホスピタリティトレーニング
・指導者としてのホスピタリティ・コミュニケーション技術・コミュニケーショントレーニング

1）あいさつ
・仲間に関心をもつこと（共感）から人間関係がスタートする。
・自分をオープンにすれば（自己開示）すればするほど自分に好意を持ってくれる。
人間関係のスタートは共感と自己開示から成り立つ。
※初対面でいきなり自己紹介はしにくい
　人と会うとまず何をする？
　挨拶は，人間関係を作る潤滑油
【あいさつのポイント】
　あ＝明るくあいさつ
　い＝生き生きとあいさつ
　さ＝さっと自分からあいさつ
　つ＝積み重ねてあいさつ
あいさつは，「あなたに近づきたい。」「私はあなたに対して心を開いています。」という意味がある。
【印象を決めるポイント】
　①　外見（表情・身だしなみ）例）笑顔・髪・服装・つめなど
　②　態度（動作）例）物を投げる　上から目線
　③　話し方　例）声のトーン　強弱　敬語
　④　話す内容　例）知識など

2）笑顔
　笑顔は人間だけにしかできない特権。笑顔を大切に。笑顔は連鎖する。
【ポイント】
　①　口角が上がっている
　②　やさしい目元であること
　③　心がこもっていること

3）コミュニケーション
　人と人との関係にある意思疎通のこと。言葉・文字・身振りなどを活用して情報を相互に伝達すること。
　人と人とをつないでいくのがコミュニケーション。

社会生活を送る為には不可欠である。
　双方向のコミュニケーション直接的な対面による二者間のコミュニケーションは支援の効果や参加者の安心や自信にもつながっている。
【コミュニケーションの手段】
　①　言語的コミュニケーション
　　日常生活に中で最も多く使われる。記述された文字や点字，手話。人の感覚機能のなかでも視野や聴覚，触角を使う。
　②　非言語的コミュニケーション
　　態度や表情，姿勢，身振り手振りなど言葉にそのまま直訳できない身体的な表現を指す。
　非言語コミュニケーションは，言語的コミュニケーションに比べ，本音や細かな感情が表現されやすい。無意識に行っている場合は，いつのまにか習慣可しているものが多い為に注意が必要。
　参加者の非言語的コミュニケーション
膝や肩に手をおく。（−）　笑っていたり，ガッツポーズ（＋）
服の色が綺麗（＋）口に手をあてる　化粧をする
言葉にできない非言語的コミュニケーションを大切にする。

2．モジュール7―レクリエーションの理論
・レクリエーションとは
・4つの分類からのレクリエーションの理解　福祉レクリエーションの考え方

1）レクリエーションとは
　「レクリエーション」のイメージについて
　例）・皆であそぶ　・何かと何かのクッション
　　　・交流　・○○ゲーム　・元気になる
　『広辞苑』（岩波書店）＝仕事や勉強などの疲れを，休養や娯楽によって精神的・肉体的に回復すること。またそのために行う休養や娯楽。
由来…レクリエーションという外来語は，英語のRECRETIONを取り入れたものである。この語は，create（つくる）という語にre（再び）という接頭辞を加えたものである。意味は「再び創る」こと，すなわち「創り直し」ということになる。壊れた状態（怪我や病気）が癒えることになる。後には用例が日常的に広がって，一般的に疲労から元気を回復することを意味するようになり，さらには，元気回復のための遊びや楽しい活動自体を呼ぶことになった。[5]
定義…元気になる為の気晴らしや休養や楽しみの活動

２）レクリエーションの４つの分野

楽しい・嬉しい・気持ち良い・さっぱり・すっきりなどのプラスの感情にする。

気持ちが上向きになるように支援することが指導者として大切。

① スポーツ（楽しむことを目的として行われるスポーツ）ニュースポーツ

② カルチャー（創造・文化的な活動）クラフト・折り紙

③ ネイチャー（自然と親しむ活動）キャンプ・ウォークラリー

④ 福祉（全ての人に対して）

一人ひとりに合わせたレクリエーションの提供が大切。

例）・友達とお話しをする・カラオケ歌を歌う・好きな音楽を聴く・部屋の模様替えをする

３）ニュースポーツとは？

いつでも・どこでも・だれもが楽しむことを目的として考案されたスポーツ（ルールも技術も簡単）例）フットサル　インディアカ　ミニバレーボール　ドッチビー　ラケットテニス・雪合戦　カバディ　ゲートボール　ユニホック　キンボールなど百種類以上ある。

（ルールや道具等は参加者に合わせて変えている。）

４）福祉レクリエーションとは？

身体的・精神的・社会的に不利な条件を負っている人を主な対象として展開される多様なレクリーション・

・基礎生活…生命維持に必要な生活領域（睡眠・食事・排泄・入浴など）

・社会生活…自分と自分以外の人と組織の関わり関わりからなる生活領域（家庭　学校　会社）

・余暇生活…個人の裁量で自由にふるまうことのできる生活領域（人生80年のうち，余暇生活は約30万時間である）

※基礎生活　社会生活　余暇生活の３つ生活がバランスよくとれていることが大切であり，余暇生活の中で行われるのがレクリエーションである。

（１）生活のレクリエーション化

衣食住に関連する基礎生活場面にレク的，遊び感覚的な工夫をすることでより生活を心地よくすること。

（２）レクリエーションの生活化

個人の内発的な動機づけのもとに実践されるレクリエーション活動が自分の生活の中に大切な生活の一部として意識され，組み込まれること。

５）セラピューティックレクリエーション

アメリカで発展。治療で使用されるレクリエーション

付　記

本研究は，「平成26年度北翔大学北方圏生涯スポーツ研究センターの研究費」の助成を受けて実施されたものである。

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５）財団法人　日本レクリエーション協会編：レクリエーション支援の基礎. pp10～11, 2007.

介護予防運動プログラム指導者への研修プログラムの作成

Study on Development of a Training Program for Care Prevention Exercise Instructors

上　田　知　行[1)]　　井　出　幸二郎[1)]　　小坂井　留　美[2)]　　小　田　史　郎[2)]
小田嶋　政　子[3)]　　本　間　美　幸[2)]　　本　多　理　紗[3)]　　相　内　俊　一[3,4)]

Tomoyuki UEDA[1)]　　　Kojiro IDE[1)]　　　Rumi KOZAKAI[2)]　　　Shiro ODA[2)]
Masako ODAJIMA[3)]　　Miyuki HONMA[2)]　　Risa HONDA[3)]　　Toshikazu AIUCHI[3,4)]

キーワード：介護予防，指導者，養成，運動プログラム

Ⅰ．はじめに

　これまで産学官で協働した地域におけるソーシャルビジネスの研究として，「地域まるごと元気アッププログラム」を実施してきた。「地域まるごと元気アッププログラム」は行政・民間企業・非営利団体・大学の協働によって行なわれている。60歳以上の高齢者を中心とした一般町民に向けた体力測定会の結果による市民の生活体力の把握をはじめ，比較的低体力者から中程度の体力者までの3クラスの高齢者対象の運動教室，中学生と高齢者の世代間交流，高齢者向け音楽会などを平成22年から実施している[1) 2) 3) 4)]。

　これまで行なってきた「地域まるごと元気アッププログラム」（以下「まる元」）では，体力測定会や健康講演会，運動教室を実施してきた。そのうち運動教室については，自治体の介護予防事業のうち，地域支援事業にも位置づけられ一定の成果を果たしている[2) 3) 4)]。高齢化が進む北海道の地域市町村では，介護予防のための運動教室が実施されている。しかし，「まる元」運動教室のように，健康づくりのための運動指導の専門家である健康運動指導士に運動指導を任せるには，人的資源が不足しており，保健師や管理栄養士などが運動指導を担うケースも少なくない。さらに，継続した運動により，介護予防のための体力が維持されることは明らかになっているが，多くの事業では3か月間や半年間など，運動習慣を身に着けることまでは至っておらず，通年型の「まる元」運

動教室を導入しようとする自治体が増加することが予測される。こういったニーズに対応するには，体力の維持向上を図り，楽しく運動指導を行える運動指導者の輩出が不可欠であるが，この人的資源を輩出するための研修プログラムが整備されているとは言い難い。そこで本研究では，より質の高い運動指導者を輩出するために，健康運動指導士を取得して，地域における介護予防運動プログラムを指導しようとする運動指導者を育成するため研修プログラムを策定し実施した。

Ⅱ．概　要

1．目的

　高齢者の健康づくりや介護予防の運動指導者として，教室の指導能力，市町村の課題に合ったプログラムを展開する能力，教室運営上の他業種との連携等が必要となる。この研修では，健康運動指導士としての指導上の知識，ホスピタリティ，運動の動機づけ，リスク管理等を身につけ，地方市町村での運動指導ができることを目的とする。

