Disclosure Statement

The authors declare that there are no conflicts of interest.

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HEPATOLOGY

Moderate to Vigorous Physical Activity Volume Is an Important Factor for Managing Nonalcoholic Fatty Liver Disease: A Retrospective Study

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Recently, the beneficial effects of increased physical activity (PA) on nonalcoholic fatty liver disease (NAFLD) in obese subjects were reported. However, the optimal strength and volume of PA in lifestyle modification to improve NAFLD pathophysiology and be recommended as an appropriate management of this condition are unclear. The primary goal of this retrospective study was to estimate the beneficial effects of a varying volume of moderate to vigorous intensity PA (MVPA) on the improvement of NAFLD. A total of 169 obese, middle-aged men were enrolled in a 12-week weight reduction program through lifestyle modification consisting of dietary restrictions plus aerobic exercise. Among these obese subjects, 40 performed MVPA for <150 min·wk^{-1}, 42 performed MVPA for 150-250 min·wk^{-1}, and 87 performed MVPA for >250 min·wk^{-1}. The subjects in the MVPA ≥250 min·wk^{-1} group, in comparison with those in the MVPA <250 min·wk^{-1} group, showed significantly attenuated levels of hepatic steatosis (−31.8% versus −23.2%). This attenuation was likely independent of the detectable weight reduction. MVPA for ≥250 min·wk^{-1} in comparison with that for <150 min·wk^{-1} led to a significant decrease in the abdominal visceral adipose tissue severity (−40.6% versus −12.9%), levels of ferritin (−13.6% versus +1.5%), and lipid peroxidation (−15.1% versus −2.8%), and a significant increase in the adiponectin levels (+17.1% versus +5.6%). In association with these changes, the gene expression levels of sterol regulatory element-binding protein-1c and carnitine palmitoyltransferase-1 in peripheral blood mononuclear cells also significantly decreased and increased, respectively. *Conclusion:* MVPA for ≥250 min·wk^{-1} as part of lifestyle management improves NAFLD pathophysiology in obese men. The benefits seem to be acquired through reducing inflammation and oxidative stress levels and altering fatty acid metabolism. (HEPATOLOGY 2014;00:000-000)

With the increasing prevalence of overweight and obese middle-aged men in Japan, nonalcoholic fatty liver disease (NAFLD) is commonly diagnosed in daily clinical practice; analysis of data from cross-sectional health examination surveys in the general population shows the prevalence of NAFLD in middle-aged men to be >40% in Japan.[1]

Abbreviations: ACC, acetyl-CoA carboxylase; ACS, acyl-CoA synthetase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; CPT1, carnitine palmitoyltransferase I; FAS, fatty acid synthase; FFAs, free fatty acids; FPG, fasting plasma glucose; Fpn1, ferroportin-1; γGT, gamma glutamyl transpeptidase; HDL-C, high-density lipoprotein-cholesterol; HO1, heme oxygenase 1; HOMA-IR, insulin resistance by homeostasis model assessment; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; Mac2bp, mac-2 binding protein; MVPA, moderate to vigorous intensity physical activity; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NQO1, NADH quinone oxidoreductase; Nrf2, nuclear factor E2-related factor 2; SAT, subcutaneous adipose tissue; SCD1, stearoyl-CoA desaturase-1; SREBP1c, sterol regulatory element-binding protein 1c; PBMCs, peripheral blood mononuclear cells; TBARS, thiobarbituric acid reactive substances; TEI, total daily energy intake; TF, total fat; TG, triglycerides; TNF-α, tumor necrosis factor alpha; VAT, visceral adipose tissue; WC, waist circumference.

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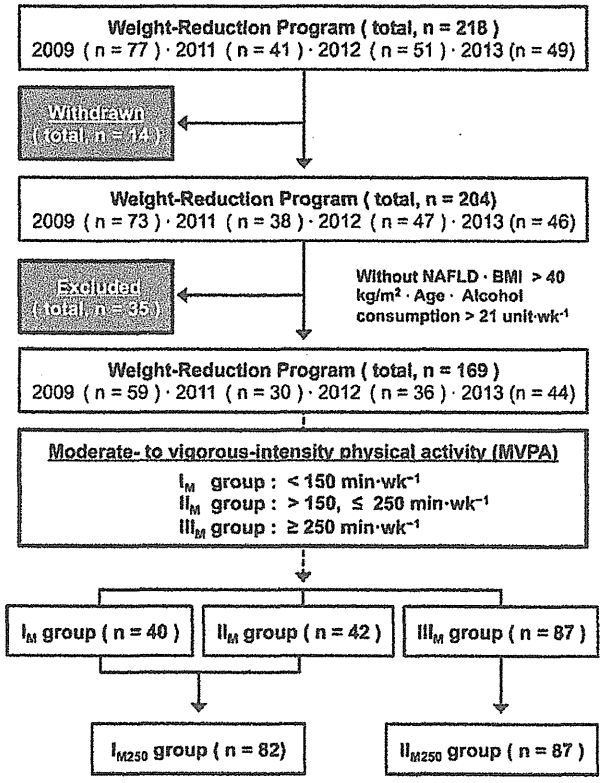

Fig. 1. Flow diagram of the enrollment and classification of study participants.

Weight reduction has been the only strategy established thus far to reduce hepatic lipid levels[2]; thus, dietary restrictions focused on weight reduction is recommended as the cornerstone for managing NAFLD.[3] With respect to physical activity (PA), recent reports on clinical outcomes have indicated that physical inactivity and low aerobic fitness are underlying reasons for the increased number of NAFLD cases,[4,5] and that both aerobic and resistance exercises have specific effects on NAFLD treatment in the absence of weight reduction.[6-8] We have recently proven the therapeutic effects of increased PA with or without dietary restriction in greatly reducing hepatic inflammation and the related oxidative stress levels outweigh those achieved by dietary restriction alone.[9,10] Thus, although there is an overall paucity of evidence on the benefits of PA as a treatment for NAFLD, management should include assessing PA levels and setting of lifestyle goals based on adopting regular exercise, with a focus on attaining sustainable PA habits.

Diet and PA interventions are important for NAFLD management, and there is increasing evidence that exercise beneficially modulates liver fat content. However, at present, clear guidelines for such a "lifestyle PA" for NAFLD management are lacking, and the dose (i.e., intensity and volume) of PA required to reduce liver fat content remains unclear.[11]

Considering this issue, we conducted a retrospective analysis of a large number of obese, middle-aged men with NAFLD who completed a 12-week supervised exercise plus dietary restriction program to determine the benefits of a varying PA dose (intensity and volume) in lifestyle modification on improving the pathophysiology of NAFLD.

Materials and Methods

Subjects. Figure 1 depicts the workflow of enrollment to the program, which was carried out in 2009 and 2011-2013 at the University of Tsukuba (Tsukuba, Japan). A total of 218 obese, middle-aged men (body mass index [BMI] 25-40 kg/m^2)[12] were recruited from Ibaraki prefecture through advertisements of weight reduction by means of a lifestyle management program by way of dietary restriction and exercise. The diagnosis of NAFLD was based on the diagnostic guidelines for NAFLD in the Asia-Pacific region.[13] For a comparative and thorough analysis, we excluded participants who withdrew, those without NAFLD, those <35 or >65 years old, those with BMI >40 kg/m^2, and those with an alcohol consumption of >21 units·wk^{-1}. Finally, of the initial 218 applicants, 169 subjects were enrolled and their data were analyzed for this retrospective study. Participants who performed moderate to vigorous intensity PA (MVPA) per week at PA level 4-9, estimated by using a uniaxial accelerometer, were classified into three groups: I_M group, <150 min·wk^{-1} (mean 101.6 ± 15.8 min·wk^{-1}, n = 40); II_M group, 150-250 min·wk^{-1} (mean 216.0 ± 15.4 min·wk^{-1}, n = 42); and III_M group, ≥250 min·wk^{-1} (mean 409.7 ± 10.7 min·wk^{-1}, n = 87) (for details, see Table 1). For further analysis, we created two subgroups by using

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MVPA for 250 min·wk^{-1} as the cutoff (I_{M250} group: <250 min·wk^{-1} [mean 160.3 ± 7.20 min·wk^{-1}, n = 82]; II_{M250} group: ≥250 min·wk^{-1} [mean 409.7 ± 10.7 min·wk^{-1}, n = 87]). This allowed us to obtain more concrete data on the effects of a larger volume of MVPA on the risk factors for fibrosis progression and the changes of total fatty composition and related genes. The study protocol was approved by the Institutional Review Board of the University of Tsukuba. All participants provided written informed consent before their participation in the study.

Diet Restriction and Exercise Programs. The subjects were provided with a dietary program restricting their dietary intake to ~1,680 kcal·d^{-1}. The dietary intake per meal (three times per day) was 80 kcal from eggs and/or dairy products; 80 kcal from vegetables and fruits; 160 kcal from meat, fish, and/or soybean products; and 240 kcal from carbohydrates and oils. During the 12 weekly lectures, the subjects mainly learned methods for calculating dietary calories by weighing food and planned a dietary program for maintaining 1,680 kcal·d^{-1}. After each lecture, dietitians checked the subjects' food diaries (on which daily dietary calories were recorded) and provided face-to-face dietary behavioral counseling.

The subjects also underwent an aerobic exercise program of 90 min·d^{-1}, 3 days·wk^{-1}. This program consisted of 40-60 minutes of walking and/or light jogging sessions and 15-25 minutes each of warm-up and cool-down sessions. Although they were given a few recommendations targeted to the Borg[14] scale ranging from 11 (light) to 13 (fairly hard), the subjects were free to determine the intensity and volume of exercise appropriate for their health condition. By taking part in the above sessions, the subjects learned about exercise methods, such as how to increase the exercise intensity, volume, and frequency according to their physical condition. On the basis of this education, the subjects were advised to keep performing aerobic exercise at or around their homes on days.

Energy Intake and PA Analysis. At baseline and at week 12, the study subjects maintained daily food-intake records for 3 consecutive days. A dietician used the food-intake records to estimate the total daily energy intake (TEI) and macronutrient composition (carbohydrate, protein, and fat intake), by using Excel Eiyo-Kun v. 4 software (Kenpakusya, Tokyo, Japan).

Daily PA monitoring in the aerobic exercise program and in free-living conditions was conducted using a uniaxial accelerometer (Lifecorder; Suzuken, Nagoya, Japan). As this device continuously measures the intensity, duration, and frequency of PA, it is useful for obtaining objective data on PA patterns and for estimating the related energy expenditure. Such PA information taken in 4-second sampling intervals was categorized into one of nine PA levels (1.0-9.0). Each PA level can be classified into a category of metabolic equivalents (METs) according to Kumahara et al.[15] PA levels estimated according to METs were also categorized on the basis of the intensity cutoff by Pate et al.,[16] and a PA level from 4.0 to 9.0 was used as the MVPA (for details, see Table 1).

