Korean J Physiol Pharmacol 2016;20(3):287-295
http://dx.doi.org/10.4196/kjpp.2016.20.3.287

KJPP

Original Article

Voluntary stand-up physical activity enhances endurance exercise capacity in rats

Dae Yun Seo[1,#], Sung Ryul Lee[1,#], Hyo-Bum Kwak[2], Kyo Won Seo[1], Robin A McGregor[1], Ji Young Yeo[1], Tae Hee Ko[1], Saranhuu Bolorerdene[1], Nari Kim[1], Kyung Soo Ko[1], Byoung Doo Rhee[1], and Jin Han[1,*]

[1]*National Research Laboratory for Mitochondrial Signaling, Department of Physiology, Department of Health Sciences and Technology, BK21 Project Team, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan 47392,* [2]*Department of Kinesiology, Inha University, Incheon 22212, Korea*

ARTICLE INFO

Received December 11, 2015
Revised March 2, 2016
Accepted March 2, 2016

***Correspondence**
Jin Han
E-mail: phyhanj@inje.ac.kr

Key Words
Body weight
Exercise endurance capacity
Voluntary physical activity

[#]Both authors contributed equally to this work.

ABSTRACT Involuntary physical activity induced by the avoidance of electrical shock leads to improved endurance exercise capacity in animals. However, it remains unknown whether voluntary stand-up physical activity (SPA) without forced simulating factors improves endurance exercise capacity in animals. We examined the effects of SPA on body weight, cardiac function, and endurance exercise capacity for 12 weeks. Twelve male Sprague-Dawley rats (aged 8 weeks, n=6 per group) were randomly assigned to a control group (CON) or a voluntary SPA group. The rats were induced to perform voluntary SPA (lifting a load equal to their body weight), while the food height (18.0 cm) in cages was increased progressively by 3.5 every 4 weeks until it reached 28.5 cm for 12 weeks. The SPA group showed a lower body weight compared to the CON group, but voluntary SPA did not affect the skeletal muscle and heart weights, food intake, and echocardiography results. Although the SPA group showed higher grip strength, running time, and distance compared to the CON group, the level of irisin, corticosterone, genetic expression of mitochondrial biogenesis, and nuclei numbers were not affected. These findings show that voluntary SPA without any forced stimuli in rats can effectively reduce body weight and enhance endurance exercise capacity, suggesting that it may be an important alternative strategy to enhance endurance exercise capacity.

INTRODUCTION

The health benefits of regular physical activity for prevention of chronic diseases are well recognized. Exercise affects multiple physiological systems that over time leads to changes in body composition, skeletal muscle function, and exercise capacity [1-3]. Many previous experiments in animals have attempted to determine the biological and molecular mechanisms underlying the beneficial effect of physical activity on health [4]. However, many studies have used an involuntary animal exercise model, in which physical activity is induced by external stimuli. Involuntary exercise in animal models are not representative of normal exercise or physical activity in humans which is usually voluntary or motivated by positive stimuli. Thus, it is important to develop an alternative animal exercise model that avoids forced or involuntary physical activity and therefore can be translated into humans.

Regular exercise training protects against obesity as well as cardiac and skeletal muscle dysfunction [5,6]. Maximum endurance exercise capacity can be improved through regular physical activity, which is determined according to environmental factors [7-9]. Evidence from animal exercise studies using different exercise modalities including treadmill running, swimming, ladder climbing, and standing indicate a strong association between physiological changes in body weight, cardiac function, and endurance exercise capacity [10-12]. In

Author contributions: D.Y.S., S.R.L., and J.H. designed and performed the experiments. J.Y.Y., T.H.K., and S.B. analyzed the data. H.B.K., K.W.S., R.A.M., N.K., K.S.K., and B.D.R. contributed critical discussion. D.Y.S., S.R.L., and J.H. wrote the manuscirpt.

forced exercise models, exercise intensity can be maintained or increased using external stimuli, such as electrical shock and surgical ablation [11]. However, involuntary animal exercise models have several disadvantages including suppression of natural behaviours in the animal [13]. Therefore, it is important to develop and validate a voluntary animal exercise model, which results in physiological and functional changes such as enhanced endurance exercise capacity that is more representative to exercise and physical activity in humans. The development and validation of a voluntary animal exercise model is important to enable different exercise, diet or pharmaceutical interventions to improve exercise training-induced health benefits to be assessed in the future.

One recent study reported that involuntary stand-up physical exercises induced by electronic stimuli could cause skeletal muscle hypertrophy by enhancing protein synthesis in rats [12]. In this study, we determined whether voluntary stand-up physical activity (SPA) without electronic stimuli can effectively lead to similar benefits in terms of endurance exercise capacity and can help identify how primary exercise can be useful in involuntary exercise models. Thus, we assessed the effects of voluntary SPA on body composition, skeletal muscle tissue histology, running time, distance, echocardiography results, and hormone levels including irisin and corticosterone in rats.

METHODS

Animals and experimental design

Eight-week-old male Sprague-Dawley (n=12) rats (Orient Bio company, Daejeon, Korea) were maintained in an environmentally controlled facility (12:12-h light/dark cycle, 22°C). The rats were randomly assigned to two groups: sedentary control (CON, n=6) or voluntary SPA (n=6) for 12 weeks. All experimental procedures were approved by Inje Medical University Animal Care and Use committee for standard ethics in animal studies.

