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Original Research

Effects of aerobic exercise training on circulating angiopoietin-like protein 2 in overweight and obese men: a pilot study

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Abstract

Background and objective: Angiopoietin-like protein 2 (ANGPTL2) is a pro-inflammatory adipokine that is upregulated in obesity and plays a role in the progression of cardiometabolic diseases, including diabetes and atherosclerosis. Aerobic exercise is one of the effective strategies for reducing the levels of various pro-inflammatory biomolecules in obese individuals. However, the effects of aerobic exercise training on circulating ANGPTL2 levels in obese individuals remain unclear. The objective of this study was to investigate the effect of aerobic exercise training on serum ANGPTL2 levels in overweight and obese men. **Material and methods**: Twenty overweight and obese men (age, 49 $\pm$ 10 years; body mass index, 27.4 $\pm$ 2.2 kg/m^2) completed a 12-week aerobic exercise training program (60–85% Heart rate$_{max}$, 40–60 min/day, 3 days/week). Before and after the exercise program, serum ANGPTL2 levels were measured using the enzyme-linked immunosorbent assay. Daily step counts and the different physical activities based on the intensity were assessed using a triaxial accelerometer. **Results**: Serum ANGPTL2 levels were significantly decreased after the 12-week aerobic exercise training program ((3.0 $\pm$ 0.6) vs. (2.7 $\pm$ 0.7) ng/mL, $P < 0.05$). Daily step counts ((8362 $\pm$ 4551) vs. (10357 $\pm$ 3168) steps/day, $P < 0.05$) and moderate- to vigorous-intensity physical activity (MVPA) time ((58 $\pm$ 45) vs. (76 $\pm$ 37) min/day, $P < 0.001$) were significantly increased after the exercise intervention. The changes in serum ANGPTL2 levels were negatively correlated with corresponding changes in daily step counts (partial $r = –0.49$, $P < 0.05$) and MVPA time (partial $r = –0.47$, $P < 0.05$) after adjustment for age and accelerometer wear time. **Conlcusion**: These findings collectively suggest that aerobic exercise training, in particular an increase in MVPA, can be associated with decreased circulating levels of ANGPTL2 in overweight and obese men.

Keywords: Angiopoietin-like protein 2; Inflammation; Aerobic exercise; Obesity

1. Introduction

Obesity has become a major health concern and is closely linked to the pathogenesis of several chronic diseases, such as type 2 diabetes mellitus, hypertension, dyslipidemia, cardiovascular disease, and some cancers [1, 2]. Accumulating evidence indicates that obesity causes chronic and systemic inflammation [3]. Although adipose tissue plays an essential role in maintaining lipid and glucose homeostasis, excess adipose tissue in obesity releases a variety of adipokines and bioactive mediators [3]. These processes promote a chronic low-grade inflammatory environment that contributes to the development of obesity-related clinical disorders.

Angiopoietin-like protein 2 (ANGPTL2), pro-inflammatory adipokine, belongs to the angiopoietin-like family (ANGPTL1-8), which is characterized by a coiled-coil domain at the N-terminus and a C-terminal fibrinogen-like domain [4]. ANGPTL2 is abundantly expressed in the adipose tissue of obese mice [5]. ANGPTL2 expression in adipose tissues increases under obese conditions, and increased ANGPTL2 secretion from adipose tissues promotes pathological adipose tissue remodeling by macrophage recruitment, resulting in metabolic disease, such as obesity-related insulin resistance [5]. Circulating levels of ANGPTL2 have been reported to be positively correlated with body mass index (BMI), body mass, visceral fat area, and other adiposity parameters in the normal-weight population [5,6], as well as in overweight or obese populations [7,8]. Several observational studies have demonstrated that circulating levels of ANGPTL2 increase with chronic inflammation and are associated with diabetes, cardiovascular disease, chronic kidney disease, and some cancers [6,9,10]. Collectively, these findings suggest that circulating levels of ANGPTL2 can be a key inflammatory mediator of obesity-induced metabolic abnormalities.