Hepatic Stiffness and Steatosis. During 2011-2013, a clinical gastroenterologist assessed hepatic stiffness by using a Fibroscan device (Echosens, Paris, France) with the 3.5-MHz standard probe. The principles and examination procedures for such an assessment have been previously published.[17] In addition, the hepatic steatosis levels in 2012 and 2013 were determined by using a controlled attenuation parameter (CAP) designed to measure the liver ultrasonic attenuation at 3.5 MHz by using signals acquired with Fibroscan. Detailed descriptions of the CAP have also been previously published.[18]

Anthropometric Parameters. The body weight was measured with a digital electronic scale (TBF-551; Tanita, Tokyo, Japan). Their standing height was measured with a wall-mounted stadiometer (YG-200; Yagami, Nagoya, Japan). These data were used to calculate the BMI (kg/m^2). Total fat (TF) was assessed by using dual-energy x-ray absorptiometry (QDR 4500; Hologic, Bedford, MA). Abdominal adipose tissue was determined by using magnetic resonance imaging (3.0-Tesla system, Achieva R3.2; Philips, Best, the Netherlands) in which the visceral adipose tissue (VAT) area and subcutaneous adipose tissue (SAT) area were measured at the umbilicus level. Waist circumference (WC) was measured by using a fiberglass tape at the umbilicus level.

Blood Test. The levels of high-density lipoprotein-cholesterol (HDL-C) and triglycerides (TG) were analyzed enzymatically as follows: fasting plasma glucose level with a hexokinase-G-6-PDH method; fasting plasma insulin level with a chemiluminescent immunoassay method; aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transpeptidase (γGT) levels with the Japan Society of Clinical Chemistry transferable method; hyaluronic acid (HA) and ferritin levels with the latex agglutination method; and high-sensitivity C-reactive protein (hs-CRP) level with a fixed time assay method. The platelets were counted on an automated analyzer (XE-2100; Sysmex, Kobe, Japan). We used these data to calculate the surrogate marker levels for insulin resistance by homeostasis model assessment (HOMA-IR) according to Matthews et al.[19] and for NAFLD fibrosis scores (NFS) according to the equation of Angulo et al.[20]

Table 1. PA Data (Intensity and Volume) Recorded With the Accelerometer for the 12-Week Weight Reduction Program in a Total of 169 Obese, Middle-Aged Men With NAFLD, With Stratification According to the Time of Moderate to Vigorous Intensity PA

		I_M	II_M	III_M	I_M vs. II_M	I_M vs. III_M	II_M vs. III_M
PA Intensity*	PA level [METs]†	N = 40	N = 42	N = 87		*P* Value	
Light intensity, *min·wk*$^{-1}$	1.0 [1.8]	87.2 ± 6.61	100.5 ± 6.46	100.8 ± 4.49	0.327	0.211	0.999
	2.0 [2.3]	189.8 ± 13.0	215.5 ± 12.7	216.9 ± 8.82	0.338	0.200	0.995
	3.0 [2.9]	90.2 ± 7.15	108.6 ± 6.98	115.6 ± 4.85	0.158	<0.05	0.691
Moderate intensity, *min·wk*$^{-1}$	4.0 [3.6]	61.1 ± 7.77	100.8 ± 7.58	156.1 ± 5.27	<0.01	<0.01	<0.01
	5.0 [4.3]	25.6 ± 9.03	62.5 ± 8.81	122.3 ± 6.12	<0.01	<0.01	<0.01
	6.0 [5.2]	10.9 ± 7.52	30.3 ± 7.33	78.7 ± 5.01	0.158	<0.01	<0.01
Vigorous intensity, *min·wk*$^{-1}$	7.0 [6.1]	2.55 ± 3.31	8.16 ± 3.23	20.4 ± 2.25	0.385	<0.01	<0.01
	8.0 [7.1]	1.20 ± 3.42	11.8 ± 3.03	24.8 ± 2.32	<0.05	<0.01	<0.01
	9.0 [>8.3]	0.26 ± 1.82	2.64 ± 1.78	7.35 ± 1.24	0.622	<0.05	0.078
LPA (PA level 1.0-3.0), *min·wk*$^{-1}$		367.2 ± 23.3	424.6 ± 22.8	433.3 ± 15.8	0.187	0.061	0.947
MVPA (PA level 4.0-9.0), *min·wk*$^{-1}$		101.6 ± 15.8	216.0 ± 15.4	409.7 ± 10.7	<0.01	<0.01	<0.01
Energy expenditure, *kcal·d*$^{-1}$		2388.3 ± 42.5	2437.1 ± 41.0	2576.1 ± 28.8	0.205	<0.01	<0.01
Step frequency, *step·d*$^{-1}$		7367.1 ± 382.0	9075.5 ± 372.8	11779.4 ± 259.7	<0.01	<0.01	<0.01

Values are presented as the group means ± SE. To compare between groups, all dependent variables in PA levels were analyzed by using one-way ANOVA. *PA levels estimated by using METs were categorized according to the intensity cutoff of Pate et al.[16] (1.0 [PA level] = 1.8 [MET], 2.0 = 2.3, 3.0 = 2.9, 4.0 = 3.6, 5.0 = 4.3, 6.0 = 5.2, 7.0 = 6.1, 8.0 = 7.1, 9.0 = >8.3). †Each PA level measured with the accelerometer was classified into the category of METs according to Kumahara et al.[15] (light: <3.0 [MET], moderate: 3.0-6.0 and vigorous: >6.0).

PA, physical activity; METs, metabolic equivalents; LPA, light intensity PA; MVPA, moderate to vigorous intensity PA; I_M, the group who performed moderate to vigorous intensity PA for <150 min·wk^{-1}; II_M, the group who performed moderate to vigorous intensity PA for 150-250 min·wk^{-1}; III_M, the group who performed moderate to vigorous intensity PA for ≥250 min·wk^{-1}.

Serum fatty acid compositions were analyzed with gas chromatography-mass spectrometry. Commercial enzyme-linked immunosorbent assay kits were used to measure the serum levels of thiobarbituric acid reactive substances (TBARS; Cayman Chemical, Ann Arbor, MI); tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and leptin (R&D Systems, Minneapolis, MN); M30 and M65 (Peviva; Bromma, Sweden); WFA^+-Mac-2 binding protein (WFA^+-M2BP; Sysmex, Kobe, Japan); M2BP (Immuno-Biological Lab, Kunma, Japan), and adiponectin (Sekisui Medical, Tokyo, Japan).

PBMC Isolation, RNA Isolation, and Reverse-Transcription, Real-Time Quantitative Polymerase Chain Reaction (PCR). The gene expression levels in peripheral blood mononuclear cells (PBMCs) can reflect those in the liver.[21] PBMCs are considered a good model to reflect important metabolic changes in the liver.[22]

PBMCs were isolated from LSM density gradients (MP Biomedical, Santa Ana, CA). From cell pellets containing the PBMCs, RNA was extracted. Subsequently, first-strand complementary DNA was synthesized by using a PrimeScript RT reagent kit (Takara Bio, Shiga, Japan). The complementary DNA templates were added to Fast SYBR Green Master Mix (Applied Biosystems, Santa Ana, CA). PCR was performed on a CFX384 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Foster City, CA). The primers (FASMAC, Tokyo, Japan) are shown in Supporting Material 1.

Statistics. Statistical analysis was performed using SPSS 20.0 (IBM, Armonk, NY). Descriptive parameters were given as mean ± SE or log transformations for skewed variables, and as percentages for categorical variables. To compare groups for all dependent variables at baseline and PA data, we performed one-way analysis of variance (ANOVA). Categorical variables, the chi-square test, or Fisher's exact test was used. To compare intra- and intergroup changes over time (at baseline and the 12th week), all dependent variables of the pathophysiological factors of NAFLD were subjected to a repeated-measure ANOVA with/without the change in weight as a covariate. $P < 0.05$ was considered significant.

Results

Baseline Characteristics. There were no significant differences in age, BMI, PA, alcohol intake, smoking, or medication use of subjects among the three groups (Table 2). The mean values of all parameters including dietary intake (Table 3), body adiposity (Table 4), and blood test (Table 4) were not significantly different between the three groups. The attendance rate was 81.2% in the I_M group, 80.3% in the II_M group, and 85.3% in the III_M group. The difference was not statistically significant.

PA Data. Table 1 shows the results of PA recorded with the accelerometer during the

Table 2. Baseline Characteristics of a Total of 169 Obese, Middle-Aged Men With NAFLD Who Participated in a Weight Reduction Program, With Stratification According to the Time of Moderate to Vigorous Intensity PA

	I_M	II_M	III_M	
Parameters	Baseline	Baseline	Baseline	*P* Value
n	40	42	87	
Age, *year*	52.6 ± 1.30	49.0 ± 1.27	51.9 ± 0.88	0.096
BMI, kg/m^2	29.4 ± 0.51	28.8 ± 0.50	29.2 ± 0.35	0.673
Physical activity				
Energy expenditure, $kcal \cdot d^{-1}$	2354.3 ± 40.1	2351.4 ± 40.0	2425.3 ± 27.8	0.182
Step frequency, $steps \cdot d^{-1}$	7277.9 ± 312.4	7497.7 ± 304.9	7944.9 ± 204.8	0.151
Alcohol intake, $unit \cdot wk^{-1}$	7.68 ± 1.46	8.92 ± 1.34	7.92 ± 1.04	0.785
Smoking, % *subject*	15.0	19.0	17.2	0.888
Medication				
Hyperglycemic agents, % *subject*	7.5	2.4	5.7	0.569
Hypertensive agents, % *subject*	32.5	26.2	32.2	0.759
Hyperlipidemia agents, % *subject*	15.0	19.0	18.4	0.869

Values are presented as the group means ± SE and as percentages. To compare between groups, all dependent variables at baseline were analyzed by using one-way ANOVA. In the case of categorical variables, the chi-square test or Fisher's exact test was used.

BMI, body mass index; I_M, the group who performed moderate to vigorous intensity PA for <150 $min \cdot wk^{-1}$; II_M, the group who performed moderate to vigorous intensity PA for 150-250 $min \cdot wk^{-1}$; III_M, the group who performed moderate to vigorous intensity PA for ≥250 $min \cdot wk^{-1}$.

intervention periods. When a comparison was made between the groups, the mean values of the energy expenditure and step frequency were greater in the III_M group than those in the I_M and II_M groups ($P < 0.01$). A comparison between I_M and II_M revealed that the magnitude of the change in step frequency was greater in the II_M group ($P < 0.01$) than in the I_M group. The mean value of the light-intensity PA was not significantly different between the three groups, whereas there was a statistical difference in the MVPA between each group ($P < 0.01$).

Dietary Intake. Table 3 shows the outcomes of dietary intake during the intervention period. According to the daily food-intake records, all groups had significantly reduced TEI and carbohydrate, protein, and fat intake between baseline and week 12 ($P < 0.01$). When an analysis with group-by-time interactions was made the magnitude of change in all outcomes showed no significant differences between the groups.

Body Weight. Weight and BMI (Table 4) were significantly reduced at week 12 in each group ($P < 0.01$). When comparing group-by-time interaction between groups, these parameters were significantly reduced for the II_M and III_M groups compared with the I_M group ($P < 0.01$). However, a comparison between II_M and III_M found no statistically significant difference.

Body Adiposity. The results of the evaluations of body composition and abdominal distribution (Table 4) revealed that all parameters were improved at week 12 in each group ($P < 0.01$). When comparing group-by-time interactions between groups with adjustment for change in weight, all body adiposity parameters significantly improved in the II_M and III_M groups compared with those in the I_M group ($P < 0.01$), except for the SAT area. A comparison between II_M and III_M with adjustment for change in weight revealed that the magnitude of change in the VAT area was greater in the III_M group ($P < 0.05$) than in the II_M group.