2．到達目標

・健康運動指導士として，安心・安全・楽しく効果的で継続できる運動指導ができる。

・参加者のQOLを向上できるような動機づけができる。

・多様な体力の方々に対する運動プログラムの構成ができる。

・リスクを予測し，リスク管理ができる。

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）NPO）ソーシャルビジネス推進センター

・ホスピタリティを理解し，コミュニケーションがとれる。
・評価についての知識と方法を習得する。

3．参加条件

健康運動指導士または健康運動指導士と同等の知識や技能を有する者

4．開催時期および講義時間

研修は1モジュールを3時間とし16モジュールに加えて，特別講義8回からなる60時間の計画とした。実施した期間は，平成26年4月7日（月）から平成26年5月26日（月）の毎週月曜日に，各モジュール180分および特別講座90分を実施した。各モジュールと特別講義のテーマ

表1：各モジュールの概要と特別講義のテーマ

モジュール	内容
1	「テーマ」オリエンテーション 健康運動指導士としての基本的知識の確認。まる元各クラスの目的や内容の確認。指導者・支援者としての目標設定。
2	「テーマ」コミュニケーションワーク ホスピタリティについての理解とホスピタリティトレーニング。 指導者としてのホスピタリティについて。コミュニケーション技術について。コミュニケーショントレーニング。
3	「テーマ」アイスブレーキング アイスブレーキングの意義と基本技術　様々なアイスブレーキングの体験とアレンジ方法。
4	「テーマ」アイスブレーキング アイスブレーキングの基本プログラミングと実践。（アセスメントの理解）
5	「テーマ」トレーニング トレーニング論を踏まえた運動プログラムの構成 ストレッチング・整理体操の確認（注意点なども含めて）と実践
6	「テーマ」トレーニング まる元で行っている筋力トレーニングの確認と実践　ボールを使用しての軽体操の実践
7	「テーマ」レクリエーションの理論 レクリエーションとは？　4つの分類からのレクリエーションの理解　福祉レクリエーションの考え方
8	「テーマ」レクリエーションの実際① 虚弱な高齢者へのレクリエーションの実際（Aクラスの想定） レクリエーション実施中に予測されるリスクと回避方法
9	「テーマ」レクリエーションの実際②③ 元気な高齢者へのレクリエーションの実際（B・Cクラスの想定） 身近な素材や道具を使用した様々なレクリエーションやリズム体操
10	「テーマ」レクリエーションの計画・実践 レクリエーションの指導案作成の説明と指導案作成をする。実際に実践をし，お互いに評価を行う。
11	「テーマ」ケーススタディ① 様々な事例からプログラムを構成できるようにする。指導案作成の説明
12	「テーマ」ケーススタディ② 様々な事例からプログラムを構成できるようにする。指導案作成の説明。
13	「テーマ」プログラム実践発表 様々なケースを想定し，プログラムの説明を行い発表する。（時間管理も含む。）自己の評価・反省と他者からの評価を行う。（Aクラス想定）
14	「テーマ」プログラム実践発表 様々なケースを想定し，プログラムの説明を行い発表する。（時間管理も含む。）自己の評価・反省と他者からの評価を行う（Aクラス想定）
15	「テーマ」プログラム実践発表 様々なケースを想定し，プログラムの説明を行い発表する。（時間管理も含む）自己の評価・反省と他者からの評価を行う。（混合クラス想定）
16	「テーマ」プログラム実践発表 様々なケースを想定し，プログラムの説明を行い発表する。（時間管理も含む）自己の評価・反省と他者からの評価を行う。（混合クラス想定）
特別講座	内容
1	「バランスト・スコアカードと顧客志向」
2	「健食プログラム」
3	「行動変容を促す基礎要因」
4	「体力行動面から捉えた事業評価」
5	「睡眠力」
6	「老年学」
7	「運動と認知機能」
8	「地域の介護認定者の現状　介助の基本など」

を表1に掲載する。

Ⅲ．指導プログラムの内容

　各モジュールのうち，モジュール2およびモジュール7について次のようにまとめた。

1．モジュール2─コミュニケーションワーク

・ホスピタリティについての理解とホスピタリティトレーニング
・指導者としてのホスピタリティ・コミュニケーション技術・コミュニケーショントレーニング

1）あいさつ
・仲間に関心をもつこと（共感）から人間関係がスタートする。
・自分をオープンにすれば（自己開示）すればするほど自分に好意を持ってくれる。
人間関係のスタートは共感と自己開示から成り立つ。
※初対面でいきなり自己紹介はしにくい
　　人と会うとまず何をする？
　　挨拶は，人間関係を作る潤滑油
【あいさつのポイント】
　　あ＝明るくあいさつ
　　い＝生き生きとあいさつ
　　さ＝さっと自分からあいさつ
　　つ＝積み重ねてあいさつ
あいさつは，「あなたに近づきたい。」「私はあなたに対して心を開いています。」という意味がある。
【印象を決めるポイント】
　　① 外見（表情・身だしなみ）例）笑顔・髪・服装・つめなど
　　② 態度（動作）例）物を投げる　上から目線
　　③ 話し方　例）声のトーン　強弱　敬語
　　④ 話す内容　例）知識など

2）笑顔
　　笑顔は人間だけにしかできない特権。笑顔を大切に。笑顔は連鎖する。
【ポイント】
　　① 口角が上がっている
　　② やさしい目元であること
　　③ 心がこもっていること

3）コミュニケーション
　　人と人との関係にある意思疎通のこと。言葉・文字・身振りなどを活用して情報を相互に伝達すること。
　　人と人とをつないでいくのがコミュニケーション。

社会生活を送る為には不可欠である。
　　双方向のコミュニケーション直接的な対面による二者間のコミュニケーションは支援の効果や参加者の安心や自信にもつながっている。
【コミュニケーションの手段】
　　① 言語的コミュニケーション
　　　　日常生活に中で最も多く使われる。記述された文字や点字，手話。人の感覚機能のなかでも視野や聴覚，触角を使う。
　　② 非言語的コミュニケーション
　　　　態度や表情，姿勢，身振り手振りなど言葉にそのまま直訳できない身体的な表現を指す。
　　非言語コミュニケーションは，言語的コミュニケーションに比べ，本音や細かな感情が表現されやすい。無意識に行っている場合は，いつのまにか習慣可しているものが多い為に注意が必要。
　　参加者の非言語的コミュニケーション
膝や肩に手をおく。（−）　笑っていたり，ガッツポーズ（＋）
服の色が綺麗（＋）口に手をあてる　化粧をする
言葉にできない非言語的コミュニケーションを大切にする。

2．モジュール7─レクリエーションの理論

・レクリエーションとは
・4つの分類からのレクリエーションの理解　福祉レクリエーションの考え方

1）レクリエーションとは
「レクリエーション」のイメージについて
　例）・皆であそぶ　・何かと何かのクッション
　　　　・交流　・○○ゲーム　・元気になる
『広辞苑』（岩波書店）＝仕事や勉強などの疲れを，休養や娯楽によって精神的・肉体的に回復すること。またそのために行う休養や娯楽。
由来…レクリエーションという外来語は，英語のRECRETIONを取り入れたものである。この語は，create（つくる）という語にre（再び）という接頭辞を加えたものである。意味は「再び創る」こと，すなわち「創り直し」ということになる。壊れた状態（怪我や病気）が癒えることになる。後には用例が日常的に広がって，一般的に疲労から元気を回復することを意味するようになり，さらには，元気回復のための遊びや楽しい活動自体を呼ぶことになった。[5]
定義…元気になる為の気晴らしや休養や楽しみの活動

２）レクリエーションの４つの分野

楽しい・嬉しい・気持ち良い・さっぱり・すっきりなどのプラスの感情にする。

気持ちが上向きになるように支援することが指導者として大切。

① スポーツ（楽しむことを目的として行われるスポーツ）ニュースポーツ

② カルチャー（創造・文化的な活動）クラフト・折り紙

③ ネイチャー（自然と親しむ活動）キャンプ・ウォークラリー

④ 福祉（全ての人に対して）

一人ひとりに合わせたレクリエーションの提供が大切。

例）・友達とお話しをする・カラオケ歌を歌う・好きな音楽を聴く・部屋の模様替えをする

３）ニュースポーツとは？

いつでも・どこでも・だれもが楽しむことを目的として考案されたスポーツ（ルールも技術も簡単）例）フットサル インディアカ ミニバレーボール ドッチビー ラケットテニス・雪合戦 カバディ ゲートボール ユニホック キンボールなど百種類以上ある。

（ルールや道具等は参加者に合わせて変えている。）

４）福祉レクリエーションとは？

身体的・精神的・社会的に不利な条件を負っている人を主な対象として展開される多様なレクリーション

・基礎生活…生命維持に必要な生活領域（睡眠・食事・排泄・入浴など）

・社会生活…自分と自分以外の人と組織の関わり関わりからなる生活領域（家庭 学校 会社）

・余暇生活…個人の裁量で自由にふるまうことのできる生活領域（人生80年のうち，余暇生活は約30万時間である）

※基礎生活 社会生活 余暇生活の３つ生活がバランスよくとれていることが大切であり，余暇生活の中で行われるのがレクリエーションである。

（１）生活のレクリエーション化

衣食住に関連する基礎生活場面にレク的，遊び感覚的な工夫をすることでより生活を心地よくすること。

（２）レクリエーションの生活化

個人の内発的な動機づけのもとに実践されるレクリエーション活動が自分の生活の中に大切な生活の一部として意識され，組み込まれること。

５）セラピューティックレクリエーション

アメリカで発展。治療で使用されるレクリエーション

付 記

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１年間の地域まるごと元気アッププログラム参加が高齢者の認知機能に及ぼす影響

Influence of Participation in Chiiki Marugoto Genki Up Program for an Year on Cognitive Function in the Elderly.