It has been established that lifestyle modifications (e.g., regular exercise or dietary modification) are effective in reducing the levels of various pro-inflammatory biomolecules in obese individuals [11–13]. In this context, we recently demonstrated that a dietary modification program was associated with reduced circulating ANGPTL2 levels in overweight and obese men [14]. Furthermore, a previous study showed that lifestyle interventions (combining nutritional and physical activity counseling programs) reduced plasma ANGPTL2 levels in overweight men [7]. In addition, higher plasma ANGPTL2 levels were reported to be associated with lower aerobic fitness in patients with coronary artery disease (CAD) and young or age-matched healthy controls [15]. Therefore, these findings suggest that habitual exercise can be effective in reducing circulating ANGPTL2 levels. However, it remains unknown whether aerobic exercise training decreases circulating ANGPTL2 levels in the obese population.

The purpose of this study was to investigate the effects of a 12-week aerobic exercise training program on serum ANGPTL2 levels in overweight and obese men. We hypothesized that aerobic exercise would lower serum ANGPTL2 levels, and that changes in physical activity during the exercise training program could be associated with corresponding changes in serum ANGPTL2 levels.

2. Material and methods

2.1 Participants

Participants were recruited through a local newspaper advertisement, and the inclusion criteria were men aged 30–64 years with a BMI of $\geq$25 kg/m^2. A total of 20 overweight and obese men (with a mean age and BMI of 49 $\pm$ 10 years and 27.4 $\pm$ 2.2 kg/m^2 respectively) completed a 12-week aerobic exercise training program. All participants had no apparent cardiovascular disease, as assessed by the medical history and physical examination results. All participants were sedentary or moderately physically active, and did not participate in any other vigorous exercise or sports activity. This study included two current smokers, and seven participants were taking antihypertensive, anti-diabetic, or anti-hyperlipidemic medication. The present study was reviewed and approved by the Institutional Review Board of the University of Tsukuba (Tai28-144) and was registered in the UMIN (University Hospital Medical Information Network) clinical trials registry (UMIN000027711). All participants provided written informed consent prior to enrolment in the present study.

2.2 Study design

Antropometric measures, blood pressure, resting heart rate, blood chemistry and VO_{2peak} were determined in all the participants before and after the 12-week aerobic exercise intervention. Daily step counts and physical activities based were measured before and throughout the exercise intervention period. Dietary intake was estimated using a questionnaire before and after the exercise intervention. All measurements were taken 48-hour after their last exercise training session to avoid the acute effects. Participants fasted overnight for 12-hour, duing which time they abstained from caffeine, alcohol, medications and smoking. Blood sampling was completed between 8:00–11:59 AM, and the other measurements were completed latest before 2:00 PM. Immediately before assessment, participants rested in the supine position for a minimum of 20-minute. These measurements were performed in a quiet and temperature-controlled room (25 °C).

2.3 Aerobic exercise intervention

The participants underwent an aerobic exercise training program for 12-week (3 days/week). In present study, the protocol of aerobic exercise training program for obese individuals reported by our laboratory's previous study was used [16–18]. The exercise program included a 15-minute warm-up session followed by 40–60 minutes of walking and/or light jogging session and concluded with a 15-minute cool-down session [19]. The exercise intensity was gradually increased between 60% and 85% of the participant's age-predicted maximum heart rate (target Borg's scale ranging from 11 to 15) during the exercise intervention period using a potable heart rate monitor (Fitbit charge HR, Fitbit Japan, Tokyo, Japan). In the first 2-month, exercise consisted only of walking, with the 60%–75% of maximum heart rate (target Borg's scale ranging from 11 to 14). In the last month, the participants performed a combination of walking and light jogging, with 75%–80% of maximum heart rate (target Borg's scale ranging from 14 to 15). Moreover, the participants were encouraged to continue performing aerobic exercise in their homes on other days. During the intervention, the participants were instructed to maintain their current eating habits.

2.4 Clinical measurements

Body mass was determined to the nearest 0.1 kg on a calibrated digital scale (InBody 770, InBody Japan, Tokyo, Japan), and height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Digital height meter AD-6227; A&D, Tokyo, Japan). Body mass index was calculated by dividing the weight (kg) by the height (m^2). Total body fat and skeletal muscle mass were determined by using bioelectrical impedance (InBody 770, InBody Japan, Tokyo, Japan). Waist circumference was measured in duplicate directly on the skin at the level of the umbilicus in a standing position to the nearest 0.1 cm. The abdominal visceral fat area was measured using the dual-impedance analysis method (HDS-2000; Omron Healthcare, Kyoto, Japan). This method is useful for a simple measurement, and there were high correlations between abdominal visceral fat area determined using the dual-impedance analysis method (HDS-2000) and computed tomography (r = 0.888, $P < 0.001$) [20]. Systolic blood pressure, diastolic blood

Table 1. Participants characteristics.