Voluntary stand-up physical activity

The rats were adapted to physical activity conditions (5 times/day, 60 min/times, with food maintained at a height of 1 stage: 21.5 cm) for 1 week (Fig. 1), without any stimulating factors, including electronic shock, light, and sound. As a result, the rats learned to stand, while consuming food and water. After the 1 week adaptation period voluntary exercise overload was increased progressively in three stages, using only body weight load, by increasing the height of the food cage up to a height of 28.5 cm. The voluntary SPA model was designed to be representative of daily voluntary standing type exercise and to mimic resistance exercise in humans.

Measurement of organ tissue weight and food intake

We recorded body weight and food intake weekly for 12 weeks. After these protocols, the animals were sacrificed by using a standard anesthetic protocol [14]. We immediately dissected and weighed the gastrocnemius and soleus, as described earlier [14].

Micro computed tomography

To determine the size of the lower limbs, we evaluated the lower limbs of the rats at 12 weeks with a SkyScan 1076 Micro computed tomography scanner (SkyScan, Kontich, Belgium) high-resolution system in vivo micro-computed tomography, as previous described [15]. A single representative CT image was taken for each group.

Echocardiography

To determine the cardiac function using transthoracic echocardiography results, rats were anesthetized with 2% isoflourane. We evaluated the complete 2-dimensional M-mode echocardiogram using the VIVID 7 dimension system (General Electric-Vingmed Ultrasound, Horton, Norway) and obtained images with a high spatial and temporal resolution by using a 10S transducer (5.5~12 MHz) [16]. The following data were recorded: heart rate (beats/min), stroke volume (ml/min), cardiac output (ml/min), fractional shortening (%), ejectional fraction (%), and left ventricle mass (mm). Ejection fraction was calculated as (end-diastole volume – end-systole volume) / end-diastole volume × 100%. Fractional shortening was calculated as (left ventricular

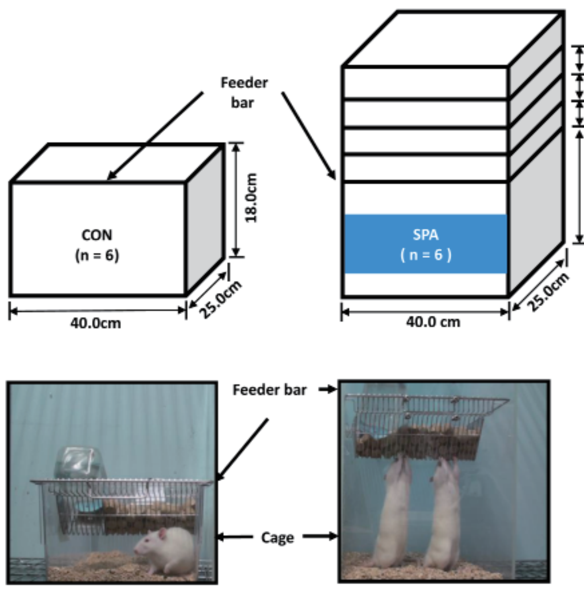

Fig. 1. Experimental device for control (CON) and voluntary stand-up physical activity (SPA).

diameter at diastole – left ventricular diameter at systole)/left ventricular diameter at diastole × 100%.

Grip strength

Grip strength was assessed in each rat using a grip strength meter (MK-380M; Muromachi Kikai Co., Tokyo, Japan) 3 days prior to the sacrifice [17]. Values were recorded as the average of three measurements.

Exercise endurance capacity

To determine the maximal exercise endurance capacity, treadmill running at 10 m/min for 5 min (grade 15°) was performed with an electronic shock stimulus and the speed was increased gradually at 2 m/min every minute, until the animals reached exhaustion. We evaluated exercise endurance capacity in terms of running time and distance [18].

Blood parameters

The concentrations of blood irisin (Phoenix Pharmaceuticals, Inc., Burlingame, USA) and corticosterone (Demeditec Co., Kiel-Wellsee, German) were measured using enzyme-linked immunosorbent assay kits.

Blood samples were taken from the heart and were drawn into heparinized tubes. The heparinized tubes were centrifuged for 10 min at 10,000 g to obtain plasma samples. All tissues were frozen in liquid nitrogen and blood samples were stored at –80oC for next analysis.

Electron microscopy

To determine mitochondrial integrity, transmission electron microscopy was used. Samples from the gastrocnemius muscles were carefully dissected from the rats and analyzed as previously described [19]. Representative mitochondrial morphology of the gastrocnemius muscle was recored for each group.

Hematoxylin and eosin staining

The gastrocnemius muscles were embedded in liquid nitrogen and stained with hematoxylin and eosin, as previously described [20]. The number of nuclei of the gastrocnemius muscle was evaluated using Image J software (NIH, USA). A single representative cross sectional area of the gastrocnemius muscle was taken for each group.

Quantitative PCR analysis

Total RNA from gastrocnemius muscle was extracted with Trizol (Invitrogen, Cergy Pontoise, France), and single-stranded cDNA was synthesized from 10 μg of total RNA with random hexamer primers (Applied Biosystems, Courtaboeuf, France). Real-time RT-PCRs were performed to measure PGC-1α, PGC-1β, Tfam, NRF-1, NRF-2a, and NRF-2b, as previously described [21]. Sequences of all primers are listed in Table 1.