井　出　幸二郎[1]　　上　田　知　行[1]　　小坂井　留　美[1]
本　多　理　沙[2]　　小　田　史　郎[1]　　相　内　俊　一[2]

Kojiro IDE[1]　　Tomoyuki UEDA[1]　　Rumi KOZAKAI[1]
Risa HONDA[2]　　Shiro ODA[1]　　Toshikazu AIUCHI[2]

キーワード：認知機能，身体機能

はじめに

　北翔大学生涯スポーツ学部は，小樽商科大学大学院ビジネススクール，コープさっぽろ，赤平市や余市町等の自治体の４者による産学官連携の協同プロジェクトとして，「地域まるごと元気アッププログラム」を運営している。本事業は，地域活性化を目指し，「行政，医療機関，大学，民間の協同プロジェクトの構築」，「高齢者に対する健康増進プログラムの実施」，「地域住民の中に運動指導層を構築するサポーター育成事業」等をコンセプトに行われている。本プログラムの具体的内容は，赤平市や余市町において，体力測定会，健康講演会，運動教室の運営，地域住民のサポーター養成等である。これまで，本事業における高齢者を対象にした健康増進プログラムを通して参加者の平衡機能や下肢パワー等の体力が改善することを報告し，転倒予防とその先にある介護予防に有効であることが示唆されてきた[1,2]。一方，本事業における健康増進プログラムが認知症発症予防対策としても有効であるかは明らかでない。本報では，１年間の地域まるごと元気アッププログラムが参加者の認知機能に及ぼす影響について報告する。

Ⅰ．健康増進プログラム

　プログラムは，１回60分間とし，週１回の頻度で開催した。健康増進プログラムは，対象者の体力レベルから３つのクラスに分けられ，それぞれ，低体力者向け運動プログラム（Aクラス），中等度の体力者向け運動プログラム（Bクラス），指導者層向け運動プログラム（Cクラス）の３クラスとした。Aクラスでは，仲間づくり・仲間意識を通じて閉じこもり予防につなげることを目標とし，椅子での体操を中心とした運動プログラムで，レクリエーションや椅子を用いた下肢の筋力トレーニング，簡単な軽体操などを展開した。Bクラスでは，日常生活動作が維持するのに必要な体力を維持することを目標とし，椅子と足踏みでの体操を中心とした運動プログラムで，レクリエーションや椅子を用いた筋力トレーニング，ラダー等での足踏みなどを展開した。Cクラスでは，教室以外で自立して運動できるようになることを目標とし，筋力トレーニングや歩行の正しい方法を学び，レクリエーションやニュースポーツ，筋トレーニング，ウォーキングや体操などを展開した。運動指導は健康運動指導士が担当し，それぞれ３つのグループの体力にあったプログラムを作成し指導にあたり，当大学学生が補助にあたった。

Ⅲ．認知機能測定

　認知機能の測定には，集団で一斉に測定可能なファイブコグを用いた。対象者はスクリーンに呈示された課題を解き，ファイブコグ解答用紙に解答を記入し，測定後に検定者が採点をした。ファイブコグでは，運動課題，並行課題，再生課題，時計課題，言語課題，類似課題の６種類の課題があり，それぞれ，手指の運動機能，注意分割機能，エピソード記憶，視空間認知，言語流暢性，

１）北翔大学生涯スポーツ学部スポーツ教育学科
２）北翔大学北方圏生涯スポーツ研究センター

思考能力を評価した。測定は，余市町中央公民館の一室で行われた。解答評価は，NPO認知症予防サポートセンターに依頼した。

IV. 統 計

平成26年度プログラム開始前にIADL，年齢，教育年数についてグループ間に差が認められるか，1元配置の分散分析を用いて検討した。体力別にABCの3つのクラスに分かれて展開された1年間の元気アッププログラムが認知機能に有効か否か，ファイブコグの運動課題，並行課題，再生課題，時計課題，言語課題，類似課題の1年間のプログラム前後の差を，2元配置の分散分析（group（体力別Aクラス，Bクラス，Cクラス）及びtime（プログラム前後）を用いて検討した。

V. 結果及び考察

表1に各グループの年齢，教育年数，手段的日常生活動作（instrumental activity of daily living ; IADL）の結果を示した。分散分析の結果，年齢，教育年数，IADLにグループ間の違いは認められなかった。

図1に3つのグループ別にプログラム前後のファイブコグ課題の結果を示した。分散分析の結果，ファイブコグ課題のうち運動課題ではtimeに主効果が認められたが，その他の課題では主効果は認めらなかった。一方，ファイブコグ課題の全ての課題において，groupに主効果が認められた。交互作用はどの課題においても認められなかった。

運動と認知機能に関して，現在まで多くの介入研究により運動プログラムが認知機能の改善に有効であることが報告されている[3,4]。これらの研究では，運動習慣がない高齢者を対象者にしたのに対して，本研究では元気アッププログラムの継続参加者と新規参加者どちらも含まれていることに加え，ウォーキング等の習慣的な運動を行っている者も含まれている。運動習慣がある高齢者やプログラムの継続者では既に認知機能に対する運動の効果を得ている可能性も考えられ，今後は運動習慣を持たない高齢者を対象にした，元気アッププログラムの認知機能に対する有効性の検証が課題である。一方，運動の有効性を示した他の研究では，マシーンを用いた中高強度の抵抗運動[4]や中強度の有酸素運動[3]がトレーニング方法として用いられたのに対して，本研究ではレクリエーションやチェアエクササイズ，ラダーを用いた調整運動等，低強度の運動が主として用いられている。また，他の研究では，週に複数回以上のトレーニング頻度でトレーニングを展開したのに対して，本研究では週1回の

表1. 地域まるごと元気アッププログラム参加者の体力レベルで分けられた3クラスの対象者数、年齢、IADL、教育年数

	Aクラス；低体力者		Bクラス；中体力者		Cクラス；高体力者	
対象者数	11		9		7	
年齢（歳）	73.5	± 4.7	72.1	± 5.0	71.3	± 6.1
IADL（点）	13.3	± 1.6	12.7	± 1.3	14.0	± 1.7
教育年数（年）	10.1	± 1.5	11.1	± 2.9	12.0	± 1.9

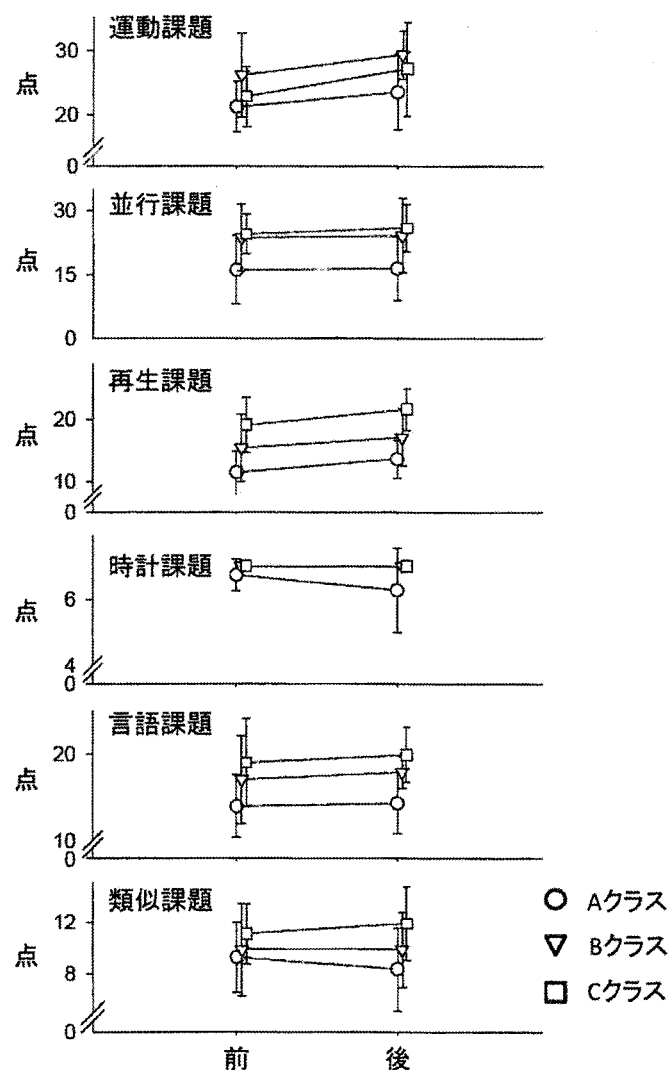IADL：instrumental activity of daily living, 手段的日常生活動作

