doubled between 1987 and 1997, and the increment has continued until recently [3, 4].

Preventing osteoporosis after menopause involves either raising the peak BMD or attenuating the decline in BMD [5–7]. Exercise, nutrition, and medication are currently recommended for the prevention and treatment of osteoporosis [8]. There is evidence supporting the effectiveness of physical activity, especially impact exercise, in the prevention and treatment of osteoporosis [9–14]. Moreover, Bassey et al. [15] have reported that a short high-impact exercise (HIE) program including 50 vertical jumps increases the BMD of the femoral neck in Caucasian premenopausal women.

Despite the widespread dissemination of information concerning the negative health consequences associated with sedentary living, adult physical activity in many industrialized nations fails to follow such regimes because of lack of time, motivation, and adherence [16, 17]. Thus, to improve exercise adherence, a simple new office-based HIE program was chosen and tested in this study.

The aim of this randomized controlled study was to examine the effects of office-based HIE on lumbar spine and femoral neck BMD among premenopausal women.

Materials and methods

Study design and participants

The Sendai Bone Health Concept Study (S-BHCS) was a 12-month randomized controlled trial conducted between November 2006 and October 2007 at the Tohoku branch of the NTT Solco Corporation (a telecom company), Japan. In this company, most of workers were premenopausal women, who were predominantly sitting at a desk all day (desk jobs). Participants were recruited for the study by the personnel department of the company between May 2006 and July 2006. One hundred eight individuals out of 373 female employees aged 25–50 years were screened for eligibility (Fig. 1). After filling out a questionnaire on their health condition, diet, medical history, lifestyle, and menstrual status, 91 women were enrolled in the study under the following inclusion criteria: (1) no intake of steroid hormones (e.g., corticosteroids or estrogen); (2) no metabolic diseases related to calcium turnover (hyper- or hypothyroidism, hyper- or hypoparathyroidism, renal or liver disease, etc.), (3) no history of rheumatoid arthritis, (4) no history of ovariectomy, (5) no current or planned pregnancy, and (6) the ability to participate in intervention and examination programs for 12 months. Bone density, physical activity level, calcium intake, leg strength, anthropometrics, blood pressure, and QOL were assessed at baseline and after 12 months. The study protocol was approved by the Local Ethical Committee. All participants provided their written informed consent for inclusion in the study.

Questionnaires

Information on the subjects' age, smoking status, drinking status, menstrual cycle, use of medication, and disease history was obtained through a questionnaire. Levels of daily physical activity were estimated using the International Physical Activity Questionnaire (IPAQ, Japanese version) [18]. Before and after the trial, we assessed the QOL on the basis of the physical and mental composite scores obtained using the Medical Outcomes 36-Item Short-Form Health Survey (MOS, SF-36, Japanese version) (both scores have a population norm of 50 points, with lower scores representing a lower QOL) [19].

Daily dietary total energy, protein, fat, carbohydrate, and calcium intake were assessed before and after the intervention using a brief self-administered diet history questionnaire (BDHQ) that included questions on 75 food items and specified their serving sizes. The reproducibility and validity of the BDHQ have been described in detail previously [20].

Bone mineral density

The BMD of the lumbar spine (L1–L4) and proximal femur (at the neck, Ward's triangle, trochanter, and intertrochanteric region) was measured at baseline and after 12 months by using a dual-energy X-ray absorptiometer (DXA, Hologic QDR 4500A, Bedford, MA). The values were expressed in terms of grams per square centimeter.

Maximal leg strength

Leg extension power in watts was measured by a well-trained physiotherapist while the subjects were sitting with the knees bent to an angle of 90° (Aneropress 3500, Combi Wellness, Tokyo) [21]. The isometric contractions of the leg muscles lasted for 5 s each and were separated by 15-s rest intervals. The average of the 2 highest measurements obtained in 5 trials was determined. To adjust for differences in the subjects' body mass, the leg extension power was expressed as the average peak value relative to the body weight (W/kg).