Statistical analysis

All data are expressed as mean±standard error of mean (SEM). Changes in body weight within and between groups were evaluated using a two-way ANOVA [group (CON and SPA)×time (before and 12 weeks)]. If a significant change was noted in the group or time interaction, paired t-test was used for post hoc comparisons. Differences in other physiological and functional parameters between the CON and SPA group were evaluated by independent t-test (SPSS 22.0, Chicago, USA). Differences were considered statistically significant at $p<0.05$.

RESULTS

Voluntary SPA reduced body weight but did not affect tissue weight or food intake

The reduction in body weight was significantly higher in the SPA group than in the CON group (Fig. 2A). Gastrocnemius and soleus weights were slightly higher in the SPA group than in the CON group, but the differences between the groups were not statistically significant (Fig. 2B, and 2C). The size of lower limb muscles were slightly bigger in the SPA group than in the CON group, but the differences between the groups were not statistically significant (Fig. 2D). Heart weight also did not significantly differ between the CON and SPA groups (Fig. 2E). To determine the changes in food intake, we measured food intake every week. There was no significant difference in food intake between groups (Fig. 2F).

Table 1. List of the RT-PCR primers

Gene		Primer (5′-3′)
PGC1- α	F	CACCAAACCCACAGAGAACAG
	R	GCAGTTCCAGAGAGTTCCACA
PGC1- β	F	CCCCAGTGTCTGAAGTGGAT
	R	TCTGGAACTGAGGCTGGTCT
Tfam	F	GGCCCTTAACAGTGAAGCTG
	R	CATCTGGGCCATTAGCATCT
NRF-1	F	TACAATTGACCAGCCTGTGC
	R	ATCCTTGGGGACCTTTGAAC
NRF-2a	F	CCCGATGGACAGCAAGTATT
	R	CCGGTTCTCAATTATTTCCA
NRF-2b	F	GAAAGCACAAATCAAGAGGAG
	R	CTGCTTTTCATCATGAGACAG
GAPDH	F	GACATGCCGCCTGGAGAAAC
	R	AGCCCAGGATGCCCTTTAGT