Variable	Before			After		
	means	±	SD	means	±	SD
Age, years	49	±	10		—	
Height, cm	170.5	±	6.7		—	
Body mass, kg	79.9	±	8.3	77.8	±	8.5**
Body mass index, kg/m^2	27.4	±	2.2	26.7	±	2.2**
Total body fat, %	27.9	±	4.2	26.4	±	3.8*
Skeletal muscle mass, kg	32.3	±	3.6	32.1	±	3.6
Waist circumference, cm	95.7	±	6.5	92.5	±	6.4**
Abdominal visceral fat area, cm^2	89.3	±	25.5	79.0	±	21.1*
Total cholesterol, mg/dL	204	±	32	204	±	33
Fasting glucose, mg/dL	102	±	13	100	±	8
Triglycerides, mg/dL	133	±	93	100	±	40*
Systolic blood pressure, mm Hg	126	±	14	128	±	17
Diastolic blood pressure, mm Hg	82	±	10	80	±	10
Resting heart rate, beats/min	61	±	6	57	±	8*
VO_{2peak}, mL (kg·min)$^{-1}$	25.7	±	3.6	29.8	±	4.3**
Total energy intake, kcal/day	1939	±	465	1902	±	674

Values are means ± SD.

*$P < 0.05$, **$P < 0.001$ vs. before the intervention.

pressure, and resting heart rate were measured using a semi-automated vascular testing device (Form PWV/ABI, Colin Medical Technology, Aichi, Japan) in the supine position.

2.5 Serum ANGPTL2 levels and blood biochemistry

Blood samples were collected from the antecubital vein after a 12-hour overnight fast. Serum concentrations of total cholesterol, fasting glucose, and triglycerides were measured using the standard enzymatic techniques. Serum ANGPTL2 levels were measured using a human ANGPTL2 enzyme-linked immunosorbent assay (ELISA) kit (Code No. 27745, Immuno-Biological Laboratories, Tokyo, Japan) according to the manufacturer's protocol.

2.6 Physical activity assessment

Daily step counts and the different physical activities based on the intensity (inactivity, light, and moderate-to-vigorous) were measured using a triaxial accelerometer (ActiveStyle Pro HJA-7501T; Omron Healthcare Co. Ltd., Kyoto, Japan) for 2 weeks before the exercise intervention (for the baseline examination) and throughout the exercise intervention period (weeks 11–12 of the intervention). The accelerometer counted daily steps and estimated the different intensities of physical activity (expressed as metabolic equivalents [METs]) with a validated algorithm [21], except when sleeping or bathing. A valid day was defined as a wear time of $\geq$10 h/day. When valid data were obtained for $\geq$3 days, daily step counts and physical activity were summarized for each participant. The physical activity levels were classified into three activity categories: light-intensity physical activity (LPA), representing 2.0–2.9 METs; moderate-intensity physical activity (MPA), representing 3.0–5.9 METs; vigorous-intensity physical activity (VPA), representing $\geq$6.0 METs [22]. Moderate to vigorous-intensity physical activity (MVPA) was calculated by summing the MPA and VPA times divided by the number of valid days.

2.7 Measurement of VO_{2peak}

The peak oxygen consumption (VO_{2peak}) was determined during a graded exercise test using a cycling ergometer using an online computerassisted circuit spirometry (AE300S; Minato Medical Science, Osaka, Japan) before and after the exercise intervention. After a 2-minute warmup at 40 W, the workload was increased by 20 W each minute until the subject felt exhausted (Borg's scale $>$19) or reached 85% of the age-predicted maximal heart rate.

2.8 Survey of dietary intake

Daily dietary intake was recorded using a food frequency questionnaire (FFQg 5.0; Kenpaku-Sha, Tokyo, Japan) before and after the intervention. FFQg is based on 29 food groups, and it can be used for a variety of clinical investigations [23]. The FFQg is composed of items on 29 food groups and 10 cooking methods and assesses the average daily intake frequency and total intake per week for each food or food group [24]. The total daily energy intake was estimated using a computer system with software using Excel-Eiyokun version 8.0 (Kenpaku Co., Ltd., Tokyo, Japan).