Blood Test. Among the 13 parameters in blood analysis (Table 4), eight parameters ($_{log}$TG, HOMA-IR, AST, ALT, γGT, $_{log}$hs-CRP, $_{log}$leptin, and $_{log}$TNF-α) in the I_M group ($P < 0.05$), nine parameters ($_{log}$TG, HDL-C, HOMA-IR, AST, ALT, γGT, $_{log}$hs-CRP, $_{log}$leptin, and $_{log}$TNF-α) in the II_M group ($P < 0.05$), and all 13 parameters in the III_M group ($P < 0.01$) improved at week 12. Parameters that did not show improvement in the I_M group were the serum levels of HDL-C, ferritin, TBARS, adiponectin, and IL-6, whereas those who failed to show improvement in the II_M group were the serum levels of ferritin, TBARS, adiponectin, and IL-6.

Analysis of group-by-time interactions with adjustment for change in weight revealed that the magnitude of change in the serum levels of $_{log}$TG was greater in the II_M group than in the I_M group ($P < 0.05$), and the magnitude of change in the serum levels of $_{log}$TG, HDL-C, ferritin, TBARS, and adiponectin was greater in the III_M group than in the I_M group ($P < 0.05$). A comparison between the II_M and III_M groups with the change in weight as a covariate revealed that only four parameters reflecting the magnitude of the increase (HDL-C and adiponectin) and the decrease (ferritin and TBARS) were greater in the III_M group than in the II_M group ($P < 0.05$).

Apoptosis and Fibrosis Biomarkers. Six molecules were selected as hepatocyte apoptosis and liver fibrosis biomarkers (apoptosis: M30, M65; fibrosis: M2BP, WFA^+-

Table 3. Outcomes of Body Weight and Dietary Intake of a Total of 169 Obese, Middle-Aged Men With NAFLD, With Stratification According to the Time of Moderate to Vigorous Intensity PA

	I_M (n = 40)			II_M (n = 42)			III_M (n = 87)			I_M vs. II_M	I_M vs. III_M	II_M vs. III_M
	Mean: 101.6 min·wk^{-1}/ 14.5 min·d^{-1}			Mean: 216.0 min·wk^{-1}/ 30.9 min·d^{-1}			Mean: 409.7 min·wk^{-1}/ 58.5 min·d^{-1}			Time × Group Interaction *P* Value		
Parameter	Baseline	After	Change	Baseline	After	Change	Baseline	After	Change			
Weight, *kg*	86.1 ± 2.15	80.6 ± 1.84	−5.5†	82.9 ± 1.39	73.0 ± 1.24	−9.9†	84.3 ± 1.18	73.4 ± 1.11	−10.9†	<0.01	<0.01	0.209
BMI, *kg/m*^{2}	29.4 ± 0.51	27.5 ± 0.48	−1.9†	28.8 ± 0.50	25.3 ± 0.47	−3.4†	29.2 ± 0.35	25.5 ± 0.33	−3.7†	<0.01	<0.01	0.227
TEI, *kcal·d*$^{-1}$	2199.1 ± 75.2	1542.2 ± 43.1	−656.8†	2177.5 ± 70.2	1490.4 ± 40.7	−687.2†	2231.0 ± 51.2	1545.5 ± 29.5	−685.6†	0.581	0.773	0.412
Carbohydrate, *g·d*$^{-1}$	295.9 ± 12.1	216.3 ± 7.05	−79.6†	278.9 ± 11.0	199.6 ± 6.42	−79.2†	299.4 ± 8.21	208.5 ± 4.56	−90.9†	0.981	0.433	0.114
Protein, *g·d*$^{-1}$	78.7 ± 3.04	68.8 ± 2.27	−9.9†	77.4 ± 3.12	65.4 ± 2.18	−12.0†	80.9 ± 2.07	72.8 ± 1.55	−8.1†	0.618	0.595	0.301
Fat, *g·d*$^{-1}$	60.5 ± 2.71	40.5 ± 1.79	−20.0†	65.4 ± 2.81	43.1 ± 1.74	−22.3†	62.5 ± 1.86	43.0 ± 1.21	−19.5†	0.602	0.864	0.902

Values are presented as the group means ± SE. To compare intergroup changes over time, from baseline to 12 weeks, all dependent variables were analyzed by using a repeated ANOVA. †$P < 0.01$, significant difference within group.

BMI, body mass index; PA, physical activity; TEI, total daily energy intake. I_M, the group who performed moderate to vigorous intensity PA for <150 min·wk^{-1}; II_M, the group who performed moderate to vigorous intensity PA for 150-250 min·wk^{-1}; III_M, the group who performed moderate to vigorous intensity PA for ≥250 min·wk^{-1}.

M2BP, HA, and NFS). Figure 2A shows the results of these parameter changes in each group from baseline to week 12. All biomarkers except NFS ($P = 0.12$) for the 82 subjects in the I_{M250} group and the 87 subjects in the II_{M250} group were decreased at week 12 ($P < 0.05$). When an analysis of group-by-time interactions with adjustment for change in weight was made between the groups, no statistically significant difference was found.

Hepatic Stiffness and Steatosis. The subjects in the 2012 and 2013 interventions were evaluated for hepatic steatosis levels by using CAP (Fig. 2B). In addition, hepatic stiffness was assessed between 2011 and 2013. From the CAP, a significant reduction in hepatic steatosis levels was seen after the 12-week program in the 31 subjects in the I_{M250} group and in the 49 subjects in the II_{M250} MVPA group (−23.2% and −31.8%, respectively). When a group-by-time interaction analysis with adjustment for change in weight was performed and compared between the groups, the magnitude of the changes in hepatic steatosis was greater in the II_{M250} group than in the I_{M250} group ($P < 0.05$). Hepatic stiffness also significantly improved in both groups (I_{M250} n = 41, II_{M250} n = 69) from baseline to week 12 (−21.0% and −21.9%, respectively). However, a comparison between these groups with the change in weight as a covariate revealed that the magnitude of the changes in stiffness was not significantly different ($P = 0.64$).

Fatty Acid Composition. The serum fatty acid compositions recorded between 2011 and 2013 are shown in Table 5. The total fatty acid parameters for the 41 subjects in the I_{M250} group and the 69 subjects in the II_{M250} group were significantly decreased at week 12 (I_{M250}: −19.8%, II_{M250}: −26.9%; $P < 0.01$). The total saturated fatty acid, total monounsaturated fatty acid, and total polyunsaturated fatty acid levels also significantly decreased in both groups ($P < 0.05$). When an analysis of group-by-time interactions was performed and compared between the groups with the change in weight as a covariate, the magnitude of change in all outcomes was not significantly different.

Expression Levels of Genes Involved in Fatty Acid Synthesis and Degradation. Table 5 shows the changes in the expression levels in the PBMCs of theeight genes involved in fatty acid synthesis ($_{log}$SREBP1c [sterol regulatory element-binding protein 1c], $_{log}$FAS [fatty acid synthase], $_{log}$SCD1 [stearoyl-CoA desaturase-1], $_{log}$ACC [acetyl-CoA carboxylase], $_{log}$EVLOVE6) and degradation ($_{log}$ACS [acyl-CoA synthetase], $_{log}$CPT1 [carnitine palmitoyltransferase I], $_{log}$acyl-CoA oxidase) in each group between baseline and week 12. Among the eight PBMC genes, three ($_{log}$FAS, $_{log}$ACC, $_{log}$ACS)in the I_{250M} group ($P < 0.05$) and seven

Table 4. Parameter Outcomes of Body Adiposity, Lipid Profiles, and Insulin Resistance, Liver Function Test, Inflammation and Oxidative Stress, and Adipocytokine Values in a Total of 169 Obese, Middle-Aged Men With NAFLD, With Stratification According to the Time of Moderate to Vigorous Intensity PA

	I_M (n = 40)			II_M (n = 42)			III_M (n = 87)			I_M vs. II_M	I_M vs. III_M	II_M vs. III_M
	Mean: 101.6 min·wk^{-1}/14.5 min·d^{-1}			Mean: 216.0 min·wk^{-1}/30.9 min·d^{-1}			Mean: 409.7 min·wk^{-1}/58.5 min·d^{-1}			Time × Group Interaction *P* Value		
Parameter	Baseline	After	Change	Baseline	After	Change	Baseline	After	Change			
Body adiposity												
WC, *cm*	101.7 ± 1.77	96.4 ± 1.62	−5.3†	98.9 ± 0.95	88.5 ± 1.00	−10.4†	99.8 ± 0.76	88.7 ± 0.95	−11.0†	< 0.01	< 0.05	0.694
TF, %	25.3 ± 0.65	23.3 ± 0.61	−2.1†	25.3 ± 0.49	21.0 ± 0.56	−4.3†	24.3 ± 0.37	19.4 ± 0.43	−4.9†	< 0.01	< 0.01	0.334
VAT area, cm^2	163.2 ± 8.06	142.2 ± 7.33	−21.0†	149.9 ± 4.89	105.5 ± 5.13	−44.4†	166.4 ± 6.68	98.8 ± 5.26	−67.6†	< 0.05	< 0.01	< 0.01
SAT area, cm^2	223.7 ± 16.1	181.1 ± 12.2	−42.6†	230.9 ± 10.1	153.9 ± 7.57	−77.0†	229.0 ± 7.37	150.2 ± 6.99	−78.8†	0.400	0.603	0.329
Lipid profiles and insulin resistance												
$_{log}$TG	2.12 ± 0.03	2.02 ± 0.03	−0.10†	2.14 ± 0.04	1.87 ± 0.03	−0.27†	2.13 ± 0.03	1.85 ± 0.02	−0.28†	< 0.05	< 0.01	0.983
HDLC, *mg/dL*	46.5 ± 1.51	47.3 ± 1.50	+0.8	47.9 ± 1.66	50.1 ± 1.66	+2.2*	50.9 ± 1.04	56.1 ± 1.13	+5.2†	0.121	< 0.05	0.069
HOMA-IR	2.61 ± 0.22	2.03 ± 0.26	−0.58*	2.64 ± 0.37	1.25 ± 0.13	−1.39†	2.39 ± 0.21	1.26 ± 0.22	−1.13†	0.595	0.412	0.256
Liver function test												
AST, *U/L*	24.8 ± 1.44	21.5 ± 0.73	−3.2†	25.5 ± 1.46	20.8 ± 0.94	−4.7†	25.9 ± 1.19	21.4 ± 0.82	−4.5†	0.894	0.294	0.630
ALT, *U/L*	34.3 ± 2.89	26.1 ± 1.70	−8.2†	36.3 ± 3.67	22.9 ± 1.58	−13.5†	33.7 ± 2.70	22.0 ± 1.18	−11.7†	0.563	0.432	0.414
γGT, *U/L*	61.6 ± 8.61	43.1 ± 4.88	−18.5†	61.9 ± 9.16	29.1 ± 2.96	−32.8†	49.4 ± 2.98	25.3 ± 1.61	−24.1†	0.440	0.892	0.095
Inflammation and oxidative stress												
$_{log}$ hs-CRP	1.81 ± 0.08	1.67 ± 0.08	−0.14*	1.78 ± 0.07	1.59 ± 0.07	−0.19†	1.81 ± 0.05	1.56 ± 0.05	−0.25†	0.489	0.155	0.315
Ferritin, μg/L	225.7 ± 16.2	229.1 ± 17.4	+3.4	203.9 ± 15.9	195.9 ± 16.9	−8.0	197.9 ± 11.0	171.0 ± 11.8	−26.9†	0.209	< 0.05	< 0.05
TBARS, μM/L	21.7 ± 1.28	21.1 ± 1.10	−0.6	23.4 ± 1.41	22.2 ± 1.41	−1.2	22.5 ± 0.97	19.1 ± 0.66	−3.4†	0.814	< 0.01	< 0.01
Adipocytokines												
Adiponectin, μg/mL	3.93 ± 0.27	4.05 ± 0.32	+0.22	4.13 ± 0.33	4.36 ± 0.32	+0.23	4.85 ± 0.25	5.68 ± 0.24	+0.83†	0.459	< 0.05	< 0.05
$_{log}$ Leptin	4.73 ± 0.04	4.48 ± 0.04	−0.25†	4.82 ± 0.04	4.46 ± 0.05	−0.36†	4.82 ± 0.26	4.43 ± 0.33	−0.39†	0.214	0.324	0.890
$_{log}$TNF-α	1.36 ± 0.04	1.94 ± 0.08	−0.42†	1.32 ± 0.04	0.99 ± 0.07	−0.33†	1.34 ± 0.03	1.12 ± 0.04	−0.22†	0.193	0.062	0.087
IL-6, *pg/mL*	1.38 ± 0.13	1.22 ± 0.13	−0.16	1.46 ± 0.15	1.21 ± 0.17	−0.25	1.65 ± 0.12	1.26 ± 0.09	−0.39†	0.865	0.099	0.371