図1. 1年間の地域まるごと元気アッププログラム前後のファイヴコグ課題成績

頻度で行われた。このような点が結果に影響している可能性も考えられ，今後考慮に入れるべき点である。

ファイブコグ課題の全ての課題において体力別に分けられたグループ間で違いが認められた。今回は認知機能に対するプログラムの明確な効果は認められなかったが，体力の高さが高齢者の認知機能の変化に影響するという報告[5]もあり，本プログラムは低強度で展開頻度も他の研究と比べ少ないが参加者の継続率が高く，長期的観点からの評価が必要である。

　地域まるごと元気アッププログラムにおける運動プログラム内容は，レクリエーションや軽運動，リズム体操等が主体であり，それらを通して参加者同士のコミュニケーションが多くの場面で生じるように指導者によって工夫されている点と楽しさやコミュニケーションを重視しプログラムに対する継続性を促進している点が大きな特徴である。個人が単独で行う運動と異なり，こういった運動プログラムに参加すると運動の効果だけでなく社会的な相互作用が生じ，認知機能に好影響を与えることが期待される。短期間で認知機能の改善効果が認められた有酸素運動[3]や抵抗運動[4]を用いたプログラムと比較して，本事業における運動プログラムは低強度に抑えられてはいるが，低強度ながらも身体機能を刺激しつつ，楽しさやコミュニケーションを取り入れており，生理学的側面だけではなく，心理学的側面からも高齢者の認知機能に働きかけがされている。このようなプログラムに長期間にわたり参加することが高齢者の認知機能と身体機能の維持・改善に有効であることを明らかにすることも，今後の課題として挙げられる。

謝　辞

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北海道の在宅高年齢者における体力測定継続に関連する身体・行動要因

Health and Behavioral Characteristics as Predictors for the Continuity of Participation in Physical Fitness Checkups among Community-Living Older People in Hokkaido

小 坂 井 留 美[1)]　　上 田 知 行[2)]　　井 出 幸 二 郎[2)]
小 田 史 郎[1)]　　本 多 理 沙[3)]　　相 内 俊 一[3,4)]

Rumi KOZAKAI[1)]　　Tomoyuki UEDA[2)]　　Kojiro IDE[2)]
Shiro ODA[1)]　　Risa HONDA[3)]　　Toshikazu AIUCHI[3,4)]

キーワード：高年齢者，体力測定，外出，追跡調査，北方圏

Ⅰ．緒　言

　定期的な健康調査や体力測定は，疾病の罹患や体力低下を予防するための有効な手段の一つである。このような調査・測定を通じ，高齢者本人に対して現在の身体状況や体調変化への気づきを促すことや地域における保健・福祉関係者が状況を把握することは，要介護予防や認知症予防につなげる意味でも重要といえる。

　北翔大学生涯スポーツ学部は，地域活性化を目指して産・学・官が協働するプロジェクトを2010年より開始し，運動教室とともに年に1回の体力測定会を実施してきた[1,2]。本体力測定会では，2012年以降体力項目だけでなく，生活習慣，運動経験や社会活動状況についても調査を行っている[3]。本体力測定会は，開始以来継続して実施してきたが，毎年新規参加者を得る一方，一度きりの参加者も多いのが現状である。心身機能の低下を未然に，あるいは早期に把握し，改善に結び付けるためには，定期的な調査・測定が必要であり，継続的な体力測定会参加者を増やすことは本研究分野における課題の一つと考える。

　そこで，本研究では，体力測定会参加者の1年間の追跡調査から，体力測定会の継続参加に関わる要因を明らかにすることを目的とした。

Ⅱ．方　法

1．対象

　本研究は，A市介護健康推進課とNPO法人ソーシャルビジネス推進センター・コープさっぽろ経営企画室・北翔大学生涯スポーツ学部が連携して行う「A・地域まるごと元気アッププログラム」，およびB町民生部と同3機関が連携して行う「B・地域まるごと元気アッププログラム」（以下，両プログラムを「まる元」と略す。）の一環として実施された。本研究の対象者は，A市・B町に在住する「まる元」参加者および，広報や地域での研修会や交流会，高齢者施設における呼びかけで実施した平成24年度体力測定会（A市：2012年8月29〜31日，B町：2012年8月20〜21日）に参加した60歳以上の高年齢者で，調査への同意の得られた男女384名である。尚，本研究では厚生労働省の表現にならい[4]，対象とした60歳以上の人について「高年齢者」という単語を用いて表す。

　体力測定の継続については，約1年後の平成25年度体力測定会（A市：2013年8月21〜23日，B町：2012年8月26日・9月2日）への参加の有無を確認した。

　「まる元」は，北翔大学大学院・北翔大学・北翔大学短期大学部研究倫理審査委員会の承認を受け，対象者全員からインフォームドコンセントの後，同意書を得て実

1）北翔大学生涯スポーツ学部健康福祉学科
2）北翔大学生涯スポーツ学部スポーツ教育学科
3）北翔大学北方圏生涯スポーツ研究センター
4）NPO法人ソーシャルビジネス推進センター

施している。

２．分析項目
１）身体・生活状況
基本的な健康状態や生活習慣，転倒や外出頻度について，自記式の調査票を用いて回答を得た。項目は次の通りである。結婚状況（未婚／既婚／別居／離婚／死別），同居家族（独居／夫婦のみ／その他），就労状況（これまでの就労年数，現在の就労：無職／主婦・夫／フルタイム／パートタイム），既往歴：高血圧，高コレステロール血症，狭心症，心筋梗塞，糖尿病，脳卒中，腰痛，関節痛，骨粗しょう症，がん，その他（あり／なし），不整脈（なし／自覚している・医師から指摘を受けた），自覚的健康度（非常に良い／良い／普通／悪い／非常に悪い），過去１年間の転倒（あり／なし），転倒恐怖（少し・とても怖い／なし），喫煙状況（以前から吸わない／やめた／現在吸っている），外出頻度（ほとんど外出しない／１週間に１回程度／2-3日に１回程度／毎日１回以上）。

血圧は，自動血圧計および血圧に心配のある場合は保健師による水銀血圧計での測定を行った。体格は，身長と体重を測定し，体重を身長の二乗で除したBody Mass Index（BMI；kg/㎡）を算出した。体力指標について，体力測定会では文部科学省新体力テストに準じた測定項目および移動・筋力項目を測定したが，本研究では握力と10m全力歩行能力について分析した。

２）活動能力
活動能力の測定には，老研式活動能力指標を用いた[5]。本指標は，地域での独立した生活を営む上で必要な活動能力を測定するために開発された尺度である。高齢者の社会的側面を含めた生活機能の把握に有用な指標と考えられている。下位尺度として，「手段的自立」（５項目），「知的能動性」（４項目），「社会的役割」（４項目）がある。計13項目からなり，２件法（できる＝１点，できない＝０点）の13点満点で，高得点程活動能力が高いことを示す。

３）社会活動状況
社会活動は，約１年程度を振り返り，人とのつながりや地域での活動等に関する９項目：「配偶者や家族とのつながり」，「友人とのつきあい」，「家事」，「社会奉仕活動」，「地域活動」，「旅行」，「学習・研究」，「運動・スポーツ」，「趣味・娯楽」の活動の有無について[6]，1）と同じ調査票を用いて回答を得た。

４）過去の運動歴
12歳から現在までに定期的な運動（１回20分・週１回・

１年以上）を行ったか（あり／なし）について[7]，「12-19歳」，「20-29歳」，「30-39歳」，「40-49歳」，「50-59歳」に分けて1）と同じ調査票を用いて回答を得た。また，現在まる元運動教室に参加しているか否かを確認した。

５）基本属性
年齢（歳：生年月日から2012年４月１日時点の年齢を算出），性（男性／女性），教育年数（年）について1）と同じ調査票を用いて回答を得た。

３．統計解析
解析では，基礎分析として平成24年度（2012年）の体力測定会のみに参加した群と平成24年度・平成25年度（2012年・2013年）の両体力測定会に参加した群の２群に分け，各測定項目についてカテゴリ変数は人数割合（％）を示しカイ二乗検定を，連続変数は平均値±標準偏差で示して一要因分散分析を行った。体力測定会の継続参加に関連する要因の検討では，１年後の体力測定会参加を目的変数，基礎分析で関連のみとめられた測定項目を従属変数，年齢，性，運動教室参加を調整変数とした多重ロジスティック回帰分析を行った。解析にはSPSS version19を用い（SPSS Inc., Chicago, IL），有意水準はすべて５％未満とした。