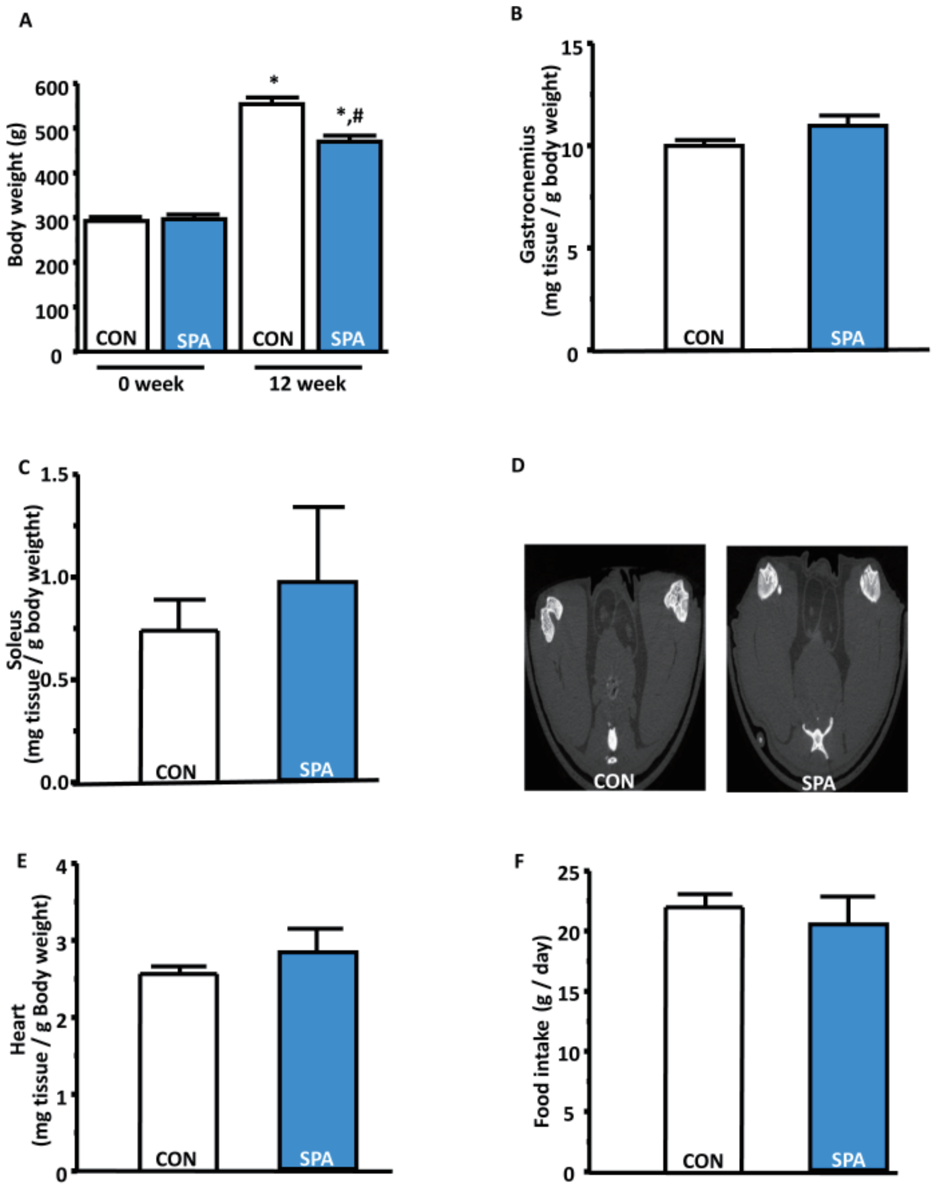

Fig. 2. The changes in body weight and organ tissues for a period of 12 weeks in the CON and SPA groups. (A) Body weight at 0 week and 12 weeks between the CON and SPA groups. (B) Gastrocnemius/body weight ratio in the groups. (C) Soleus/body weight ratio in the groups. (D) Lower limb muscle size that is only one representative CT image for each group. (E) Heart/body weight ratio in the groups. (F) Food intake/day ratio in the groups. The error bars indicate standard error of the mean (SEM) (*p<0.05; before vs after; #p<0.05; CON vs SPA). CON, control group; SPA, voluntary stand-up physical activity group.

Voluntary SPA did not change cardiac function, as determined via echocardiography

Increased physical activity typically is associated with improved cardiac function, including heart rate, left ventricle mass, cardiac output, ejection fraction, and fractional shortening [22]. However, no statistically significant differences in heart rate, stroke volume, cardiac output, fractional shortening, ejection fraction and LV mass were detectable between the CON and SPA groups in the present study (Fig. 3A-F).

Voluntary SPA improved grip strength and endurance exercise capacity

The grip strength, as measured using a grip strength meter, increased significantly in the SPA group compared to the CON group (Fig. 4A). To determine changes in the exercise endurance capacity, we examined the difference in the acute exhaustive exercise test performance between the groups and observed that running time and distance were significantly higher in the SPA group than in the CON group (Fig. 4B, and 4C).

Effects of voluntary SPA on irisin and corticosterone levels

To support the changes in body weight and exercise endurance capacity, we determined irisin levels in the blood. In addition, we determined whether the corticosterone levels in the rats changed in the study. However, the levels of irisin and corticosterone, which tipically indicate stressful condition in animals were not significantly different between the CON and SPA groups (Fig. 5A and 5B).