Values are presented as the group means ± SE. To compare intergroup changes over time, from baseline to 12 weeks, all dependent variables were analyzed by using a repeated ANOVA with the change in weight as a covariate. †$P < 0.01$ and *$P < 0.05$, significant difference within group.

PA, physical activity; WC, waist circumference; TF, total fat; VAFA, visceral abdominal fat area; SAFA, subcutaneous abdominal fat area; TG, triglyceride; HDLC, high density lipoprotein cholesterol; HOMA-IR, insulin resistance by homeostasis model assessment; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, gamma glutamyl transpeptidase; hs-CRP, high-sensitivity C-reactive protein; TBARS, thiobarbituric acid reactive substances; TNF-α, tumor necrosis factor alpha; IL-6, interleukin 6; I_M, the group who performed moderate to vigorous intensity PA for <150 min·wk^{-1}; II_M, the group who performed moderate to vigorous intensity PA for 150-250 min·wk^{-1}; III_M, the group who performed moderate to vigorous intensity PA for ≥250 min·wk^{-1}.

(except $_{log}$SCD1) in the II_{250M} group ($P < 0.05$) were found to be significantly different. When a comparison was made between the two groups with the change in weight as a covariate, only two genes showed a magnitude of decrease ($_{log}$SREBP1c) or increase ($_{log}$CPT1) that was greater in the III_M group than in the II_M group ($P < 0.05$).

Discussion

The major findings of this study are that MVPA for ≥250 min·wk^{-1} in comparison with MVPA for <250 min·wk^{-1} attenuates the degree of hepatic steatosis independent of weight reduction. In relation, MVPA for ≥250 min·wk^{-1} induced a significant improvement in the VAT severity and levels of ferritin, lipid peroxidation, and adiponectin (Fig. 3A), as well as altered fatty acid metabolism. Moreover, MVPA for 150-250 min·wk^{-1}, in comparison with MVPA for <150 min·wk^{-1}, also induced a significant improvement in the WC, TF, and VAT severity and TG level (Fig. 3B,C). Overall, the results suggest that an increase in MVPA per week benefits the management of NAFLD. To the best of our knowledge, these results provide the first clinical evidence of the benefits of an increase in MVPA, which, in turn, is used as a "lifestyle PA" in NAFLD management.

In this study, MVPA for >150 min·wk^{-1} achieved a 12.4% reduction in weight for obese, middle-aged men with NAFLD, whereas MVPA for <150 min·wk^{-1} showed a 6.4% reduction in the equivalent amount of calorie intake. This observed weight-reduction-effect of MVPA for >150 min·wk^{-1} is important for NAFLD management. Moreover, to investigate the significance of MVPA volume regardless of weight reduction on NAFLD risk factors, all parameters were analyzed with/without the change in weight as a covariate. After controlling for change in weight, the magnitude of MVPA for ≥250 min·wk^{-1} on insulin resistance and inflammatory adipokines (leptin and TNF-α) became

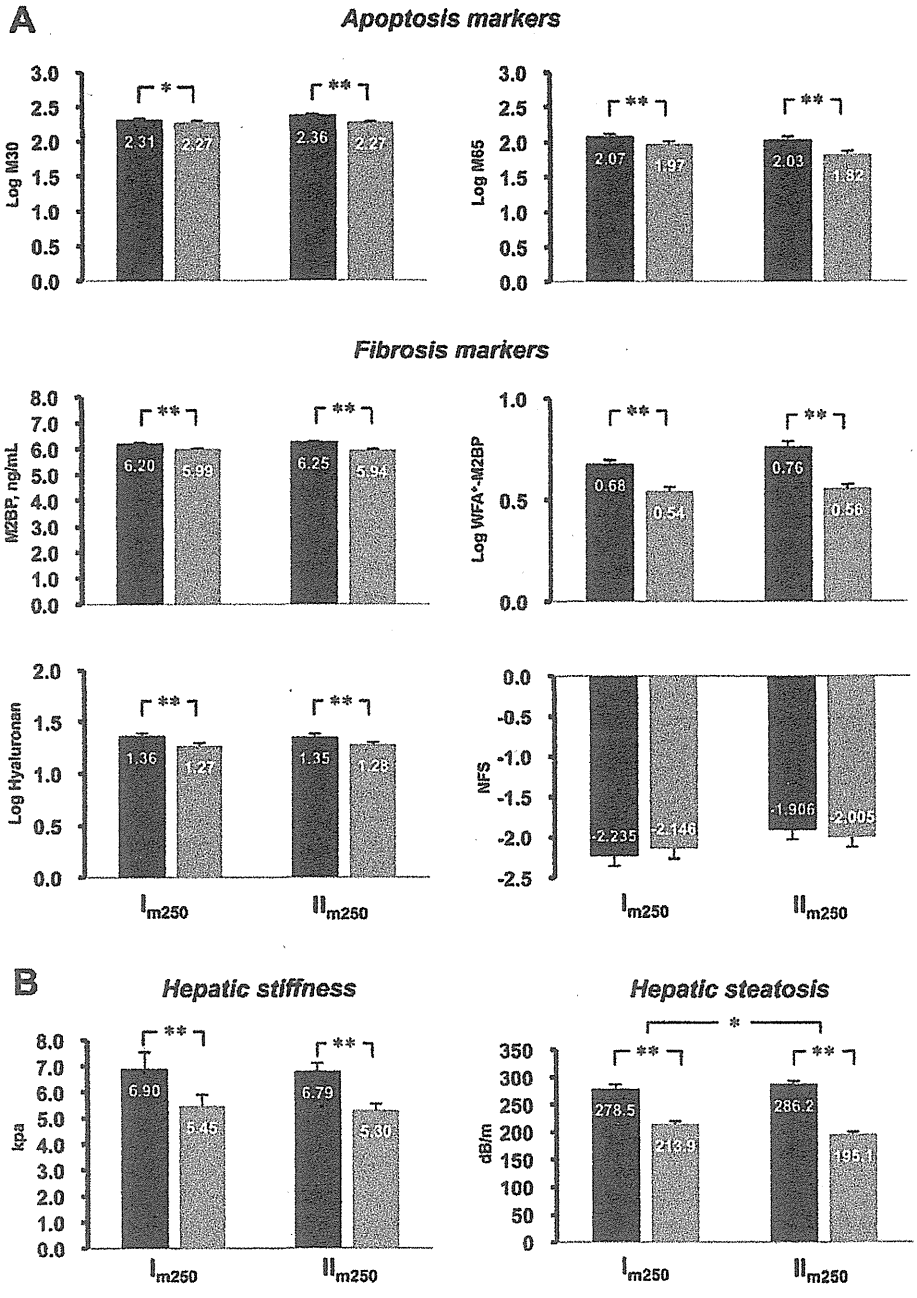

Fig. 2. Changes in the levels of apoptosis and fibrosis markers (total number of subjects = 169; I_{M250} = 76, II_{M250} = 87) (A) and the degree of hepatic steatosis (total, 80; I_{M250} = 31, II_{M250} = 49) and stiffness (total, 110; I_{M250} = 41, II_{M250} = 69) (B) from baseline to week 12 in obese, middle-aged men. Values are presented as the group means ± SE. A repeated ANOVA model with the change in weight as a covariate was used to compare the intra- and inter-group changes over time, from baseline to week 12. The black bar indicates baseline and the gray bars indicate after week 12 (means ± SE). $^{**}P < 0.01$, $^{*}P < 0.05$, significantly different between baseline and week 12; brackets $^{**}P < 0.01$, $^{*}P < 0.05$, significantly different MVPA performed per week between the groups.

statistically nonsignificant; however, that in the change in hepatic steatosis, VAT, TG, HDLC, ferritin, TBARS, and adiponectin remained significant. Collectively, the effects of MVPA seem to be associated with an improvement in the hepatic inflammatory conditions and related oxidative stress levels. These benefits might be independent of weight reduction.

In subjects with NAFLD, the clinical benefits observed in those who performed MVPA for ≥250 min·wk^{-1} are the improved inflammatory and oxidative stress levels, reflected by higher serum ferritin and TBARS levels.[23] Hepatic iron overload, reflected by elevated serum ferritin levels[24] and dysregulation of adipokine production, further increases the intrahepatic oxidative stress levels.[25] This, in turn, causes impaired nucleotide and protein synthesis, leading to apoptosis, inflammation, and liver fibrosis.[25] Therefore, the potent reduction in hepatic fat content and serum ferritin and TBARS levels in subjects who performed MVPA for ≥250 min·wk^{-1} may have contributed to improved NAFLD pathophysiology. The results indicated the advantage of adding MVPA for

Table 5. Serum Total Fatty Acid Composition and Expression Levels of Genes Involved in Fatty Acid Metabolism for a Total of 110 Obese, Middle-Aged Men With NAFLD, With Stratification According to the Time of Moderate to Vigorous Intensity PA