2.9 Statistical analysis

Data are presented as means ± standard deviation (SD). The normality of all parameters was assessed using the Shapiro-Wilk test. The paired Student's *t*-test and Wilcoxon signed-rank test were used to analyze the variables before and after the exercise intervention. The correlation between changes in serum ANGPTL2 levels and corresponding changes in physical activity levels or adiposity after the intervention were determined using the partial correlation coefficient. The partial correlation coefficient was adjusted for age or age and wear time of the device. All statistical analyses were performed using SPSS (version 24.0; IBM Inc., NY, USA), with $P < 0.05$, considered statistically significant.

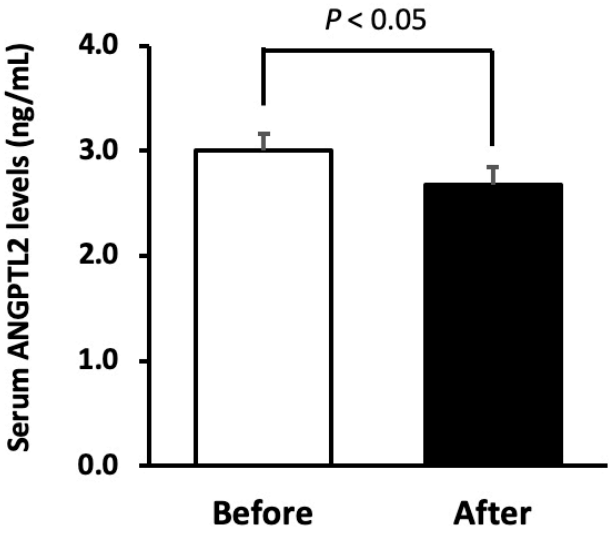

Fig. 1. Serum ANGPTL2 levels before and after a 12-week aerobic exercise training. Data are presented means ± SD.

3. Results

Participant characteristics before and after the 12-week exercise intervention are shown in Table 1. Body mass, BMI, total body fat, waist circumference, abdominal visceral fat area, triglyceride, and heart rate of the participants significantly decreased, and VO_{2peak} was significantly elevated after the 12-week exercise intervention. There were no significant differences in skeletal muscle mass, total cholesterol, fasting plasma glucose, systolic blood pressure, diastolic blood pressure, and total energy intake before and after the intervention. Fig. 1 shows the changes in serum ANGPTL2 levels before and after the 12-week exercise intervention. Serum ANGPTL2 levels were significantly decreased after the intervention compared with before the intervention ((3.0 ± 0.6) vs. (2.7 ± 0.7) ng/mL, $P < 0.05$). Fig. 2 shows the changes in physical activity during the 12-week exercise intervention period. Daily step counts ((8362 ± 4551) vs. (10357 ± 3168) steps/day, $P < 0.05$) and MVPA time ((58 ± 45) vs. (76 ± 37) min/day, $P < 0.001$) were significantly increased during the 12-week exercise intervention period. Moreover, we found significant correlation between changes in serum ANGPTL2 levels and corresponding changes in MVPA time (partial $r = –0.47$, $P < 0.05$) or daily step counts (partial $r = –0.49$, $P < 0.05$) after adjustment for age and accelerometer wear time (Fig. 3). However, there was no significant correlation between the changes in serum ANGPTL2 levels and the changes in body mass (partial $r = 0.29$, $P = 0.22$) or abdominal visceral fat area (partial $r = 0.1$, $P = 0.68$) after adjustment for age.