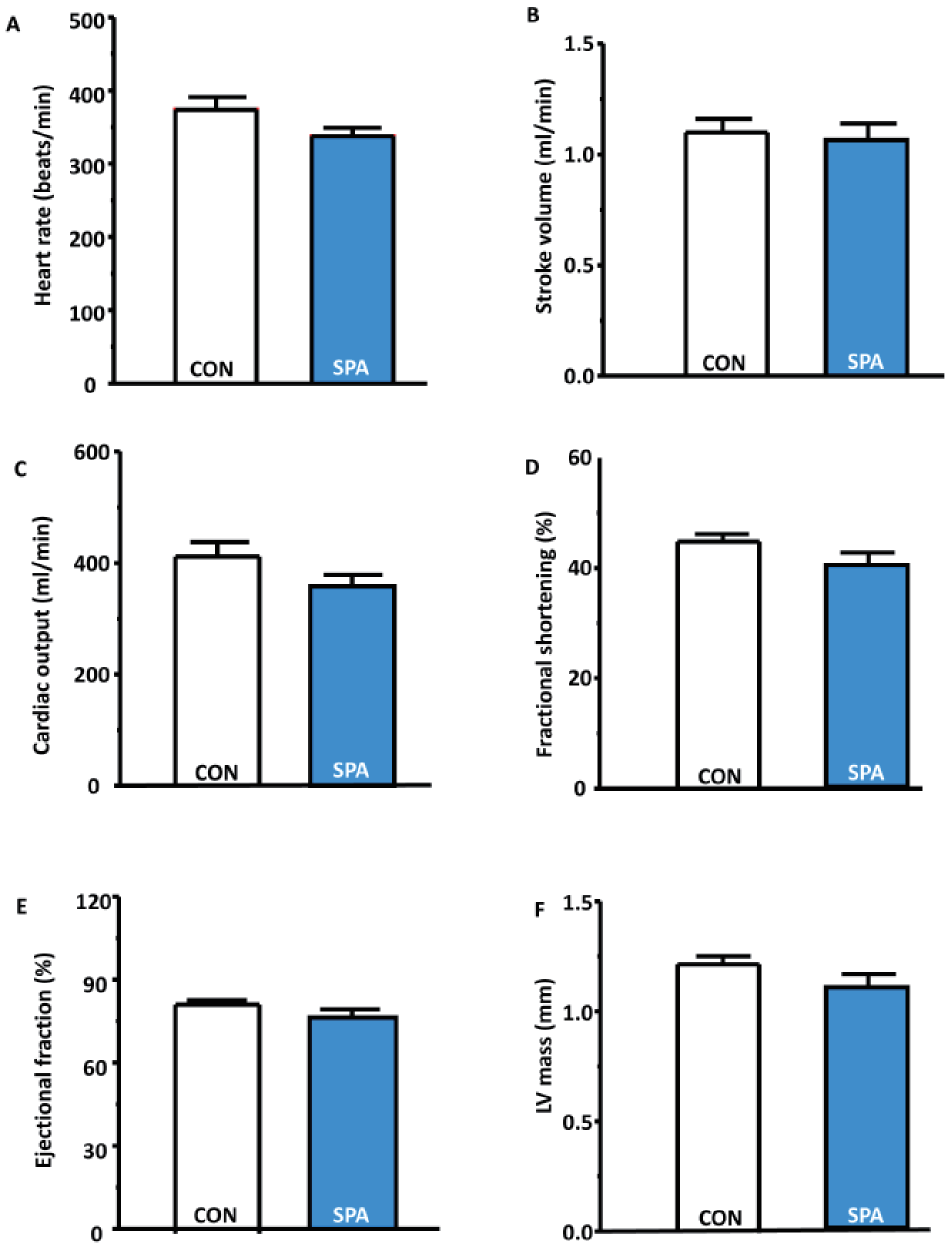

Fig. 3. Cardiac function as measured through echocardiography at 12 weeks in the CON and SPA groups. (A) Heart rate (beats/min) in the groups. (B) Stroke volume (mL/min) in the groups. (C) Cardiac output (mL/min) in the groups. (D) Fractional shortening (%) in the groups. (E) Ejection fraction (%) in the groups. (F) LV mass (mm) in the groups. CON, control group; SPA, voluntary stand-up physical activity group; Lv, left ventricle.

Effects of voluntary SPA on mitochondrial abundance, mitochondrial biogenesis, nucleus, and muscle fiber phenotype in gastrocnemius in skeletal muscle

The SPA group showed slightly increased mitochondrial abundance, mitochondrial biogenesis, nucleus numbers, and muscle fiber size in the gastrocnemius than the CON group (Fig. 6A-E), but a statistically significant difference between groups was not seen.

DISCUSSION

In the present study, we first revealed that voluntary SPA without any negative external stimuli significantly decreased body weight, which was accompanied by significantly increased endurance exercise capacity and muscle strength after 12 weeks. However, voluntary SPA was not sufficient to improve cardiac function, skeletal muscle mass as well as the levels of irisin and corticosterone.

Increased physical activity results in the control of body weight, after considering energy supply according to energy consumption and expenditure associated with hormones [23,24]. On the basis of these findings, we hypothesized that voluntary SPA would cause a decrease in body weight with a normal diet without stress. In the present study, we found that voluntary SPA was effective in decreasing body weight during 12 weeks (Fig. 2A). These findings are in agreement with previous study [25], which reported body

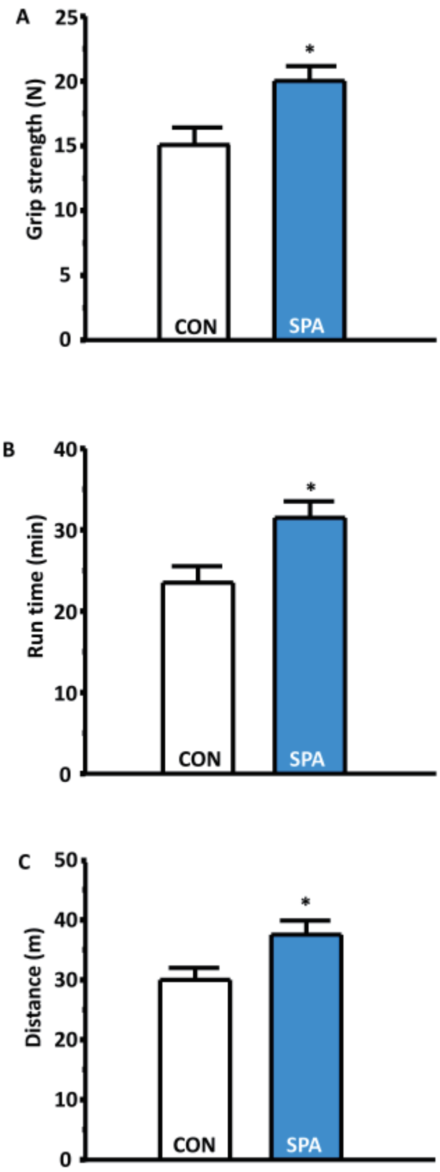

Fig. 4. Voluntary SPA shows enhancement of muscle strength grip strength and exercise capacity. (A) Grip strength (N) in the groups. (B) Run time (minutes) in the groups. (C) Distance (meters) in the groups. Error bars indicate standard error of the mean (SEM) (*p<0.05; CON VS SPA). CON, control group; SPA, voluntary stand-up physical activity group.

weight reduction with voluntary wheel running compared with non-voluntary wheel running for 12 weeks, suggesting that the reduction in body weight may remarkably inhibit food intake and promote an increase in stress hormone levels. To confirm these findings, we assessed the food intake, and investigated the changes in corticosterone hormone levels. As expected, we observed no changes in the food intake or corticosterone levels between the two groups. These results indicate that the reduction of body weight seems to be a consequence of a response to voluntary physical activity without any external stimuli during voluntary SPA.

Generally, irisin as myokine, plays a role in body weight and total energy expenditure by activating brown adipose tissue cells during or after exercise [26]. In addition, it has shown that increased serum irisin levels results in the reduction of body weight [26,27]. For these reasons, the increase of the serum irisin levels during exercise is considered a therapeutic strategy to reduce body weight. However, we showed that serum irisin levels were slightly higher in the SPA group than in the CON group, but no significant change was observed between the CON and SPA groups. These findings are in contrast to the results of many previous studies that showed that irisin levels were elevated as a reflection of the beneficial effects of metabolic response to exercise [28,29]. Unlike the previous reports, the irisin levels in this study did not change significantly in response to exercise [30,31]. Our design included a voluntary SPA protocol that inhibited body weight, but no changes in serum irisin levels were found. The present study demonstrated that voluntary SPA did not cause a change in irisin levels. Therefore, further studies on the mechanisms of irisin are necessary to determine whether voluntary SPA may be due to change in the body weight.