Ⅲ．結　果

Table 1は，体力測定会の継続参加の有無別にベースラインの対象者の基本属性および身体・生活状況等の測定項目を示した。2012年のみの参加者は206名（53.6％），2012-2013年継続参加者は178名（46.4％）であった。身体・生活状況等の各測定項目において継続参加の有無で差が認められたのは，年齢，性，就労状況，喫煙習慣，外出頻度，老研式活動能力指標下位尺度の知的能動性，運動教室への参加であった（$p<0.05$）。継続参加者では，年齢が若く，女性が多く，主婦（夫）やパート従事者が多く，喫煙習慣者が少なく，外出頻度が高く，知的能動性が高く，運動実践者が多い結果であった。その他の家族・住居形態，各疾患の既往歴，体格，体力，社会活動性，運動経験項目等では，有意な差は認められなかった。

基本属性（年齢・性）と介入である運動教室参加を調整変数とした多重ロジスティック回帰分析の結果（Model1），有意なオッズ比がみとめられたのは，外出頻度が2-3日に１回（OR 2.99, 95%CI 1.37-6.56）であった。全ての変数を投入したステップワイズ法でも分析を行ったが（Model2），外出頻度が2-3日に１回の有意性は保たれていた（OR 2.72, 95%CI 1.27-5.84）。毎日外出する人においても，オッズ比が高まる傾向ではあったが

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Table 1. Baseline characteristics of participants who did or did not attend (2012/2013, 2012) the follow-up physical fitness check-up

		2012 (n = 206)	2012/2013 (n = 178)	p value
Regidental city	%			.646
A		52.4	47.6	0.6
B		54.8	45.2	13.8
Age	year	74.2±7.0	72.2±6.3	.003
Sex	%			.018
Men		63.1	36.9	
Women		49.8	50.2	
Education	year	10.4±2.6	10.8±2.6	.110
Marital status	%			.765
Never		4.5	5.2	
Married		58.8	62.5	
Separation		2.0	0.6	
Divorce		3.0	2.3	
Bereavement		31.7	30.5	
Household membership	%			.632
Alone		31.0	35.4	
Couple		52.0	49.7	
Other (ex. with children)		17.0	14.9	
Total number of years worked	year	29.1±17.3	26.3±17.3	.162
Work status	%			.004
Unemployed		66.8	54.0	
Housewife/husband		21.6	35.6	
Full time job		7.5	3.4	
Part time job		4.0	6.9	
Prevalent diseased	%			
Hypertension		55.4	47.3	.119
High chresterol		23.9	31.7	.093
Ischemic heart disease		6.9	5.4	.542
Heart attack		4.0	4.8	.697
Diabetes		11.9	13.2	.708
Stroke		5.4	3.6	.398
Arturitic pain : back		32.3	31.1	.806
Arturitic pain : knee		13.8	14.8	.792
Osteoporosis		13.4	8.4	.135
Irregular heartbeat		16.7	15.5	.811
Muscleloskeletal pain		62.9	59.9	.554
Self-rated health	%			.330
Excellent		9.4	10.5	
Very good		28.1	23.8	
Good		51.2	58.7	
Fair		10.3	7.0	
Poor		1.0	0.0	
Fall	%			
Past year (Yes)		30.8	27.4	.468
Fear of falling (a little/very scared)		56.8	63.1	.461
Smoking habit	%			.010
Never		64.3	77.8	
Quit		23.1	16.5	
Current		12.6	5.7	
Going outdoors	%			.005
Very few		2.5	1.1	
Once a week		14.2	6.9	
2-3 days a week		30.4	46.3	
Every day		52.9	45.7	
Blood pressure (BP)	mm /H			
Systolic BP		135.9±17.6	133.1±15.8	.099
Diastolic BP		73.3±10.3	74.5±11.2	.275
Body size				
Height	cm	152.7±8.9	152.9±7.7	.794
Weight	kg	55.6±9.1	55.3±8.6	.764
BMI	kg/m²	23.9±3.3	23.7±3.2	.507
Grip strength	kg	26.4±8.6	26.2±7.3	.842
Walking speed	sec.			
Maximum		6.0±2.0	5.7±2.2	.555

Table 1. Baseline characteristics of participants who did or did not attend (2012/2013, 2012) the follow-up physical fitness check-up (continue)

		2012 (n = 206)	2012/2013 (n = 178)	p value
TMIG Index of Competence marks				
Instrumental Self-Maintenance		4.8±0.5	4.8±0.6	.758
Intellectual Activity		3.5±0.8	3.7±0.6	.023
Social Role		3.4±0.9	3.4±0.9	.921
Total		11.7±1.7	11.9±1.7	.423
Social activity	%			
With family		76.1	73.3	.530
With friends		84.9	81.8	.423
Housework		75.1	79.4	.319
Volunteer		29.3	33.5	.372
Community activities		36.6	43.2	.189
Trip		49.8	52.3	.622
Courses or lectures		24.4	25.7	.766
Sports		44.4	56.8	.016
Hobbies		67.3	61.4	.226
Exercise experiments	%			
10 - 19 years		31.2	25.7	.241
20 - 29		17.8	14.3	.352
30 - 39		14.4	15.4	.770
40 - 49		16.5	17.7	.755
50 - 59		21.9	26.9	.262
Participants in exercise class		12.1	39.9	<.0001

BMI, Body mass index; Categorical variables are presented by % and analyzed using Chi-square test. Continuous variables are presented means +/- standard decviation and analyzed one-way ANOVA. Bold represents significant p-value (<0.05).

Table 2. Odds ratio and 95% confidence interval for those who participated in the follow-up

	Model 1		Model 2	
	OR	95%CI	OR	95%CI
Smoking				
Never		ref.		
Quit	0.61	0.29 - 1.29		
Current	0.40	0.16 - 1.03		
Outgo				
1 day/1w or less		ref.		ref.
2 - 3 day/1w	2.99	1.37 - 6.56	2.72	1.27 - 5.84
Everyday	1.61	0.75 - 3.47	1.55	0.74 - 3.27
Intellectual activity				
1 mark or less		ref.		
2 - 3 marks	1.07	0.17 - 6.88.		
4 marks	1.15	0.18 - 7.20.		
Work status				
No		ref.		
House wife/husband	1.43	0.82 - 2.52		
Fulltime job	0.49	0.17 - 1.45		
Parttime job	2.05	0.72 - 5.84		

OR, odds ratio; CI, confidence interval; ref., reference. Model1: adjusted for age, sex, regular exercise. Model2: forward selection method. Bold represents significant p-value (<0.05)

有意ではなかった（Model1: OR 1.61, 95%CI 0.75-3.47, Model2: OR 1.55, 95%CI 0.74-3.27）（Table 2）。

Ⅳ．考　察

本研究では，北海道在宅高年齢者における体力測定会継続の要因について身体特性や生活習慣，社会活動性や運動経験から検討を行った。その結果，継続参加者は約46％と半数以下であり，継続参加には外出頻度が関連することが示された。

外出頻度は，高齢者において問題とされる「閉じこもり」の目安となる指標である。「閉じこもり」は，高齢者の社会的な孤立，さらに孤独死につながる可能性を含むことから老年学分野で注目されてきた[8]。本研究では，閉じこもりの目安とされる「週に1回」もしくはそれ以下の外出頻度の場合を基準にオッズ比を計算したところ，外出頻度が多いと継続参加への可能性が高まることが示された。その度合いは，週に2-3回程度の外出頻度で約3倍であった。ほぼ毎日外出する場合も継続参加への可能性は高まる傾向にあったが，有意ではなかった。この背景として毎日外出する人では，忙しいために体力測定会の参加が難しいケースのあることが考えられた。中高齢者の運動・スポーツへの対応は，1年間全く運動を行わない人と運動習慣を持ち体力の高い人との二極化傾向が指摘されているが[9]，対象地域において，日常活動性として閉じこもりを心配される高年齢者のある一方，活動的な高年齢者も一定数あることが伺われた。高齢期は社会活動性の狭まりが問題となるため[10]，外出の機会の多いことは，社会活動性が高い好ましい状況と捉えることができる。しかし，活動性の高い人では，活動範囲の広がりから転倒やケガのリスクが高まることにも注意が必要である。活動性の高い高年齢者に対しても，健康の維持増進や転倒予防への意識付けを促し，定期的な健康・体力チェックの重要性について理解を進めて行く必要があると考えられた。活動性の高い人の積極的な参加が実現できれば，こういった方々自身が体力測定会の運営や支援者となることが期待できる。「介護予防サポーター」など高齢者自身による介護予防支援体制が広まってきており[11]，高齢者自身が地域の健康づくりの担い手となる方向へも発展できる可能性がある。