Anthropometrics, blood pressure and blood samples

Body height and weight were measured and body mass index (BMI) was calculated using the following formula: weight (kg)/height^2 (m^2). Systolic (SBP) and diastolic blood pressure (DBP) in the left upper arm was measured twice using an automatic device (HEM747IC; Omron Life

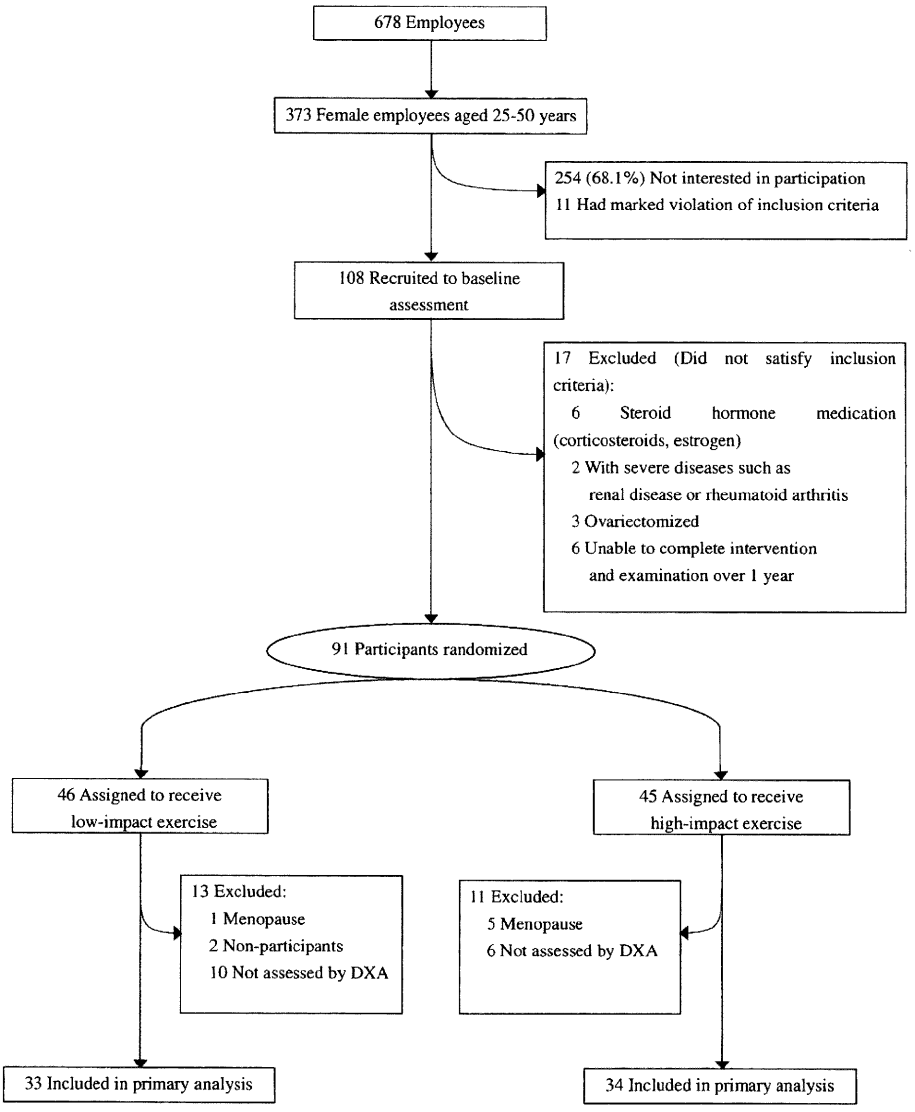

Fig. 1 Flow of participants

Science Co. Ltd., Tokyo, Japan) following a 5-min rest in the sitting position.

The blood samples were collected at the same time of the day for all participants. The total cholesterol (T-C), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) concentrations were measured by enzymatic methods using appropriate kits (T-C: Denka Seiken, Tokyo, Japan; LDL-C: Sekisui Medical Co. Ltd., Tokyo, Japan; HDL-C: Daiichi Pure Chemicals, Tokyo, Japan). The serum albumin level was also determined using standard laboratory procedures. High-sensitivity C-reactive protein (hsCRP) concentrations were determined by an immunoassay performed using a Behring BN II analyzer (Dade Behring, Tokyo, Japan. detection limit: 0.02 mg/l [22]. The serum adiponectin concentration was measured using a specific enzyme-linked immunosorbent assay (R&D Systems Inc., Minneapolis, MN; mean sensitivity: 246 pg/ml; intra- and inter-assay coefficients of variation: 3.5% and 6.5%).