There are various exercise-induced protocols such as involuntary forced treadmill running, swimming, resistance type exercise, or voluntary wheel running that have effectively caused exercise metabolism adaptation in experimental animals [11]. These studies usually reported an increase in gastrocnemius and soleus weights in rats after 12 weeks of exercise training [32]. In the present study, although voluntary SPA showed the trend of increased weight in gastrocnemius and soleus muscles, these data did not reach statistical significant. In addition, we observed that

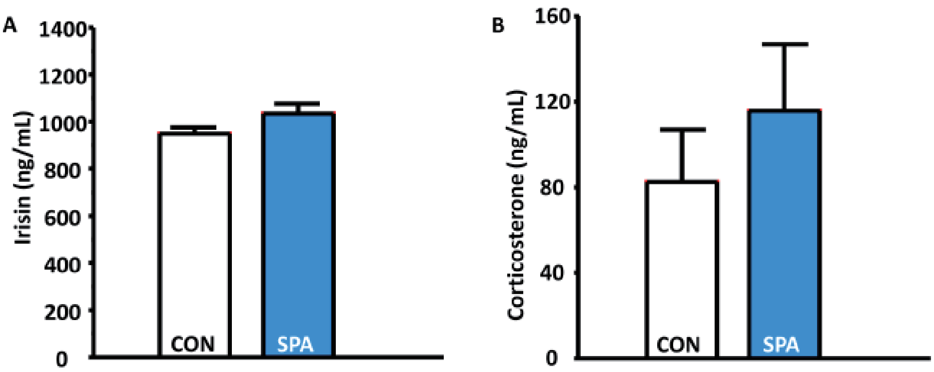

Fig. 5. Changes in the blood parameters. (A) Irisin level (ng/mL) from blood in the groups. (B) Corticosterone (ng/mL) from blood in the groups. CON, control group; SPA, voluntary stand-up physical activity group.

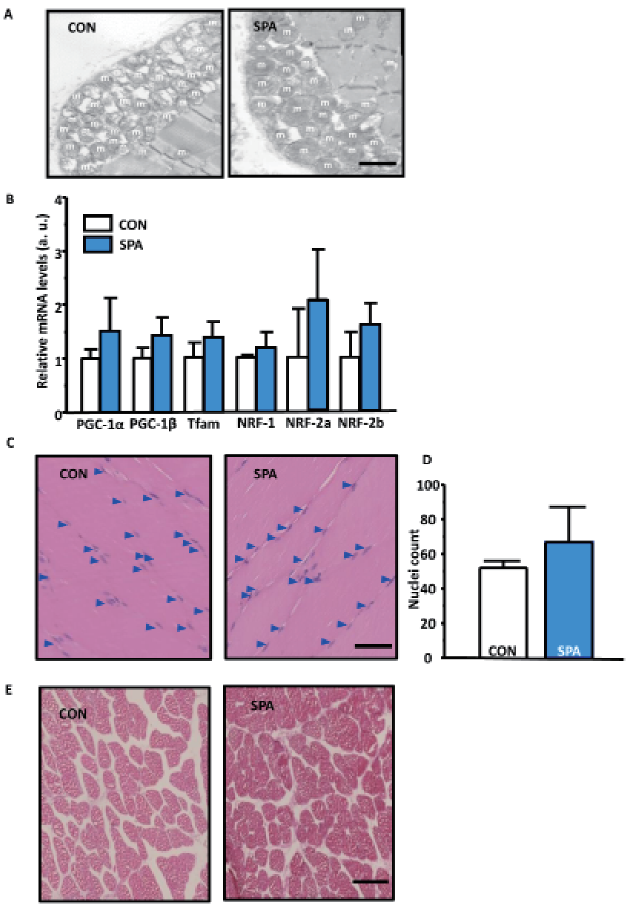

Fig. 6. Change in the histological morphology and mitochondrial biogenesis. (A) Mitochondrial abundant in the gastrocnemius in both groups. The arrow indicates the mitochondria. Scale bar indicates 500 nm. (B) Quantitative RT-PCR normalized to GAPDH mRNA. (C) Gastrocnemius hematoxylin and eosin (H&E) staining by using horizontal section in the groups. Scale bar indicates 40 μm. An arrow indicates the nucleus. (D) Counted nuclei in gastrocnemius muscles. (E) Gastrocnemius H&E staining using cross section in the groups. Scale bar indicates 25 μm. m, mitochondria; CON, control group; SPA, voluntary stand-up physical activity group.

the SPA group exhibited an increase in forelimb grip strength. This result led us to question whether the voluntary SPA enhances skeletal muscle fiber and nuclei or not. We showed that the SPA group showed slight increases in mitochondrial abundance, mitochondrial biogenesis genes, nuclei and skeletal muscle fiber size, but there were no changes in the gastrocnemius between groups. In a similarly designed study, stand-up exercise with electronic stimuli resulted in the rapid proliferation of myogenic satellite cells in atrophied muscles [12]. Our result may reflect that animals require a strong metabolic stimulus for skeletal muscle hypertrophy. Therefore, we suggest that SPA is seems more likely to be useful to augment grip strength.