一方，閉じこもりに近い外出頻度の少ない人では，1度は体力測定会に参加したものの，相対的に継続参加のされにくいことが示された。体力測定会への参加を通じた健康づくり・閉じこもり予防に向けて，外出頻度の調査結果を活用し，重点的なフォローアップや呼びかけを進めていくことが重要と考えられた。

本研究では，身体・生活状況，体格・体力，社会活動性，運動経験など多くの要因について検討したが，外出頻度以外のほとんどの項目において継続との関連はみとめられなかった。基礎分析においてのみ，継続参加の有無により喫煙習慣，知的能動性指標，就労状況に有意差がみとめられた。喫煙習慣のある人では，継続参加者の人数割合が低かった。喫煙は，死亡や疾患の最も大きな原因の一つとされている[12]。喫煙の弊害について知識の普及が進む中で，喫煙習慣は健康意識の多寡に関連すると考えられ，健康意識の低い人では，健康・体力維持に向けた取り組みへの参加も続かない可能性を示すと考えられた。知的能動性では，高い人で継続参加の人数割合が高かった。知的能動性では，質問項目に「健康についての記事や番組に関心があるか」の問いがあり，喫煙習慣とは逆に健康などへの興味関心のある人では，継続参加の多くなる可能性が確認できた。就労状況は，各要素を詳細にみるとフルタイムでの仕事のある人で継続者の人数割合が低く，パートタイムや主夫・主婦で継続者の多い傾向にあり，これは毎日外出する人で継続傾向の弱まった外出頻度の結果を補足する結果であったと考えられた。無職である人では継続参加者が少なかったことから，無職であり外出頻度が低くなっている人で，継続できていない状況が考えられた。

健康調査や体力測定などの参加者では，元気な人の参加に偏ることがしばしば指摘されるが，本研究では継続的な参加と体格や体力，疾患の有無については関連がみとめられなかった。運動・スポーツ活動の規定には，その前提，実現，強化要因として多くの要因が考えられている[13]。本研究で取り上げた身体要因以外にも，利便性などの環境要因や直接的なきっかけ（例えば，友人の誘い）など様々な要因が挙げられており，定期的な調査・測定の継続要因の検討には，さらに包括的な研究が必要と考える。

尚，地域差では調査2地域で継続参加率に差は認められなかった。両地域の背景は異なるが，本研究結果は両地域を隔てず今後の活動発展に寄与できると考える。

本研究の限界として次の点が挙げられる。第一に，本研究で用いた分析項目は主に自記式の質問紙による回答であるため，質問への理解不足やリコールバイアスの影響は避けられない。これを少しでも防ぐために，保健師および体育系大学生や教員が質問票に沿って聞き取ることや回答の確認をできる限り行った。第二に，本研究では追跡期間は1年であり，2回の体力測定会への参加を取り上げたのみである。継続性については，今後さらに長期的な観察が必要であると考える。

以上のような限界はあるが，本研究は北海道における在宅高年齢者を対象に，体力測定会への継続的な参加に関連する要因として，身体・生活状況，社会活動状況，

過去の運動経験について検討し，外出頻度との関連を示すことができた。北海道の積雪寒冷地域という気候・環境の下でも，高齢まで自立した活動を営む社会の実現に向けて，地域での実践活動の拡充と評価は重要と考える。本研究で得られた体力測定会継続参加者の特性は，その一助になると考える。

V．要　約

本研究では，北海道在宅高年齢者384名を対象に，1年間の追跡調査を行い，体力測定会への参加の有無と身体状況，社会活動状況，運動経験との関連について検討した。継続参加を目的変数とした多重ロジスティック解析を行った結果，体力測定の継続的な参加に外出頻度の高いことが関連し，週2-3回外出する人では週1回以下の外出頻度の人に比べ約3倍も参加の可能性が高まることが示された。外出頻度に着目した地域高年齢者への働きがけが，健康・体力の維持増進に繋がっていくことが示唆された。

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Abstract

The purpose of the present study was to determine the predictors for continuity of participation in physical fitness checkups in community-living older people in a 1-year follow-up study. The subjects were 384 people aged 60 years and over, who participated in the physical fitness checkups organized by local municipalities and Hokusho University in 2012. Individual attributes, health condition, lifestyle, Tokyo Metropolitan Institute of Gerontology Index of Competence (TMIG Index of Competence), social activities and exercise experience were checked by questionnaire. Body size, grip strength and maximum walking speed were measured using standard techniques. The follow-up checkup was conducted one year later. The measurements were compared between dropouts and participants in the follow-up. Multiple logistic regression models with participation in the follow-up as outcomes were used to estimate the association of baseline characteristics. The number of dropouts and participants in the follow-up was 206 (53.6%) and 178 (46.4 %), respectively. Age, sex, intellectual activity score in TMIG Index of Competence, work status, smoking habits, frequency of going outdoors and regular exercise were significantly different between dropouts and participants in the follow-up. After controlling for all potential confounders, people who go outdoors every other day were significantly more likely to participate in the follow-up (OR 2.99, 95%CI 1.37-6.56). Using the forward selection model, the association was still significant (OR 2.72, 95%CI 1.27-5.84). Our results suggest that frequent going outdoors could be an effective target for preventing health and functional decline among community-living older people in Hokkaido.

Keywords : older people, physical fitness checkup, going outdoors, follow-up study, northern region

Original Article

Inverse Relationship between Sleep Duration and Cardio-Ankle Vascular Index in Children

Noriteru Morita[1], Isao Kambayashi[2], Tomoyasu Okuda[1], Shiro Oda[3], Shingo Takada[4], Toshihiro Nakajima[5], Noriyuki Shide[1], Hisashi Shinkaiya[1] and Koichi Okita[3]

[1] Department of Sport Cultural Studies, Hokkaido University of Education, Iwamizawa, Japan
[2] Hokkaido University of Education, Sapporo, Japan
[3] Department of Sport Education, Hokusho University, Ebetsu, Japan
[4] Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[5] Hokkaido University of Science, Sapporo, Japan

Aim: Poor sleep has been shown to be associated with the development of cardiovascular risk factors, such as obesity, in both adults and children. This study aimed to investigate the relationship between sleep duration and arterial stiffness indices in Japanese children and early adolescents.
Methods: The data on 102 students (56 males, 46 females; mean age, 11.9 ± 1.8 years) were analyzed. As non-invasive arterial stiffness parameters, the cardio-ankle vascular index (CAVI) and heart-ankle pulse wave velocity (haPWV) were evaluated. Their students' sleep habits (bedtime and wake times on a usual weekday) were investigated using questionnaires, and based on these, their sleep durations were calculated.
Results: The CAVI values in the males and females were 4.8 ± 0.9 and 4.7 ± 0.9 (arbitrary unit), respectively. haPWV values in the males and females were 5.5 ± 0.6 and 5.4 ± 0.6 m/s, respectively. Sleep duration in the males, but not in the females, was negatively correlated with CAVI ($r= -0.356$) and haPWV ($r= -0.356$), suggesting that students with short sleep duration could have increased arterial stiffness. After adjusting for confounders, such as age, sex, systolic blood pressure, heart rate, adiposity, and physical fitness, the correlation of sleep duration with CAVI, but not with haPWV, was still significant (partial $r= -0.253$, $p<0.05$).
Conclusion: Our findings suggest that shorter sleep duration influences arterial stiffening even in childhood.

Key words: Primary prevention, Adolescent, Vascular stiffness, Cardiovascular risk

Introduction

Childhood cardiovascular (CV) risk factors, such as obesity, dyslipidemia, low physical fitness, and high blood pressure (BP), precede arteriosclerotic changes[1-6] and lead to increases in CV event risks and mortality rate in early adulthood[7, 8]. In Japan, the rate of overweight/obesity among children at present is higher than that in the 1980s[9], and further increases in the percentage of individuals with CV risk factors are

expected. Primary prevention in early life is thus all the more important.

Inadequate sleep is associated with both CV risk factors[10, 11] and CV events in adults[12]. Several studies have reported that children and adolescents with sleep problems, including short sleep duration, had more CV risk factors, such as overweight/obesity and high BP, than children without sleep problems[10, 13-15]. Given that arteriosclerosis progression is aggravated by these CV risk factors, we postulate that arteriosclerotic changes may occur in short sleepers, even in children and/or adolescents.

Brachial–ankle pulse wave velocity (PWV) is an indicator of systemic arterial stiffness and is shown to be related to CV events and mortality[16]. Although relationships between PWV and CV risk factors in

children have been reported[5, 17, 18], little is known about the influences of an individual's daily sleep habit on arterial stiffness. In addition, given that the PWV value depends on BP levels during the measurement time[17] and that poor and/or inadequate sleep affects BP increase and body-weight gain[10, 13, 14], we need to clarify the relationships between BP-independent arterial stiffness indicators and daily sleep habits in children and adolescents.