Physical activity measurements

An accelerometer-based physical activity recorder (Newtest Ltd., Oulu, Finland) was used to measure the daily impact loading of the subjects. All the subjects were requested to continuously carry the recorder during all waking hours for 1 week every 3 months (November, February, June, and September). The recorder was worn on a belt close to the iliac crest. The data were downloaded onto a computer after each 1-week measurement period.

The physical activity recorder has previously been described in detail [23, 24]. The device recorded the number of vertical acceleration peaks, i.e., impacts exceeding $0.3g$, where g is the acceleration due to gravity (9.81 m/s^2). The acceleration of gravity $1g$ was subtracted, i.e., $0g$ corresponded to the standing position. The average number of impacts recorded daily was calculated at 32 acceleration levels, and a 32-level histogram was obtained for each individual using these data. To assess compliance, the subjects were requested to maintain a diary noting their use of the recorder, and compliance was also checked from accelerometer data.

Exercise program

At the end of the baseline assessment, the participants were age matched and randomly allocated to the stretching exercise (SE) group ($n = 45$) or the HIE group ($n = 46$). The subjects were advised to attend video-guided exercise sessions at least 3 times a week. The sessions were scheduled when the subjects had breaks from work. The participants remained in their work clothes and were requested to wear sport shoes or light gymnastic shoes. Each exercise session included a 3-min warm-up (stretching), 10 min of stretching or HIE (SE, stretching exercise; HIE, stretching, along with up to 5×10 vertical and versatile jumps, with two legs together, using an arm swing in counter movement style, and landing with flexion of the ankles, knees, and hips), and a 3-min cool-down (stretching). The SE group continued to perform stretching and balance exercises throughout the intervention period.

The intention of the jumps in HIE program was to create nonhabitual strains in order to enhance the mechanical competence of bone. Initially, however, the primary objective of the routine was to accustom the subjects to jumping. The programs were modified monthly by increasing the number of jumps up to 50 jumps during the first 3 months. After 6 months, we ensured that the HIE program progressed in intensity by including a 10-cm step bench (Stepwell, Combi, Tokyo, Japan). The training sessions were done with the accompaniment of music and supervised at least 4 times a month by an experienced health fitness instructor.

The compliance with supervised exercise was assessed with personal diaries. Adherence to the protocol was calculated on the basis of the number of sessions attended.

Power calculation

The mean femoral neck BMD of premenopausal Japanese women was 0.763 [25]. Based on the preliminary study [26], we estimated $\geq$3% (SD 0.027 [15]) difference between the HIE and SE groups at the post-intervention femoral neck BMD measurement. To establish statistical significance (alpha) at 5%, with a statistical power of 0.8 and assuming a dropout rate of 20%, a minimum of 28 participants per group was required.

Statistical analysis

All statistical analyses were performed by using the Statistical Analysis System for Windows, version 9.1 (SAS Institute Inc., Cary, NC). For normally distributed continuous variables, the arithmetic means and standard deviations (SDs) were calculated, and for logarithmically transformed continuous variables the geometric means, and SDs were computed. For a baseline comparison between the SE and HIE groups, the Pearson method was used to analyze the categorical data, the statistical result being distributed by the χ^2 test or Fisher's exact test. For comparing the continuous variables related to the basic characteristics of the two groups, unpaired t tests were performed. Paired t tests were performed to compare the change in the outcome variables within groups, while repeated-measure ANOVA was performed to compare these changes between groups. We also applied repeated-measures analysis of covariance (ANCOVA) analyses to evaluate the BMD changes for the 2 groups after adjustment for changes in the BMI and energy-adjusted calcium intake (mg/day) during the trial. The effect size was calculated as the difference (HIE minus SE) between changes (initial minus final) in these mean values. The corresponding standardized difference was calculated by subtracting the difference (HIE minus SE) between changes (initial minus final) in these mean values divided by the pooled SD [square root of the $((N_{HIE} - 1)SD^2_{HIE} + (N_{SE} - 1)SD^2_{SE})/(N_{HIE} + N_{SE} - 2)$] of HIE and SE group SDs. The relationships between variables were assessed using Pearson's product moment correlations (or Spearman's if the data are skewed). All the tests for statistical significance were 2 sided, and $P = 0.05$ was considered statistically significant.