Improvement in endurance exercise capacity in response to exercise is a well-recognized in cardiac and skeletal muscle function and is important to prevent chronic diseases [33]. As earier study, we assessed changes in echocardiography data during chronic physical activity stimulated by voluntary SPA. Although we observed an increase in endurance exercise capacity such as running time and distance in the SPA group compared with the CON group, echocardiography data did not show significant changes between the SPA and CON groups. These results indicate that voluntary SPA did not contribute to the enhancement of cardiac function. Recently, higher exercise endurance capacity has been associated with increased mitochondrial biogenesis, suggesting that exercise training may contribute to mitochondrial biogenesis [34]. In addition, we measured mitochondrial abundance in the gastrocnemius by studying electron microscopy images. We hypothesized in the present study that abundant and biogenetic increases of mitochondria would occur in the gastrocnemius, but we were unable to show statistical significant changes. We did not explore mechanisms of mitochondrial abundance, including oxidative metabolism and respiration and production of reactive oxygen species in response to exercise training. Further studies are necessary to address and understand the inhibition of body weight during exercise, with regard to mitochondrial function.

There are some limitations in the present study. First, the group size was quite small, with only 6 rats in each group. Second, we showed that voluntary SPA decreased body weight and increases endurance exercise capacity. However, we did not measure the level of energy expenditure during voluntary SPA. Third, we did not determine mitochondrial function and oxidative metabolism in the isolated mitochondria.

In conclusion, this is the first study to show that voluntary SPA decreased body weight and increased endurance exercise performance without any forced stimuli in animals, suggesting that it may be an alternative endurance exercise model in rats.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation of Korea (NRF), and the funding was granted by the Ministry of Science, ICT & Future Planning of Korea (2015 R1A2A1A13001900, 2011-0028925) and by the Ministry of Education of Korea (2010-0020224).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Bang HS, Seo DY, Chung YM, Oh KM, Park JJ, Arturo F, Jeong SH, Kim N, Han J. Ursolic Acid-induced elevation of serum irisin