In this study, we investigated the relationship between sleep duration and arterial stiffness in Japanese children by measuring the heart-ankle PWV (haPWV) and the cardio-ankle vascular index (CAVI)[19-23], which is a modified parameter of arterial stiffness and is independent of BP levels.

Methods

Participants

The study participants were Japanese students who were fourth (15 males; 14 females) and sixth (18 males; 11 females) graders in elementary school and second year students (23 males; 21 females) of junior high school (corresponding to eight grade in the U.S.). These children and adolescents participated in the Improvement of Fitness in the Hokkaido Children Project, which was designed to identify interference factors for physical fitness, especially the influences of cold and snowfall environments, and to promote healthy growth and development in children and adolescents in Hokkaido, Japan. This research project started in October 2010 and continued until June 2013. Its study protocol was approved by the Research Ethics Committee of Hokkaido University of Education. Written informed consent was obtained from a parent or guardian of all participants.

Arterial stiffness and other physiological variables of 148 students were measured during the period from late April to early May 2012. The following criteria was applied in the present analysis: (1) the resting heart rate during arterial stiffness measurement was < 100 beats/min; (2) the brachial systolic BP during arterial stiffness measurement was < 130 mmHg; (3) there were no orthopedic injuries in the trunk or lower limbs; (4) sleep duration was > 5 h; and (5) there was no missing data, or the participants completed the fitness tests. The eligible data of 102 students (males, $n = 56$; females, $n = 46$) were analyzed in this study. All physical and physiological measurements were conducted in the morning.

Sleep Duration

On a typical weekday school day, the students' sleep duration was assessed using a survey that ques-

tioned the students "what time do you usually go to bed on weekdays" and "what time do you usually get out of bed in the morning on weekdays." These questions were used to compute the average hours of weekday sleep.

Arterial Stiffness Index

Each student's CAVI, PWV, brachial systolic BP (SBP), brachial diastolic BP, and heart rate (HR) were simultaneously measured by an automatic waveform analyzer (VaSera VS-1000; Fukuda Denshi, Tokyo). The methods used to obtain the values of these parameters were as previously described[19, 24]. Briefly, the measurements were taken with the participant lying in a supine position after resting for at least 5 min. Occlusion and monitoring cuffs were placed around both sites of the participant's upper and lower extremities. The extremity BP was measured by oscillometry. Electrocardiography electrodes were attached to the upper arm. A microphone was placed on the sternal angle for phonocardiography.

PWV was calculated by dividing the distance from the aortic valve to the ankle artery by the sum of the difference between the time the pulse waves were transmitted to the brachium and the time the same wave was transmitted to the ankle, plus the time difference between the second heart sound on the phonocardiogram and the notch of the brachial pulse waves[19]. haPWV obtained from CAVI analysis indicates the velocity of the pulse wave from the heart to the ankle artery and thus differs from the brachial–ankle PWV. CAVI was obtained from the measurement of the participant's BPs and PWV[19]. The means of the right-side and left-side values of haPWV, CAVI, and SBP were used.

Anthropometric and Fitness Measures

Each participant's height and weight were measured using a standard stadiometer and a digital scale, respectively. The students reported their birth date, and we calculated their ages (in months) at the measurement day. The body mass index (BMI) was calculated from these measures and is expressed as a standard score (z-score) using a spreadsheet program of BMI norms for the students' age (in months) and sex in accordance with the guidelines of the Japanese Society for Pediatric Endocrinology (JSPE program)[25]. The participants' waist circumference was measured at the narrowest torso using a measuring tape. Physical fitness was assessed by a 20-m shuttle run test (20 mSRT) in which the participants ran between two lines 20 m apart until they twice failed to reach the front line within the required time. The initial running speed was 8.5 km/h. The pace of the test was

Table 1. Characteristics of subjects

Variable	Male, N = 56	Female, N = 46
Age, yr	11.9 ± 1.7	11.9 ± 1.8
Height, cm	150.7 ± 12.8	148.9 ± 9.6
Weight, kg	45.8 ± 11.7	43.4 ± 9.7
BMI, kg/m²	19.9 ± 3.2	19.4 ± 3.0
BMI z-score	0.42 ± 1.05	0.22 ± 1.03
Waist, cm	68.9 ± 8.9	65.3 ± 6.9
HR, bpm	75.1 ± 10.7	77.9 ± 9.2
SBP, mmHg	114.9 ± 8.7	113.8 ± 6.0
CAVI	4.82 ± 0.90	4.71 ± 0.82
haPWV, m/s	5.51 ± 0.62	5.47 ± 0.64
20mSRT, stage	6.6 ± 2.7	4.8 ± 1.6
Sleep duration, h	8.1 ± 1.1	8.1 ± 1.1

BMI denotes body mass index; SBP, brachial systolic pressure; DBP, brachial diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; HR, heart rate; CAVI, cardio-ankle vascular index; PWV, pulse wave velocity; 20mSRT, 20-m shuttle run test.

increased by 0.5 km/h every minute, and an audio signal determined whether each lap was completed on time. Total laps were classified by 10 stages, as described by the Ministry of Education, Culture, Sports, Science and Technology of Japan[26], and we used the stage reached by the participants as a parameter of systemic physical fitness.

Statistical Analyses

We report the data as means ± standard deviations unless otherwise noted. The normality of data distributions was tested using the Kolmogorov–Smirnov test. The correlations between two variables were assessed using Pearson's correlation analyses. We performed partial correlation analyses after controlling for confounding factors to examine the relationships between arterial stiffness indices and sleep duration. Model 1 was adjusted for age, BMI z-score, HR, and SBP for each gender. In addition, in the total of male and female students (overall), sex (male = 1, female = 2) was included into the model as control variables. Model 2 was adjusted for the control variables used for model 1 and 20 mSRT. We observed multicollinearity among study variables and therefore performed partial correlation analyses. All statistical analyses were calculated with PASW statistics ver. 18.0 software (SPSS, Chicago, IL), and P-values < 0.05 were considered significant.

Results

The students' characteristics are summarized in **Table 1.** The mean CAVI values were 4.8 ± 0.9 and 4.7 ± 0.8 (arbitrary unit) for the 56 males and 46

females, respectively. haPWV was 5.5 ± 0.6 and 5.5 ± 0.6 m/s in the groups of males and females, respectively. Both males and females slept an average of 8.1 ± 1.1 h. The number (%) of students who slept less than 7.5 h per night (< 7.5 sleep duration) was two (6.9%) among the fourth graders, two (6.9%) among the sixth graders, and 25 (56.8%) among the junior high schoolers.

Table 2 provides the Pearson's correlation coefficients between arterial stiffness measures and other study variables. In the total sample of males and females, CAVI and haPWV were positively correlated with age and 20 mSRT and negatively correlated with the BMI z-score, HR, and sleep duration. SBP was significantly correlated with haPWV but not with CAVI. **Table 3** shows the correlation coefficients between sleep duration and other variables and between CAVI and other variables for students of each school grade. The correlations between age and sleep duration and between age and CAVI were not significant in any grade. The relationships between CAVI and sleep duration and between the haPWV values and sleep duration are illustrated in **Fig. 1.**

To adjust for confounding factors, we performed a partial correlation analysis using CAVI, haPWV, and sleep duration (**Table 4**). After controlling for age, sex, BMI z-score, SBP, and HR, the partial correlation coefficient of CAVI and sleep duration and of haPWV and sleep duration in overall students was not significant (CAVI, $r = -0.185$; PWV, $r = -0.101$; both $p > 0.05$). As shown in **Table 4**, model 2 analyses after controlling for control variables of model 1 and 20 mSRT demonstrated significant correlations between CAVI and sleep duration in male and overall students.

**Table 2.** Pearson's correlation coefficients between arterial stiffness indices and study variables

Variable	CAVI			haPWV		
	Male	Female	Overall	Male	Female	Overall
Age, yr	0.391**	0.085	0.252*	0.385**	0.325*	0.355**
BMI z-score	−0.529**	−0.342*	−0.441**	−0.429**	−0.278	−0.356**
Waist, cm	−0.077	−0.277	−0.132	0.066	−0.037	0.032
SBP, mmHg	−0.114	0.085	−0.041	0.239	0.439**	0.308**
HR, bpm	−0.297*	−0.221	−0.273**	−0.190	−0.201	−0.197*
Sleep duration, h	−0.395*	−0.199	−0.309**	−0.406**	−0.220	−0.319**
20 mSRT, stage	0.226	0.301*	0.249*	0.225	0.338*	0.249*

*, $p < 0.05$; **, $p < 0.01$; BMI, body mass index; SBP, systolic blood pressure; HR, heart rate; 20 mSRT, 20-m shuttle run test; CAVI, cardio-ankle vascular index; haPWV, heart-ankle pulse wave velocity.