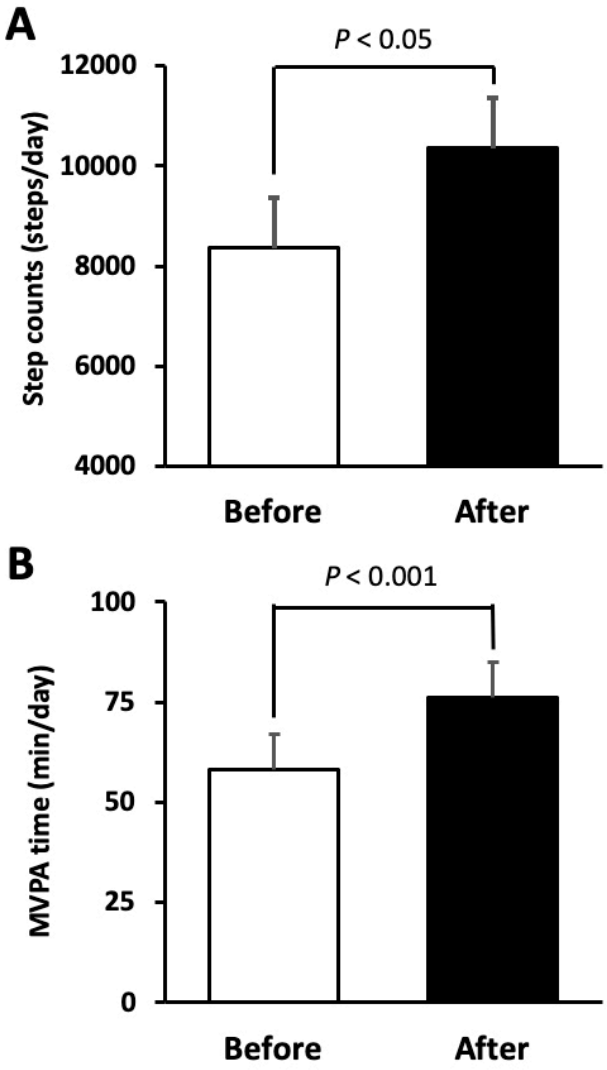

Fig. 2. Physical activity before and after 12-week aerobic exercise training. Data are presented means ± SD.

4. Discussion

In this study, we investigated whether a 12-week aerobic exercise training decreased serum ANGPTL2 levels in overweight and obese men. Our study showed that

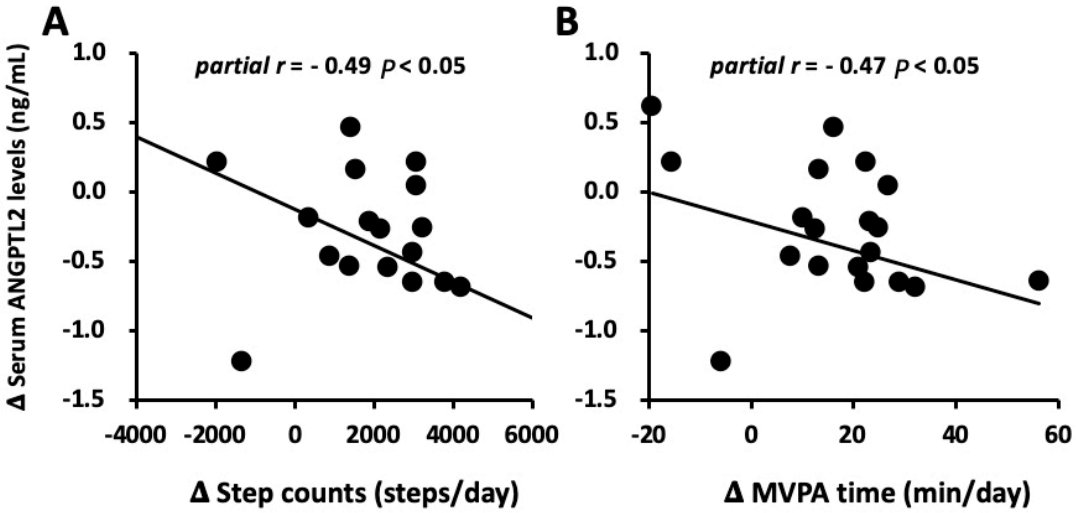

Fig. 3. Association between the changes in serum ANGPTL2 levels and Step counts (A) and MVPA time (B) following a 12-week aerobic exercise training.

serum ANGPTL2 levels significantly decreased after aerobic exercise intervention. Moreover, we found that the exercise-induced reductions in circulating ANGPTL2 were correlated with corresponding changes in daily step counts and MVPA time. These results suggest that aerobic exercise training can be beneficial in reducing circulating ANGPTL2 levels in overweight and obese men.

A previous study reported that aerobic exercise reduced plasma ANGPTL2 levels in male patients with coronary artery disease [25]. Furthermore, it has been demonstrated that weight reduction with lifestyle modification is associated with lower serum ANGPTL2 levels in overweight men [7]. These previous studies have implied that exercise intervention may decrease circulating ANGPTL2 levels in obese individuals. However, the effects of aerobic exercise training on circulating ANGPTL2 levels in obese individuals have not yet been clarified. To the best of our knowledge, this is the first study to show a significant reduction in circulating ANGPTL2 levels after aerobic exercise training in overweight and obese men.