	I_{M250} (n = 41)			II_{M250} (n = 69)			I_{M250} vs. II_{M250}
Parameter	Baseline	After	Change	Baseline	After	Change	Time x Group Interaction *P* Value
Fatty acid composition (μg/mL)							
Total SFA	1387.5 ± 84.9	1092.1 ± 59.1	−295.4†	1311.6 ± 59.1	916.2 ± 27.2	−395.4*	0.556
C14:0	36.8 ± 4.67	25.6 ± 5.32	−11.2*	33.4 ± 3.18	11.9 ± 3.62	−21.5†	0.267
C16:0	1010.0 ± 63.3	803.5 ± 35.8	−206.5†	957.0 ± 43.1	680.4 ± 24.4	−276.6†	0.620
C18:0	299.0 ± 16.3	228.5 ± 10.0	−70.5†	280.3 ± 11.1	191.5 ± 6.83	−88.8†	0.857
C22:0	21.0 ± 0.70	17.8 ± 0.61	−3.2†	20.4 ± 0.47	16.6 ± 0.41	−3.8†	0.586
C24:0	20.7 ± 0.66	16.7 ± 0.54	−4.0†	20.5 ± 0.45	15.8 ± 0.37	−4.7†	0.598
Total MUFA	1028.2 ± 65.1	750.5 ± 32.9	−277.7†	925.3 ± 43.9	623.0 ± 20.2	−302.3†	0.978
C16:1 W-7	77.6 ± 7.33	43.2 ± 3.39	−34.4†	71.9 ± 5.00	32.5 ± 2.31	−39.5†	0.623
C18:1 W-9	906.8 ± 57.8	660.7 ± 28.7	−246.1†	809.6 ± 39.4	541.3 ± 19.6	−268.3†	0.982
C20:1 W-9	6.12 ± 0.56	4.21 ± 0.18	−1.9†	5.53 ± 0.38	3.93 ± 0.13	−1.6†	0.655
C24:1 W-9	37.7 ± 1.40	42.4 ± 1.67	+4.7†	38.3 ± 0.95	45.3 ± 1.14	+7.0†	0.939
Total PUFA	1526.1 ± 53.2	1320.3 ± 39.8	−205.8†	1529.4 ± 41.5	1212.9 ± 24.6	−316.5†	0.305
C18:2 W-6	1112.5 ± 44.4	940.2 ± 28.3	−172.3†	1081.5 ± 30.2	838.1 ± 19.3	−243.4†	0.541
C20:2 W-6	7.33 ± 0.44	4.91 ± 0.23	−2.42†	7.14 ± 0.30	4.56 ± 0.16	−2.58†	0.867
C20:4 W-6	202.9 ± 10.0	187.3 ± 6.90	−15.6	207.3 ± 6.83	181.0 ± 4.70	−26.3†	0.128
C20:5 W-3	60.4 ± 9.78	55.1 ± 7.05	−8.3	76.7 ± 6.66	61.1 ± 4.80	−15.6†	0.554
C22:4 W-6	5.33 ± 0.43	3.92 ± 0.26	−1.41†	4.87 ± 0.29	3.43 ± 0.18	−1.44†	0.686
C22:6 W-3	137.6 ± 12.4	128.9 ± 8.27	−8.7	151.9 ± 8.44	124.7 ± 5.63	−27.2†	0.123
Genes involved in fatty acid synthesis							
$_{log}$SREBP1c	2.51 ± 0.06	2.58 ± 0.07	+0.07	2.52 ± 0.05	2.37 ± 0.05	−0.15*	<0.05
$_{log}$FAS	2.31 ± 0.09	2.78 ± 0.10	+0.476*	2.28 ± 0.07	2.55 ± 0.08	+0.27*	0.533
$_{log}$SCD1	1.30 ± 0.13	1.39 ± 0.12	+0.09	1.23 ± 0.09	1.32 ± 0.09	+0.09	0.990
$_{log}$ACC	2.35 ± 0.04	2.19 ± 0.05	−0.16†	2.38 ± 0.03	2.25 ± 0.04	−0.13†	0.663
$_{log}$EVLOVE6	1.96 ± 0.06	1.94 ± 0.04	−0.02	2.02 ± 0.05	1.91 ± 0.03	−0.11*	0.222
Genes involved in fatty acid degradation							
$_{log}$ACS	2.34 ± 0.07	2.75 ± 0.06	+0.41†	2.36 ± 0.05	2.79 ± 0.05	+0.43†	0.994
$_{log}$CPT1	2.22 ± 0.05	2.15 ± 0.07	−0.07	2.08 ± 0.04	2.17 ± 0.05	+0.09*	<0.05
$_{log}$ACO	2.20 ± 0.04	2.29 ± 0.04	+0.10*	2.22 ± 0.03	2.32 ± 0.03	+0.10†	0.963

Values are presented as the group means ± SE. To compare intergroup changes over time, from baseline to 12 weeks, all dependent variables were analyzed by using a repeated ANOVA with the change in weight as a covariate. †$P < 0.01$ and *$P < 0.05$, significant difference within group.

PA, physical activity; C14:0, myristic acid; C16:0, palmitic acid; C18:0, stearic acid; C22:0, behenic acid; C24:0, lignoceric acid; C16:1 W-7, palmitoleic acid; C18:1 W-9, oleic acid; C20:1 W-9, eicosenoic acid; C24:1 W-9, nervonic acid; C18:2 W-6, linoleic acid; C20:2 W-6, eicosadienoic acid; C20:4 W-6, arachidonic acid; C20:5 W-3; eicosapentaenoic acid; C22:4 W-6, docosatetraenoic acid; C22:6 W-3, docosahexaenoic acid; SREBP1c, sterol regulatory element-binding protein 1c; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; SCD1, stearoyl-CoA desaturase-1; ACS, acyl-CoA synthetase; CPT1, carnitine palmitoyltransferase I; ACO, acyl CoA oxidase; I_{M250}, the group who performed moderate to vigorous intensity PA for <250 min·wk^{-1}; II_{M250}, the group who performed moderate to vigorous intensity PA for ≥250 min·wk^{-1}.

≥250 min·wk^{-1} to dietary restriction. Because of the lack of histology data, the benefits of MVPA for ≥250 min·wk^{-1} in terms of antifibrosis effects could not be evaluated in this study. Nevertheless, the results of improvement in VAT, ferritin, TBARS, adiponectin, and other variables may indicate a potential improvement in inflammatory liver injuries associated with NASH. Recently, on the basis of liver histology, vigorous PA was related to a decrease in NASH risk found in a retrospective analysis of PA data from adult subjects with NAFLD.[26] Inflammation plays a central role in the onset of NASH.[27] Therefore, the antiinflammatory and/or antioxidative stress effects induced by MVPA for ≥250 min·wk^{-1} may be important in terms of the lifestyle management for NAFLD.

The details of such a mechanism underlying the observed clinical benefits are not fully understood; however, evidence from our laboratory and that of others may help clarify this issue. Exercise-induced oxidative stress, especially during high-intensity exercise such as MVPA for ≥250 min·wk^{-1}, triggers the activation of a redox-sensitive transcription factor known as nuclear factor E2-related factor 2 (Nrf2).[28] Nrf2 serves as an oxidative stress sensor and master regulator of the antioxidant response.[29] In this study, MVPA for ≥250 min·wk^{-1} significantly increased in obese subjects the expression levels of NADH quinone oxidoreductase (an enzyme involved in cellular detoxification) and ferropotin-1 (a transporter involved in cellular iron homeostasis), known as prototypical Nrf2 target genes (Supporting Material 2). Moreover, Nrf2 activation down-regulates the expression and activity of fatty acid synthesis enzymes,[30] which are associated with hepatic steatosis. Comparable to this, MVPA for ≥250

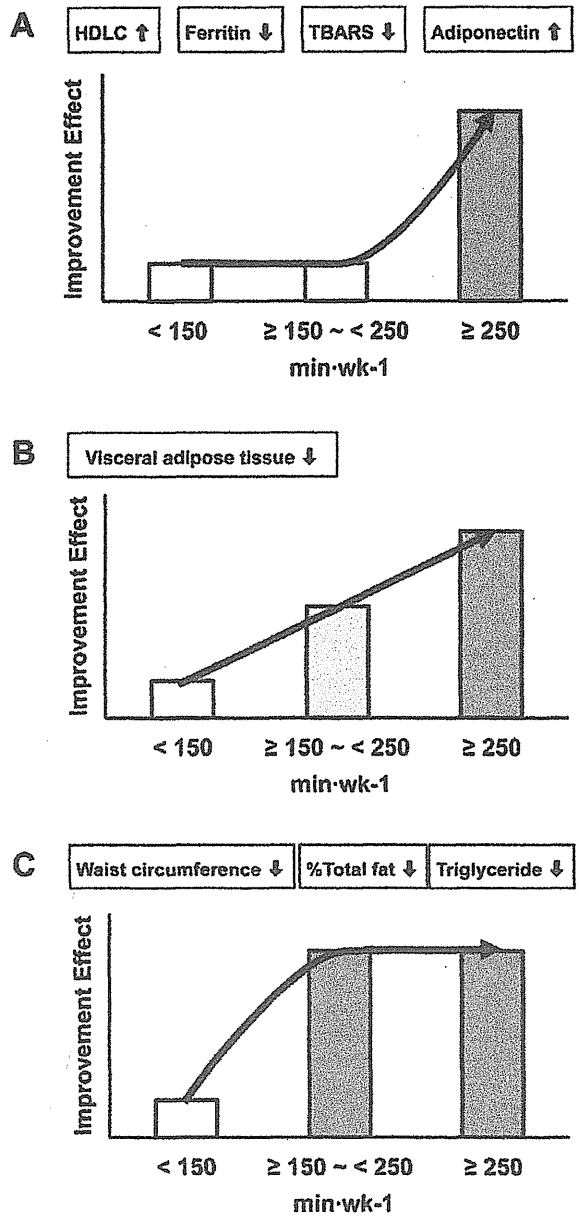

Fig. 3. Schematic summary of the beneficial effects of varying MVPA doses in lifestyle intervention on the pathophysiology of obesity-related liver disease in obese, middle-aged men. MVPA for ≥250 min·wk^{-1} combined with dietary restriction enhances the treatment effect by managing hepatic lipid levels through the modification of imbalanced adipokine levels and increased inflammation and oxidative stress levels in the liver (A). In addition, increasing the volume of MVPA contributes to improvements in obesity-related liver disease through reduction in the risk of abdominal obesity (B). Finally, MVPA for ≥150 min·wk^{-1} coupled with dietary restriction may arrest obesity and lipid profiles and thus also potentially improve obesity-related liver disease (C).

min·wk^{-1} decreased the SREBP1c expression involved in fatty acid synthesis and increased the CPT1 levels involved in fatty acid degradation (Table 5).

Adipokines, which are produced in adipose tissue, have a close relation with NAFLD pathology.[31] Therefore, it is noteworthy that MVPA for ≥250 min·wk^{-1} led to a marked increase in the adiponectin levels in comparison with MVPA for <150 min·wk^{-1} and MVPA for 150-250 min·wk^{-1} (Table 4). Thus, MVPA for ≥250 min·wk^{-1} is further beneficial as a lifestyle PA program in obese subjects. Low adiponectin levels are implicated in hepatic lipid accumulation and associated with NAFLD severity.[32] On the other hand, elevated adiponectin levels improve insulin sensitivity and enhance fatty acid oxidation, which in turn improve NAFLD pathology.[32] In addition, in one report, hepatic adiponectin signaling played a protective role against progression from simple steatosis to NASH in mice.[33] It is likely that the increased serum adiponectin level resulting from MVPA for ≥250 min·wk^{-1} contributes to the improved pathophysiology of NAFLD.