augments muscle strength during resistance training in men. *Korean J Physiol Pharmacol.* 2014;18:441-446.
2. Kim JS, Kim MG, Kang SW, Kim BR, Beak MY, Park YM, Shin MS. Association of obesity, hypertension and their impact on cardiac function and exercise capacity. *Korean Circ J.* 2016;46:e51.
3. Jung SR, Kim KJ. Exercise-induced PGC-1 transcriptional factors in skeletal muscle. *Integrative Medicine Research.* 2014;3:155-160.
4. Constable SH, Favier RJ, McLane JA, Fell RD, Chen M, Holloszy JO. Energy metabolism in contracting rat skeletal muscle: adaptation to exercise training. *Am J Physiol.* 1987;253:C316-322.
5. Takada S, Kinugawa S, Matsushima S, Takemoto D, Furihata T, Mizushima W, Fukushima A, Yokota T, Ono Y, Shibata H, Okita K, Tsutsui H. Sesamin prevents decline in exercise capacity and impairment of skeletal muscle mitochondrial function in mice with high-fat diet-induced diabetes. *Exp Physiol.* 2015;11:1319-1330.
6. Edelmann F, Gelbrich G, Dungen HD, Frohling S, Wachter R, Stahrenberg R, Binder L, Topper A, Lashki DJ, Schwarz S, Herrmann-Lingen C, Loffler M, Hasenfuss G, Halle M, Pieske B. Exercise training improves exercise capacity and diastolic function in patients with heart failure with preserved ejection fraction: results of the Ex-DHF (Exercise training in Diastolic Heart Failure) pilot study. *J Am Coll Cardiol.* 2011;58:1780-1791.
7. Lim CL, Mackinnon LT. The roles of exercise-induced immune system disturbances in the pathology of heat stroke: the dual pathway model of heat stroke. *Sports Med.* 2006;36:39-64.
8. Cook BJ, Hausenblas HA. The role of exercise dependence for the relationship between exercise behavior and eating pathology: mediator or moderator? *J Health Psychol.* 2008;13:495-502.
9. Sprangers RL, Wesseling KH, Imholz AL, Imholz BP, Wieling W. Initial blood pressure fall on stand up and exercise explained by changes in total peripheral resistance. *J Appl Physiol (1985).* 1991;70:523-530.
10. Ubeda Tikkanen A, Opotowsky AR, Bhatt AB, Landzberg MJ, Rhodes J. Physical activity is associated with improved aerobic exercise capacity over time in adults with congenital heart disease. *Int J Cardiol*. 2013;168:4685-4691.
11. Seo DY, Lee SR, Kim N, Ko KS, Rhee BD, Han J. Humanized animal exercise model for clinical implication. *Pflugers Arch.* 2014;466:1673-1687.
12. Itoh Y, Hayakawa K, Mori T, Agata N, Inoue-Miyazu M, Murakami T, Sokabe M, Kawakami K. Stand-up exercise training facilitates muscle recovery from disuse atrophy by stimulating myogenic satellite cell proliferation in mice. *Physiol Rep.* 2014. doi: 10.14814/phy2.12185.
13. Helmreich DL, Parfitt DB, Lu XY, Akil H, Watson SJ. Relation between the hypothalamic-pituitary-thyroid (HPT) axis and the hypothalamic-pituitary-adrenal (HPA) axis during repeated stress. *Neuroendocrinology.* 2005;81:183-192.
14. Seo DY, McGregor RA, Noh SJ, Choi SJ, Mishchenko NP, Fedoreyev SA, Stonik VA, Han J. Echinochrome A improves exercise capacity during short-term endurance training in rats. *Mar Drugs.* 2015;13:5722-5731.
15. Jeffery NS, Stephenson RS, Gallagher JA, Jarvis JC, Cox PG. Micro-computed tomography with iodine staining resolves the arrangement of muscle fibres. *J Biomech.* 2011;44:189-192.
16. Bruns RF, Menegatti CM, Martins WP, Araujo Junior E. Applicability of pocket ultrasound during the first trimester of pregnancy. *Med Ultrason.* 2015;17:284-288.
17. Fukada S, Morikawa D, Yamamoto Y, Yoshida T, Sumie N, Yamaguchi M, Ito T, Miyagoe-Suzuki Y, Takeda S, Tsujikawa K, Yamamoto H. Genetic background affects properties of satellite cells and mdx phenotypes. *Am J Pathol.* 2010;176:2414-2424.
18. Brenner DA, Apstein CS, Saupe KW. Exercise training attenuates age-associated diastolic dysfunction in rats. *Circulation.* 2001;104:221-226.
19. Reznik M. Electron microscopy and histochemistry of regenerating skeletal muscle. *Neurol India.* 1972;20:71-74.
20. Ahmet I, Tae HJ, de Cabo R, Lakatta EG, Talan MI. Effects of calorie restriction on cardioprotection and cardiovascular health. *J Mol Cell Cardiol.* 2011;51:263-271.
21. Zhang B, Wang B, Cao S, Wang Y. Epigallocatechin-3-Gallate (EGCG) attenuates traumatic brain injury by inhibition of edema formation and oxidative stress. *Korean J Physiol Pharmacol.* 2015;19:491-497.
22. Andersson C, Lyass A, Larson MG, Spartano NL, Vita JA, Benjamin EJ, Murabito JM, Esliger DW, Blease SJ, Hamburg NM, Mitchell GF, Vasan RS. Physical activity measured by accelerometry and its associations with cardiac structure and vascular function in young and middle-aged adults. *J Am Heart Assoc.* 2015;4:e001528.
23. Harris L, Hankey C, Murray H, Melville C. The effects of physical activity interventions on preventing weight gain and the effects on body composition in young adults with intellectual disabilities: systematic review and meta-analysis of randomized controlled trials. *Clin Obes.* 2015;5:198-210.
24. Chen N, Li Q, Liu J, Jia S. Irisin, an exercise-induced myokine as a metabolic regulator: an updated narrative review. *Diabetes Metab Res Rev.* 2016;32:51-59.
25. Mifune H, Tajiri Y, Nishi Y, Hara K, Iwata S, Tokubuchi I, Mitsuzono R, Yamada K, Kojima M. Voluntary exercise contributed to an amelioration of abnormal feeding behavior, locomotor activity and ghrelin production concomitantly with a weight reduction in high fat diet-induced obese rats. *Peptides.* 2015;71:49-55.
26. Mahajan RD, Patra SK. Irisin, a novel myokine responsible for exercise induced browning of white adipose tissue. *Indian J Clin Biochem.* 2013;28:102-103.
27. Bluher S, Panagiotou G, Petroff D, Markert J, Wagner A, Klemm T, Filippaios A, Keller A, Mantzoros CS. Effects of a 1-year exercise and lifestyle intervention on irisin, adipokines, and inflammatory markers in obese children. *Obesity (Silver Spring).* 2014;22:1701-1708.
28. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Bostrom EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Hojlund K, Gygi SP, Spiegelman BM. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature.* 2012;481:463-468.
29. Al-Daghri NM, Alokail MS, Rahman S, Amer OE, Al-Attas OS, Alfawaz H, Tripathi G, Sabico S, Chrousos GP, McTernan PG, Piya MK. Habitual physical activity is associated with circulating irisin in healthy controls but not in subjects with diabetes mellitus type 2. *Eur J Clin Invest*. 2015;45:775-781.
30. Hew-Butler T, Landis-Piwowar K, Byrd G, Seimer M, Seigneurie N, Byrd B, Muzik O. Plasma irisin in runners and nonrunners: no favorable metabolic associations in humans. *Physiol Rep.* 2015. doi: 10.14814/phy2.12262.

31. Hecksteden A, Wegmann M, Steffen A, Kraushaar J, Morsch A, Ruppenthal S, Kaestner L, Meyer T. Irisin and exercise training in humans results from a randomized controlled training trial. *BMC Med.* 2013;11:235.
32. Gomez-Merino D, Drogou C, Guezennec CY, Chennaoui M. Effects of chronic exercise on cytokine production in white adipose tissue and skeletal muscle of rats. *Cytokine.* 2007;40:23-29.
33. Yang X, Zhou Y, Wang P, He C, He H. Effects of whole body vibration on pulmonary function, functional exercise capacity and quality of life in people with chronic obstructive pulmonary disease: A systematic review. *Clin Rehabil.* 2015. doi: 10.1177/02692155155-89202. [Epub ahead of print]
34. Nishikawa M, Ishimori N, Takada S, Saito A, Kadoguchi T, Furihata T, Fukushima A, Matsushima S, Yokota T, Kinugawa S, Tsutsui H. AST-120 ameliorates lowered exercise capacity and mitochondrial biogenesis in the skeletal muscle from mice with chronic kidney disease via reducing oxidative stress. *Nephrol Dial Transplant.* 2015; 30:934-942.