AGNTPL2 is abundantly expressed in the white adipose tissue, especially in the visceral adipose tissue in mice, and ANGPTL2 overexpression in adipose tissue leads to an increase in adipose tissue inflammation [5]. These results suggest that adipose tissue is the main source of ANGPTL2 [5]. Muramoto *et al.* [7] reported that circulating ANGPTL2 levels decreased with a reduction in body weight and visceral fat in overweight men. This suggests that circulating ANGPTL2 levels can reflect adiposity. In present study, adiposity parameters, such as body mass, BMI, total body fat, waist circumference and abdominal visceral fat area significantly decreased after the exercise intervention. However, there was no significant correlation between changes in serum ANGPTL2 levels and adiposity parameters after aerobic exercise training. It is well established that aerobic exercise improves chronic inflammatory states, especially in obese individuals with high levels of pro-inflammatory biomarkers. Moreover, several studies have reported that the protective effects of aerobic exercise on inflammation are independent of weight loss [13,26]. These data might explain why the present study failed to observe significant associations between changes in serum ANGPTL2 levels and adiposity parameters after aerobic exercise training. It has also been reported that moderate- to vigorous- intensity physical activity is associated with a more favorable profile of inflammatory markers [27]. Together, these findings suggest that aerobic exercise training-induced increase in MVPA effects circulating ANGPTL2 levels, independent of adiposity.

Zheng *et al.* [28] showed that TNF-α treatment increased the expression of the ANGPTL2 gene in adipocytes. This means that the adipocyte production of ANGPTL2 may be stimulated by TNF-α. These results indicate that the reduction of TNF-α through aerobic exercise training may inhibit the expression of ANGPTL2. Meng *et al.* [29] reported that epinephrine, which was massively released during exercise, inhibited the ANGPTL2 expression. These results indicate that a higher concentration of exercise-induced epinephrine may affect a reduction in circulating ANGPTL2 levels. It has been reported that epinephrine itself inhibits TNF-α expression [30]. Therefore, changes in TNF-α and epinephrine during aerobic exercise training may affect changes in circulating ANGPTL2 levels. However, we did not measure these parameters in this study; therefore, we could not confirm the association between changes in circulating ANGPTL2 levels and TNF-α or epinephrine.

The present study has several limitations. First, this study is a pilot study with a small sample size, and we did not use any comparable control group. Future randomized

controlled trials with larger sample sizes and wider population of subjects are needed to establish the effectiveness of aerobic exercise training on circulating ANGPTL2 levels in overweight and obese individuals. Second, as mentioned above, we did not measure other parameters (e.g., adipokines or adioponectin, myokines, some hormones, ect.) in blood sample which is may affect changes in circulating ANGPTL2 levels, and did not investigate the mechanisms underlying the effects of aerobic exercise on circulating ANGPTL2 levels. Therefore, further research is warranted to better understand the mechanisms responsible for the observed changes in overweight and obese individuals.

5. Conclusions

In this study, we have demonstrated that a 12-week aerobic exercise training decreased serum ANGPTL2 levels in overweight and obese men. Moreover, aerobic exercise training-induced reduction in serum ANGPTL2 levels was associated with increased daily step counts and MVPA time. These findings collectively suggest that aerobic exercise training can be associated with decreased circulating levels of ANGPTL2 in overweight and obese men.

Abbreviations

ANGPTL2, Angiopoietin-like protein 2; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; PA, physical activities; METs, metabolic equivalents; LPA, light-intensity physical; MPA, moderate-intensity physical activity; VPA, vigorous-intensity physical activity; MVPA, moderate- to vigorous- intensity physical activity.

Author contributions

JP, KM, NS and SM conceived and designed this study. JP, KK, YC, KM, and TT performed experiments and analyzed the data. JP, KK, and SM made the first draft of the manuscript and all authors contributed to editorial changes in this manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of the University of Tsukuba (Tai28-144) and was registered in the UMIN (University Hospital Medical Information Network) clinical trials registry (UMIN000027711). All the experiments were carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki), and all participants provided written informed consent before participating.

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Conflict of interest

The authors declare no conflict of interest.

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