Concerning clinical relevance, increasing exercise frequency and dose, to achieve an MVPA of ≥250 min·wk^{-1}, is difficult for most obese subjects with NAFLD. In some obese subjects, continuous training with any type of exercise with less frequency and dose per week is recommended. The results of this study could address this critical issue. MVPA for 150-250 min·wk^{-1} yielded similar health benefits to those performing MVPA for ≥250 min·wk^{-1}, in terms of hepatic steatosis and the measured apoptosis and fibrosis markers. However, MVPA for <250 min·wk^{-1} did not affect HDL-C, ferritin, TBARS, adiponectin, VAT, SREBP1c, and CPT1 levels (Tables 3 and 4; Fig. 2B). Several studies have reported indications for obtaining the direct effect of exercise on hepatic steatosis[6-8]; exercise with less frequency and dose was found to be effective in both light and moderate volume exercise programs,[34] the PA volume of which was below the current guidelines for health promotion[35] and for managing body weight.[36] At present, scientific evidence for the benefits of PA and the optimal dose and modality for PA in NAFLD management is limited.[11]

In summary, the results of this study show the beneficial effects of increasing the volume of MVPA combined with dietary restriction on managing NAFLD in obese, middle-aged men. In the near future, prospective studies are needed to improve the objective criteria for the optimal intensity, duration, or total volume of MVPA; this is necessary to obtain beneficial effects in the management of NAFLD. Clarification of the criteria will enable the formulation of an effective and time-efficient PA program for improved outcomes, participation, and adherence of obese, middle-aged men.

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Longitudinal association between habitual walking and fall occurrences among community-dwelling older adults: Analyzing the different risks of falling

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ABSTRACT

The purpose of this longitudinal study was to examine the association between habitual walking and multiple or injurious falls (falls) among community-dwelling older adults, by considering the relative risk of falling. A cohort of Japanese community-dwelling older adults ($n = 535$) aged 60–91 years (73.1 ± 6.6 year, 157 men and 378 women) who underwent community-based health check-ups from 2008 to 2012 were followed until 2013. Incidence rate of falls between walkers and non-walkers was compared separately by the number of risk factors (Groups R0, R1, R2, R3 and R4+). The Cox proportional hazard model was used to assess the association between habitual walking and falls separately by lower- ($R < 2$) and higher- ($R \geq 2$) risk groups. In Groups R0 and R1, the incidence of falls was lower in walkers than non-walkers; however, in Groups R2, R3, and R4+, the incidence of falls was higher in walkers. The Cox proportional hazard model showed that habitual walking was not significantly associated with falls (hazard ratio (HR): 0.88, 95% confidence interval (CI): 0.48–1.62) among the lower risk group but that it was significantly associated with increased falls (HR: 1.89, 95% CI: 1.04–3.43) among the higher risk group. The significant interaction between habitual walking and higher risk of falling was found ($P < 0.05$). When individuals have two or more risk factors for falling, caution is needed when recommending walking because walking can actually increase their risk of experiencing multiple or injurious falls.

1. Introduction

Approximately 30% of community-dwelling elderly individuals experience falls each year (Tinetti, Speechley, & Ginter, 1988). In Japan, falls and consequent fractures are the fifth most common cause of functional dependence (Japanese Ministry of Health, Labour and Welfare, 2007). In the rapidly aging Japanese society, approximately one in four people are now 65 years of age or older (Japanese Ministry of Health, Labour and Welfare, 2013). The number of falls and their seriousness are expected to dramatically increase as the number of older adults increases worldwide (World Health Organization, 2008). The increasing age of the worldwide population has led to a corresponding need for fall prevention programs and solution-oriented approaches. Several attempts to develop national and community-wide approaches have recently been reported (Campbell & Robertson, 2010; McClure et al., 2005; Tinetti et al., 2008). Appropriate fall prevention programs that can benefit the greater community are urgently needed.

Recommended walking regimens have the potential to serve as effective community-wide fall prevention programs because such regimens can be implemented regardless of the time, location, previous sports experience of the participants, or the presence of instructors. Furthermore, walking is the most prevalent type of exercise (Japanese Ministry of Education, Culture, Sports Science and Technology, 2013; Morris & Hardman, 1997). However, the effects of walking as a part of fall prevention programs remain unclear (Gregg, Pereira, & Caspersen, 2000). A meta-analysis of randomized controlled trials (RCTs) reported that the inclusion of a

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walking program significantly increased the pooled fall rate by 32% (Sherrington et al., 2008). One study that reported an increase in the rate of falls with a walking-related intervention suggested that increased exposure to environmental hazards was likely to be the cause of the increased fall incidence (Vetter, Lewis, & Ford, 1992). Our previous cross-sectional study showed that among higher-risk, community-dwelling, older adults, habitual walking was significantly correlated with a greater number of falls (Okubo et al., 2011). In contrast, among lower-risk participants, habitual walking was significantly correlated with a history of fewer falls (Okubo et al., 2011). Although the above study suggested both the possible fall prevention effects of habitual walking among the lower-risk older population and the need for caution among higher-risk individuals, the cause–effect relationship between walking and falls requires re-examination in a longitudinal study.

Therefore, the purpose of this study was to examine the longitudinal association between habitual walking and falls among community-dwelling older adults, by considering the relative risk of falling.

2. Methods

2.1. Participants

The study participants included community-dwelling older adults who participated in health checkups. These checkups were organized by their municipalities as part of a nursing care prevention program in Ibaraki, Chiba, and Fukushima from 2008 to 2012. Follow-up checkups continued until 2013. Almost all of the participants were recruited through local advertisements and flyers. The eligibility criteria were as follows: (1) community dwellers aged 60 years or older and (2) individuals who were able to understand the instructions on the performance tests and the questionnaires. In total, 1474 individuals (448 men and 1026 women) aged 60–91 participated in the health checkups. We excluded 773 individuals (247 men and 526 women) from the analysis due to incomplete follow-up from 2009 to 2013. We also excluded 49 individuals (12 men and 37 women) who were under the age of 60 and 117 individuals (32 men and 85 women) from whom we collected incomplete data. Thus, a total of 535 individuals (157 men and 378 women) were enrolled in the present study. All participants provided their written informed consent for participation. We conducted this study in accordance with the guidelines proposed in the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of the University of Tsukuba, Japan.

2.2. Baseline measurements

At baseline, the participants completed self-reported health status questionnaires that included items related to age, gender, frequency of outings, fear of falling (yes/no), self-rated health (good/bad), medical history during the previous year (e.g., history of stroke, hypertension, diabetes, heart disease, osteoporosis, glaucoma/cataracts), and functional status using the Tokyo Metropolitan Institute of Gerontology Index of Competence (TMIG-IC) (Koyano, Shibata, Nakazato, Haga, & Suyama, 1991). Height (cm) and weight (kg) were measured using a stadiometer and a calibrated scale, respectively. The presence of scoliosis (yes/no) was determined when measuring height. Body mass index (kg/m^2) was calculated by dividing the weight by the height. Trained researchers measured the participants' one-leg balance with open eyes, tandem balance and functional reach, five repetitions of sitting-to-standing, alternate step ability, timed up and go, 5-m habitual walk, and 3-m tandem walk. All of the performance tests used in this study have been described in detail elsewhere (Kim et al., 2010).

2.2.1. Habitual walking

The duration (min), frequency (times/week), and number of years that each participant engaged in habitual walking were ascertained in an interview. The participants were classified as walkers if, for more than 1 year, they reported walking for at least 30 min a day two times a week (Japanese Ministry of Health, Labour and Welfare, 2012) or if their total walking amounted to more than 60 min a week. Those who walked for shorter periods of time were classified as non-walkers.

2.2.2. Risk of falling

Of the measurements collected during the health checkups, seven risk factors for falling had been previously identified (American Geriatrics Society et al., 2001). All risk factors can be easily assessed in a community or home setting (Okubo et al., 2011). The fall risk factors were as follows:

2.2.2.1. Poor balance. To measure balance, participants were asked to stand on their preferred leg in a standard position for a maximum of 60 s with their eyes open. A one-leg standing time of less than 10 s was considered as indicative of poor balance (Okubo et al., 2011; Vellas et al., 1997).

2.2.2.2. Mobility limitation. The participants who reported difficulty in climbing 10 steps or walking 400 m without resting were defined as having a mobility limitation (Guralnik et al., 1993; Seino et al., 2010).

2.2.2.3. Knee pain. The participants who experienced knee pain or underwent treatment for knee osteoarthritis were defined as having knee pain.

2.2.2.4. Depressive symptoms. The participants who reported "I felt everything I did was an effort" or "I could not get going" during the past week were defined as having depressive symptoms (Fried et al., 2001; Radloff, 1977).

2.2.2.5. Assistive device. The participants who regularly used a walking cane, walker, or wheelchair were defined as requiring an assistive device.

2.2.2.6. Polypharmacy. Participants who were taking four or more medications were defined as requiring polypharmacy (Robbins et al., 1989).

2.2.2.7. Previous fall history. The participants who experienced an injurious fall or multiple falls within the year prior to entry in the study were defined as having a previous fall history (Delbaere et al., 2010; Okubo et al., 2011).

2.3. Follow-up surveillance and end point determination

For the purposes of this study, a fall was defined as "unintentionally coming to rest on the ground, floor, or other lower level due to reasons other than sudden-onset paralysis, epileptic seizures, or overwhelming external forces." The fall frequency for the past year and sustained injuries (e.g., contusions, incised wounds, abrasions, and fractures) were ascertained at the annual health checkup. When the participants reported falls, both the activities being performed when the falls occurred and the causes of the falls were recorded only for the most serious falls. The "fallers" in this study included both participants who suffered multiple falls within 1 year during the follow-up period and

participants who suffered a fall with an injury (Delbaere et al., 2010; Okubo et al., 2011). The "non-fallers" in this study included participants who did not fall and participants who experienced one non-injurious fall. The participants were followed with an annual health checkup until an injurious fall occurred, multiple falls occurred, the participant missed the annual health checkup, or the end of the study in 2013.

2.4. Sample size calculation

To detect significant differences in the fall incidences between the walkers and non-walkers (lower-risk group: 7.5% and 15%; higher-risk group: 25% and 16%) (Okubo et al., 2011) with a 5% alpha level, 80% power, and 1.5 years of follow-up, a total of 406 participants (610 person-years) (lower-risk group: $n = 187$, 280 person-years; higher-risk group: $n = 220$, 330 person-years) were determined to be the required sample size.

2.5. Statistical analyses

To examine the point at which the association between habitual walking and falls was modified, the participants were first classified into five different risk levels for falling (R0, R1, R2, R3, and R4+) according to the numerical value of the positive score for the fall risk factors. The incidence of falls (n/100 person-years) of the walkers and non-walkers was calculated according to the five risk levels for falling. An unadjusted Cox proportional hazard model was used to examine the statistical significance of the changes in the fall incidence between the walkers and non-walkers, according to the five levels of falling risks. Then, R0 and R1 were grouped into the lower-risk ($R < 2$) group, and groups R2, R3, and R4+ were grouped into the higher-risk ($R \geq 2$) group. An analysis of the covariance (ANCOVA) adjusted for the gender and age (60–64, 65–69, 70–74, 75–79, and ≥80 years) was used for the continuous variables, and a χ^2 test was used for the binomial variables to examine the statistical significance of differences in the baseline characteristics between the walkers and non-walkers separately for the lower- and higher-risk groups. The same methods were applied to examine the statistical significance of any differences between the lower- and higher-risk groups. The logrank test was used to examine the differences in the time to the falls among the walkers and non-walkers in the lower- and higher-risk groups. The HR of the falls, with their corresponding 95% CI for habitual walking, were calculated using the Cox proportional hazards regression model. This analysis was conducted as a subgroup analysis that was stratified according to the risk of falling (lower/higher). The covariates included the baseline age, presence of depressive symptoms (yes/no), poor balance (yes/no), polypharmacy (yes/no), use of an assistive device (yes/no), mobility limitations (yes/no), and previous fall history (yes/no). These covariates were chosen because they are all related to falls (American Geriatrics Society et al., 2001). The interaction between habitual walking and a higher risk of falling (yes/no) was examined using the Cox proportional hazards regression model adjusted for the above covariates plus habitual walking (yes/no) and a risk of falling (lower/higher). $P < 0.05$ was considered to be statistically significant. All statistical analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC, USA).