Table 3. Pearson's correlation coefficients between sleep duration or CAVI and study variables in each grade

	Sleep duration			CAVI		
	4th graders	6th graders	Second year students of junior high school	4th graders	6th graders	Second year students of junior high school
Age	−0.261	0.169	0.134	−0.007	−0.104	0.050
BMI z-score	0.207	−0.204	−0.091	−0.296	−0.427*	−0.409**
20 mSRT	−0.060	0.132	−0.129	0.021	0.089	0.206
SBP	−0.041	−0.060	−0.259	−0.333	−0.344	0.063
HR	−0.221	0.215	−0.019	0.056	−0.337	−0.354*
CAVI	−0.378*	−0.172	−0.155	-	-	-
haPWV	−0.145	−0.071	−0.272	0.699**	0.832**	0.922**

*, $p < 0.05$; **, $p < 0.01$; BMI, body mass index; SBP, systolic blood pressure; HR, heart rate; 20 mSRT, 20-m shuttle run test; CAVI, cardio-ankle vascular index; haPWV, heart-ankle pulse wave velocity.
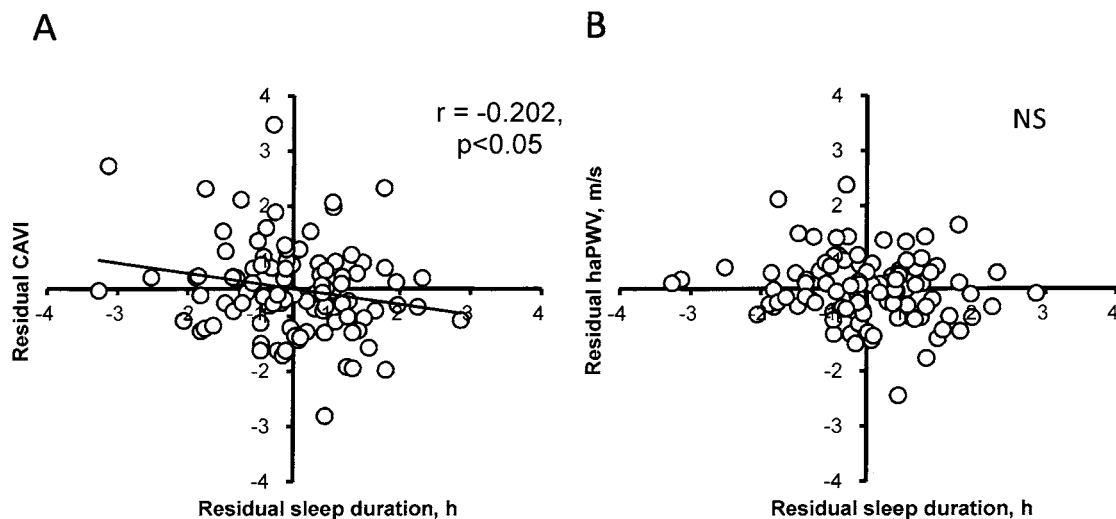
Fig. 1. Correlations between the arterial stiffness parameters and sleep duration in the 102 Japanese children and adolescents.

A: The correlation between the residual CAVI and residual sleep duration after adjusting for age and sex. B: The correlation between residual PWV and residual sleep duration after adjusting for age and sex. NS, no significant.

Table 4. Partial correlation analyses

		Sleep duration					
		Males		Females		Overall	
		partial r	p value	partial r	p value	partial r	p value
Model 1	CAVI	−0.226	0.106	0.123	0.439	−0.185	0.061
	haPWV	−0.232	0.098	0.085	0.594	−0.101	0.310
Model 2	CAVI	*−0.414*	*0.003*	−0.135	0.401	*−0.250*	*0.014*
	haPWV	*−0.378*	*0.006*	0.081	0.615	−0.137	0.180

CAVI, cardio-ankle vascular index; PWV, pulse wave velocity; model 1 was adjusted by BMI z-score, HR, age, and SBP; model 2 was adjusted by model 1 and 20mSRT. Italic letters are statistically significant.

In model 2, haPWV was negatively associated with sleep duration in male students. After adjusting for age, sex, BMI z-score, HR, and SBP, the 20 mSRT was not significantly correlated with CAVI and haPWV (data not shown).

Discussion

The main finding from the present study is that there was a significant inverse association between sleep duration and arterial stiffness in Japanese children and early adolescents, even after adjusting for age, HR, BP, adiposity, and physical fitness. Our results suggest that inadequate sleep duration could enhance arterial stiffening in healthy children and may be a behavioral target for primary prevention of early vascular aging.

PWV measurement is a non-invasive and useful tool for assessing arterial stiffness, based on the increasing evidence of PWV as a significant predictor of CV events[16] and CV mortality[27]. However, PWV is affected by BP levels at the time of measurement, and thus, when PWV of sensitive persons (e.g., some children and individuals with white coat hypertension) is evaluated, there is a possibility of obtaining an overestimated PWV value. CAVI showed no correlation with BP in previous studies[19, 20] or in the present study, suggesting that CAVI measurement is a suitable method for assessing the arterial stiffness of such individuals. Although Philip *et al.* (2016)[20] examined CAVI values in children, recent papers have shown relationships between PWV and intima-media thickness or flow-mediated dilation even in children[28-30]. Based on the results of these previous studies, it appears that a non-invasive evaluation of arterial stiffness in childhood would be a surrogate marker for vascular health.

On the other hand, poor sleep habits, including

short sleep duration and low sleep quality, are associated with high BP and increased CV risk factors in children and adolescents[10, 13, 15, 31-34]. To our knowledge, the present study is the first report of an inverse association between sleep duration and arterial stiffness evaluated using CAVI after controlling for potential confounding factors in 9- to 13-year-old children. Our present findings support the previous studies' results regarding abnormalities in great artery elasticity early in life and also imply that unhealthy sleep habits in childhood can accelerate the process of arteriosclerosis.

We found no correlation between CAVI and physical fitness after adjusting for confounders. Another study also showed no significant association between physical fitness and PWV after adjusting for age, sex, SBP, HR, mean arterial pressure, and adiposity[5]. With the use of the second derivative of the finger photoplethysmogram, it was demonstrated that low physical fitness was related to higher arterial stiffness parameters in children[35]. A recent study has revealed that the correlations between arterial stiffness parameters assessed by PWV and the second derivative of the finger photoplethysmogram were only mild[36], and the parameters reflected different physiological properties at central and peripheral sites in the arterial system[37, 38]. In addition, systemic physical fitness assessed using the 20 mSRT is influenced by adiposity because greater body fat would be a load on skeletal muscles of the lower limbs during the repeated stopping and running in this test. Although it is possible that there is an association between arterial stiffness and physical fitness in children, further studies are needed to clarify the influences of physical fitness on arterial stiffness using more precise evaluation methods and/or a longitudinal study design.

The underling mechanisms whereby short sleep duration may lead to arterial stiffening cannot be

determined using our cross-sectional data. However, one possible explanation is that short sleep duration could induce a sustained activation of the neuroendocrine system. Earlier studies showed that low sleep quality was related to an unhealthy pattern of sympathetic and parasympathetic nervous system activity in 5- to 11-year-old children[32] and that children with short sleep duration had higher salivary cortisol levels as a biomarker of sympathoadrenal system activity than children with average or long sleep duration[39]. It was also shown that CAVI was mediated by the α 1-adrenergic receptor pathway[40, 41].

Given the results of these previous studies, it appears that short sleep duration could be related to higher sympathetic nervous activity, which may concomitantly mediate alterations in arterial function in children with habitual short sleep durations. On the other hand, CAVI increases with age, especially after middle age, which would be attributed to structural changes in the vascular wall as an inevitable consequence of aging[40]. Considering that our present participants were 9- to 13-year-old children, there is only a slight possibility of the structural alteration in the vascular wall in children with short sleep duration.

There are several limitations to this study. It had a cross-sectional study design, and thus, our results do not provide a cause–effect relationship between predictors and outcomes. In addition, sleep duration was assessed only by a self-reported questionnaire and not by objective sleep monitors. In previous studies, sleep quality and quantity were assessed using actigraphy monitors worn on the wrist[33, 42, 43]. Actigraphy methods for sleep assessment showed a tentative low to moderate correlation with sleep polysomnography[44], which is a gold standard for evaluating sleep quantity and quality. Given that it is important for students to have self-awareness regarding their sleep habits to improve their daily lifestyle, our results based on self-reported sleep duration could have clinical and/or educational implications. Our results do not show the true effects of childhood obesity and low fitness on arterial stiffness in children and early adolescents because the study's design was cross-sectional. Further research should be performed to determine the relationship between sleep status and arterial stiffness in children and early adolescents in a longitudinal study design using larger sample sizes.

In conclusion, we found that sleep duration was inversely associated with arterial stiffness after adjusting for confounders. Our data suggest that inadequate sleep duration could enhance the process of arterial stiffening in healthy children who are $\geq$ 10 years old. Our findings also suggest that sleep assessments, even by a self-reported questionnaire, may be a useful

screening tool for identifying children with early vascular aging and future CV risks.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgement

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