3. Results

At baseline, the age of the study participants was 73.1 ± 6.6 years (range: 60–91 years; men: 73.2 ± 6.2 years; women: 73 ± 6.7 years). The median (interquartile range) duration of walking, frequency of walking, and number of years of walking were 40 (30–60) min, 6 (3–7) times/week, and 5 (3–10) years, respectively. The weekly total amount of walking was 210 (120–300) minutes. Compared with the participants who were followed for the full study period, those who were lost to follow up were significantly younger (71.6 ± 6.9 years) and had fewer risk factors for falling (1.1 ± 1.2); however, no differences were observed in the gender, prevalence of walkers, or fall history between the groups.

Table 1 shows the prevalence of walkers at baseline and the incidence rate of fallers in the follow-up period. The prevalence of habitual walking was 30.6% in men and 18.5% in women. During the follow-up period, which lasted through 2013 and consisted of a mean period of 1.7 (1–5) years (1.9 years for men and 1.6 years for women), a total of 916 person-years (295 person-years for men and 621 person-years for women) and 112 fallers (26 for men and 86 for women) in a group of 535 older adults (157 men and 378 women) were observed. The incidence of falls was 8.8% in men and 13.8% in women. The falls ($n = 112$) occurred during walking (58.3%), descending stairs (7.1%), ascending stairs (2.4%), standing up (2.4%), standing (1.2%), running (1.2%), playing sports (1.2%), bicycling (13.1%), and doing other tasks (13.1%). The causes of the falls were tripping (48.5%), slipping (21.1%), misstepping (12.4%), staggering (3.1%), dizziness (2.1%), and other reasons (10.8%).

Table 2 shows the prevalence of positive scores for the risk factors, the number of risk factors for falling at baseline, and the fall status during the follow-up period among the walkers and non-walkers. The prevalence of mobility limitations and an R0 categorization were significantly higher in the non-walkers than in the walkers. In contrast, the prevalence of an R4+ categorization was significantly lower in the walkers than in the non-walkers. The incidence of multiple or injurious falls was 13.5% in the walkers compared with 11.8% in the non-walkers; thus, no significant difference was observed.

Fig. 1 shows the incidence of falls during the follow-up period using the five risk levels for falling and the presence of habitual walking. The incidence of falls did not differ significantly between the walkers and non-walkers in the R0 and R1 levels or between the walkers and non-walkers in the R2 and R3 levels; however, the incidence of falls was significantly higher in the walkers in the R4+ level compared with the non-walkers. Despite the lack of statistical significance, the direction of the differences in the incidence of falls (i.e., lower among walkers in R0 and R1 groups but higher among walkers in R2, R3, and R4+ groups) led to the simplification of these five categories into two groups. According to these results, R0 and R1 were grouped as a lower-risk group ($n = 336$, 594 person-years), and R2, R3, and R4+ were grouped as a higher-risk group ($n = 199$, 322 person-years) for subsequent subgroup analyses.

Table 3 shows the baseline characteristics of the walkers and non-walkers in the lower- and higher-risk groups. Among the

Table 1
Prevalence of walkers at baseline and the incidence of multiple (≥2) or injurious (≥1) falls in the follow-up period ($n = 535$).

Age (years)	n	Walkers n (%)	Multiple or injurious falls n (n/100 person-years)
Men			
60–64	12	3 (25.0)	0 (0.0)
65–69	37	13 (35.1)	3 (3.8)
70–74	38	16 (42.1)	4 (6.3)
75–79	42	15 (35.7)	9 (12.7)
≥80	28	8 (28.6)	10 (18.2)
Total	157	55 (35.0)	26 (8.8)
Women			
60–64	40	10 (25.0)	7 (10.1)
65–69	89	21 (23.6)	19 (12.9)
70–74	89	27 (30.3)	27 (19.0)
75–79	89	22 (24.7)	16 (11.7)
≥80	71	11 (15.5)	17 (13.5)
Total	378	91 (24.1)	86 (13.8)

Table 2
Prevalence of positive scores for the risk factors, the number of risk factors for falling at baseline, and the fall status during the follow-up period among walkers and non-walkers ($n = 535$).

Variables	Non-walkers ($n = 389$)	Walkers ($n = 146$)	All participants ($n = 535$)
Risk factors for falling			
Poor balance, yes	106 (27.2)	37 (25.9)	143 (26.7)
Mobility limitation, yes	133 (34.2)	27 (18.9)**	160 (29.9)
Knee pain, yes	101 (26.0)	37 (25.9)	138 (25.8)
Depressive symptoms, yes	41 (10.5)	11 (7.7)	52 (9.7)
Use of assistive device, yes	30 (7.7)	11 (7.7)	41 (7.7)
Polypharmacy, yes	86 (22.1)	28 (19.6)	114 (21.3)
Previous fall history, yes	57 (14.7)	12 (8.4)*	69 (12.9)
Number of risk factors for falling	1.42 ± 1.46	1.12 ± 1.38	1.34 ± 1.44
0	136 (35.0)	63 (44.1)	199 (37.2)
1	94 (24.2)	43 (30.1)	137 (25.6)
2	77 (19.8)	19 (13.3)	96 (17.9)
3	43 (11.1)	9 (6.3)	52 (9.7)
4+	39 (10.0)	12 (8.4)	51 (9.5)
Fall status during the follow-up period			
Any falls (≥1), yes	124 (20.0)	45 (18.3)	169 (19.5)
Multiple falls (≥2), yes	34 (4.9)	22 (8.1)	56 (5.8)
Injurious falls (≥1), yes	68 (10.0)	24 (9.3)	92 (9.8)
Multiple or injurious falls, yes	80 (12.1)	32 (12.6)	112 (12.2)

Notes: * $P < 0.05$, ** $P < 0.01$ versus non-walkers. *n* (%) or mean ± standard deviation, *n* (*n*/100 person-years) for fall status.

lower-risk group, the walkers showed significantly better performance in the open-eye one-leg balance test and the alternate step test and a higher prevalence of diabetes than did the non-walkers. In the higher-risk group, the walkers had significantly higher weights and BMIs than did the non-walkers. The higher-risk group showed significantly worse values in all variables except sex, fieldwork, TMIG-IC, outing frequency, and the prevalence of diabetes compared with the lower-risk group.

Fig. 2 shows the Kaplan–Meier curve illustrating the incidence of falls among the walkers and non-walkers in the lower- and higher-risk groups. A significant difference in at least one of the four groups was observed ($P < 0.0001$).

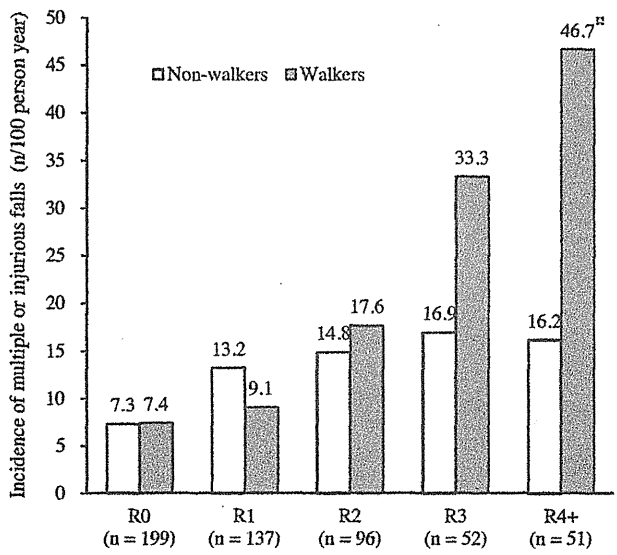

Fig. 1. Incidence of multiple (≥2) or injurious (≥1) falls during the follow-up period among the walkers and non-walkers, who were categorized into five levels based on the risk of falling ($n = 535$). *Notes:* * $P < 0.05$ versus non-walkers. *R* = Number of risk factors for falling. The risk factors include depressive symptoms, poor balance, polypharmacy, the use of an assistive device, knee pain, mobility limitation, and previous fall history.

Table 4 shows the HR (95% CI) of habitual walking for falls during the follow-up period for the lower- and higher-risk groups. In the lower-risk group, no significant associations between habitual walking and falls were observed (HR: 0.90, 95% CI: 0.50–1.64). In contrast, in the higher-risk group, a positive association between habitual walking and falls was observed (HR: 1.66, 95% CI: 0.94–2.94). In model 5, which was adjusted for all covariates, no associations between habitual walking and falls were observed in the lower-risk group (HR: 0.88, 95% CI: 0.48–1.62). In the higher-risk group, a significant positive association between habitual walking and falls was observed (HR: 1.89, 95% CI: 1.04–3.43).

A statistical interaction between habitual walking and a higher fall risk ($R \geq 2$) was observed in the Cox proportional hazard model adjusted for all covariates ($P < 0.05$).

4. Discussion

The present longitudinal study showed that habitual walking significantly increased the incidence of falls by a factor of two among higher-risk community-dwelling older adults (HR: 1.89, 95% CI: 1.04–3.43). This result confirmed the finding of a previous cross-sectional study conducted by the same research group (Okubo et al., 2011), which showed that habitual walking among higher-risk community-dwelling older adults was significantly associated with an increased history of falls (adjusted odds ratio: 4.61, 95% CI: 1.32–16.09). These results were also consistent with the meta-analysis of 44 RCTs assessing fall prevention programs (Sherrington et al., 2008), which reported that the inclusion of a walking program significantly increased the fall incidence (fall rate ratio: 1.32, 95% CI: 1.11–1.58). In that meta-analysis, 29 (59%) of the 44 RCTs examined recruited higher risk populations and included aged care facility residents of 75 years of age or older.

The higher-risk participants in the current study scored significantly worse in all of their physical performance characteristics, including their dynamic balance, static balance, strength, gait, and agility, compared to the lower risk participants. In addition, the higher-risk participants were significantly older, had higher BMIs, had greater fears of falling, and had poorer vision than did the lower-risk participants, which indicated that the higher-risk participants were generally more susceptible to falling

Table 3
Comparisons of the baseline characteristics of walkers and non-walkers in the lower- and higher-risk groups (n = 535).[a]

Variables	Lower risk (R<2) (n=336)		Higher risk (R≥2) (n=199)	
	Non-walkers (n=230)	Walkers (n=106)	Non-walkers (n=159)	Walkers (n=40)
Sociodemographics & anthropometrics				
Age, year	71.1±0.4	71.0±0.6	76.6±0.5	77.1±1.0††
Gender, female	166 (72.2)	66 (62.3)	121 (76.1)	25 (62.5)
Height, cm	152.4±0.3	153.4±0.5	149.2±0.4	149.3±0.9††
Weight, kg	53.9±0.5	54.6±0.7	53.7±0.6	56.7±1.3*††
BMI, kg/m^2	23.1±0.2	23.1±0.3	24.0±0.3	25.3±0.6*††
Scoliosis, yes	11 (4.8)	1 (0.9)	22 (13.8)	6 (15.0)††
Performance tests				
+ One-leg balance with eyes open, s	39.2±1.2	44.0±1.8*	16.4±1.3	13.5±2.5††
+ Tandem stance, s	28.4±0.3	28.8±0.4	23.8±0.7	21.7±1.4††
+ Functional reach, cm	28.1±0.3	28.1±0.5	24.6±0.5	24.0±1.0††
− 5-repetition sit-to-stand, s	6.9±0.1	6.7±0.2	9.6±0.3	9.7±0.5††
− Alternate step, s	4.5±0.1	4.2±0.1*	5.7±0.2	5.7±0.4††
− Timed up & go, s	6.1±0.1	6.0±0.1	8.5±0.2	8.9±0.5††
− 5-m habitual walk, s	3.7±0.1	3.8±0.2	4.9±0.2	5.0±0.3††
− Tandem walk, s	11.6±0.2	11.1±0.3	15.4±0.5	16.6±0.9††
Lifestyle factors				
Field work, day/week	4.3±0.2	3.7±0.3	3.9±0.3	3.0±0.5
House work, day/week	6.0±0.2	5.8±0.2	4.6±0.2	4.1±0.5††
Frequency of outings, day/week	6.3±0.1	6.5±0.1	5.9±0.1	6.3±0.3
Psychological factors				
Fear of falling, yes	68 (29.7)	29 (27.4)	97 (61.0)	23 (57.5)††
Self-rated health, good	209 (90.9)	99 (93.4)	112 (70.4)	34 (85.0)††
Functional status				
+TMIG-IC[b], 0–13	10.9±0.2	10.8±0.2	10.8±0.2	10.8±0.4
Medical history in 1 year				
Stroke, yes	3 (1.3)	1 (1.0)	9 (5.7)	5 (12.5)††
Hypertension, yes	82 (35.7)	40 (38.1)	81 (50.9)	20 (50.0)††
Diabetes, yes	9 (3.9)	12 (11.4)**	12 (7.6)	4 (10.0)
Heart disease, yes	21 (9.1)	6 (5.7)	26 (16.4)	9 (22.5)†
Osteoporosis, yes	13 (5.7)	6 (5.7)	21 (13.2)	5 (12.5)††
Glaucoma/cataract, yes	8 (3.5)	7 (6.7)	17 (10.7)	6 (15.0)†

Notes: n (%) or adjusted mean ± standard error. R = the number of risk factors for falling. † P<0.05, †† P<0.01 versus lower risk. * P<0.05, ** P<0.01 versus non-walkers. Analysis of covariance (ANCOVA); continuous variables were adjusted by age and gender. BMI = body mass index.

[a] Less than 5% missing data, except in the categories of field work (n=328) and house work (n=325).

[b] TMIG-IC. +: Higher values signify a better performance, −: Lower values signify a better performance.

(American Geriatrics Society et al., 2001). Vision problems such as decreased contrast sensitivity and depth perception could impede the detection of environmental hazards when walking outside

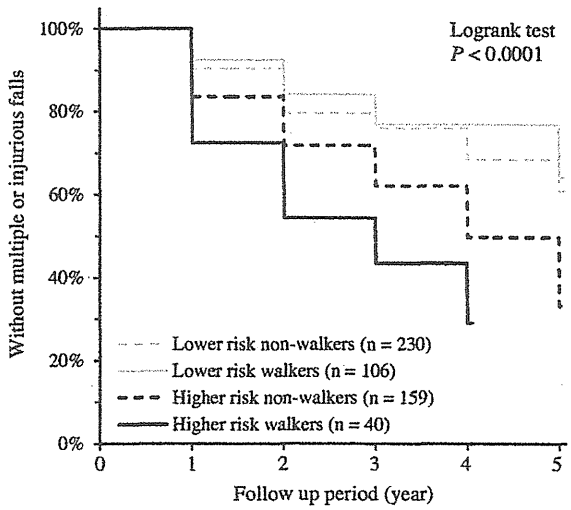

Fig. 2. A Kaplan–Meier curve illustrating the incidence of multiple (≥2) or injurious (≥1) falls among walkers and non-walkers in the lower- and higher-risk groups (n = 535).

(Lord, 2006). Murray, Kory, & Clarkson (1969) reported that the walking pattern of the elderly is characterized by decreases in the step length, range of motion in hip flexion and extension, dorsiflexion of the ankle, and toe elevation during the swing phase of walking. Kaneko, Morimoto, Kimura, Fuchimoto, & Fuchimoto (1991) reported that the decrease in toe elevation during the swing phase of walking increases the risk of stumbling over obstacles. Berg, Alessio, Mills, & Tong (1997) reported that tripping was the most prevalent cause of falls that occurred while walking. It has been suggested that older adults tend to reduce their level of physical activity as they become afraid of falling (Wijlhuizen, de Jong, & Hopman-Rock, 2007), and the decrease in physical activity (e.g., avoiding hazards) in older adults can generally be interpreted as a behavioral response to perceived difficulty in balance control (Etman, Wijlhuizen, van Heuvelen, Chorus, & Hopman-Rock, 2012). Therefore, if the higher-risk walkers in our current study had a walking pattern based on their own perceived weaknesses, they may have had a greater chance of trips and falling. However, though the 58.3% of fallers experienced a fall during walking (which was not necessarily intended as exercise), accidental falls unrelated to habitual walking were included. Thus, habitual walking by the high-risk older adults might be related to other factors such as risk-taking behaviors in daily life (Kloseck, Crilly, & Gibson, 2008).

The significant interaction found in this study between habitual walking and the risk of falling suggests that the observed effect of habitual walking on falls was modified when individuals had two

Table 4
HR (95% CI) of habitual walking for multiple (≥2) or injurious (≥1) falls during the follow-up period in the lower- and higher-risk groups (n=535).

	Lower risk (R<2)		Higher risk (R≥2)	
	Non-walkers (n=230)	Walkers (n=106)	Non-walkers (n=159)	Walkers (n=40)
Model 1	1.00 (reference)	0.90 (0.50–1.64)	1.00 (reference)	1.66 (0.94–2.94)
Model 2	1.00 (reference)	0.89 (0.49–1.62)	1.00 (reference)	1.64 (0.93–2.90)
Model 3	1.00 (reference)	0.89 (0.48–1.62)	1.00 (reference)	1.68 (0.95–3.00)
Model 4	1.00 (reference)	0.84 (0.46–1.55)	1.00 (reference)	1.67 (0.94–3.00)
Model 5	1.00 (reference)	0.88 (0.48–1.62)	1.00 (reference)	1.89 (1.04–3.43)*

Notes: * P<0.05.
Model 1: Adjusted by gender; Model 2: Model 1 adjusted by age; Model 3: Model 2 adjusted by depressive symptoms, poor balance, polypharmacy; Model 4: Model 3 adjusted by use of assistive device, knee pain, mobility limitation; Model 5: Model 4 adjusted by previous fall history.
R=the number of risk factors for falling. The risk factors include depressive symptoms, poor balance, polypharmacy, the use of an assistive device, knee pain, mobility limitation, and a history of previous falls.

or more risk factors for falling. This result suggests that when individuals have two or more risk factors for falling, caution is warranted when recommending habitual walking because walking may actually put these individuals at a greater risk of multiple or injurious falls. Similar modification effects were reported with habitual walking (Okubo et al., 2011) and high levels of physical activity (Faulkner et al., 2009; Stevens, Powell, Smith, Wingo, & Sattin, 1997). In a case-control study (Stevens et al., 1997), high levels of physical activity (exercise, heavy housecleaning, and other hard labor) were associated with a reduction in the number of fractures occurring in participants with no ADL limitations (odds ratio: 0.6, 95% CI: 0.5–0.8), but they were also associated with more fractures in participants with at least one ADL limitation (odds ratio: 3.2, 95% CI: 1.1–9.8). Thus, a more comprehensive examination of which types of physical activity, in addition to habitual walking, are most associated with falls is needed.

In this longitudinal study, the habitual walking observed among 336 lower-risk participants was not significantly associated with a reduction in the number of falls (HR: 0.88, 95% CI: 0.48–1.62). This result did not confirm the cause-effect relationship of our previous cross-sectional study (Okubo et al., 2011), in which habitual walking for at least 30 min twice per week for 1 year among 585 lower-risk participants was associated with 56% fewer falls. This finding represented a significant reduction in the number of falls (adjusted odds ratio: 0.44, 95% CI: 0.20–0.97). The inconsistency between the current longitudinal study and previous cross-sectional studies may be explained by the following reasons (Okubo et al., 2011). First, although the sample size goal determined by the previous results (Okubo et al., 2011) was fulfilled (goal: 280 person-years; analyzed data: 594 person-years), the statistical power may not have been sufficient to detect smaller differences. Second, because we did not collect data related to the intensity of walking, the inclusion of both moderate-to-vigorous intensity walkers and older adults who merely walk for leisure might have weakened the association. However, despite the lack of statistical significance, the direction of the association between lower-risk walkers and falls was positive, as observed in the previous cross-sectional study (Okubo et al., 2011). In Table 3, the lower-risk walkers performed significantly better on the one-leg balance (static balance) and alternate step (stepping agility) tests than did the lower-risk non-walkers. Murray et al. (1969) reported that walking could be characterized as the "continuous process of recovery from a loss of balance". Habitual walking may be effective in maintaining balance if it is continued with sufficient intensity for a long enough period of time. Indeed, Brown and Holloszy (1993) reported that endurance training consisting of brisk walking, cycling, and jogging significantly improved one-leg balance after 15 months. Because the effects of walking on falls have been inconclusive for many years (Gregg et al., 2000), a RCT with a larger cohort is needed to definitively re-examine the effects of habitual walking on falls in a lower-risk population (Voukelatos et al., 2011).

Our study has several limitations. First, it may not be widely generalizable for the following reasons. (1) The follow-up rate was low (36.3%), and the mean follow-up period was relatively short (1.7 years) because the participants could choose to participate or not participate in the annual health checkup, and a one-time absence was sufficient to terminate follow up. (2) The participants were relatively healthy community-dwelling older adults who volunteered to participate in the health checkups. Second, there is the potential for unmeasured confounding variables that we could not assess, such as total physical activity, cognitive function, and risk-taking behavior. Third, although the reliability of a retrospective fall survey in Japanese community-dwelling elderly individuals has been confirmed (Haga et al., 1996), prospective surveillance using a monthly fall calendar would be a more reliable measurement method.

In conclusion, this longitudinal study showed that the effects of walking on multiple or injurious falls are modified by the presence of risk factors for falling. When individuals have two or more risk factors for falling, caution is warranted when recommending walking because walking can actually increase their risk of experiencing multiple or injurious falls. Further research should therefore focus on safe walking programs for higher-risk, community-dwelling, older adults and on the positive effects of habitual walking among lower-risk, community-dwelling, older adults.

Conflict of interest statement

The authors have no conflicts of interest to report.

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