Results

Figure 1 shows the flow of participants from the time they were screened to the end of the study, i.e., after 12 months. Ninety-one women met the inclusion criteria and consented to participate in the trial. Their baseline characteristics are presented in Table 1. There were no significant differences between the SE and HIE groups at baseline.

Both the SE and the HIE intervention programs were tolerated well by the study participants, and no treatment-related adverse events occurred. Twenty-four subjects were excluded from the primary analysis (16 were not assessed

Table 1 Baseline characteristics of trial participants

Variables	Stretching exercise ($n = 46$)	High-impact exercise ($n = 45$)	P values[a]
Age (years)	38.1 (1.2)	39.7 (1.2)	0.35
BMI (kg/m^2)	22.3 (1.2)	21.9 (1.2)	0.69
Weight (kg)	56.5 (9.9)	55.6 (12.7)	0.71
Height (cm)	158.3 (5.3)	157.6 (5.8)	0.53
SBP (mmHg)	113.8 (13.4)	116.4 (17.9)	0.44
DBP (mmHg)	74.6 (10.9)	77.4 (13.1)	0.26
Total serum protein (g/dl)	7.5 (1.1)	7.4 (1.1)	0.32
Albumin (g/dl)	4.7 (1.1)	4.6 (1.0)	0.46
Total cholesterol (mg/dl)	185.8 (26.9)	190.7 (27.4)	0.39
TG (mg/dl)	78.7 (1.6)	85.2 (1.9)	0.51
LDL (mg/dl)	96.7 (30.6)	102.8 (29.9)	0.34
HDL (mg/dl)	65.9 (14.6)	64.5 (17.0)	0.69
CRP (ng/ml)	212.4 (3.1)	282.5 (4.4)	0.30
Adiponectin (μg/ml)	8.6 (1.8)	7.7 (2.0)	0.43
Smoker			
Current smoker	32.6	28.9	0.70[b]
Ex-smoker	8.7	13.3	0.52[c]
Physical activity (METs h/week)	11.7 (3.3)	11.5 (2.9)	0.94
SF-36 (total scores)	86.2 (1.2)	80.4 (1.3)	0.15
Leg-power (W/kg)	8.6 (3.5)	8.2 (3.4)	0.61
Energy-adjusted Ca intake (mg/d × 2,000 kcal)	463.3 (164.6)	454.3 (169.0)	0.80
BMD by DXA (g/cm^2)			
Spine (L1–L4)	0.980 (0.128)	0.976 (0.132)	0.88
Proximal femur			
Femoral neck	0.767 (0.092)	0.749 (0.128)	0.45
Trochanter	0.627 (0.077)	0.622 (0.101)	0.79
Intertrochanteric	0.997 (0.108)	0.983 (0.141)	0.59
Femoral total	0.852 (0.087)	0.842 (0.126)	0.65
Ward's triangle	0.651 (0.122)	0.626 (0.168)	0.42

Convert from milligram per deciliter (mg/dl) to millimole per liter (mmol/l), divide by 38.67 (for total cholesterol, LDL, and HDL), or 88.57 (for TG)

Values are mean (standard deviation) or %

BMD bone mineral density, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *TG* triglyceride, *LDL* low density lipoprotein cholesterol, *HDL* high-density lipoprotein-cholesterol, *CRP* C-reactive protein, *SF-36* MOS short-form 36-item health survey, *DXA* dual energy X-ray absorptiometry

[a] *t* test

[b] Chi-squared test

[c] Fisher's exact test

during the final measurement because of job-based reasons, such as job transfer or resignation, 2 dropped out of the study, and 6 naturally attained menopause); of the remaining 67 women, 33 (71.7%) were classified into the SE group and 34 (75.6%) into the HIE group. The dropout rate was 26.4%. There were no significant differences in any of the baseline variables of the dropout subjects and the subjects who completed the study. For the women who completed the study, compliance, defined as the number of exercise sessions attended, had a median (interquartile range) value of 2.4 (0.8–3.2) times per week. There were no significant differences in compliance between the SE and HIE groups.

The accelerometer-based method enabled differentiation between the exercise intensities used. The average numbers of impacts recorded daily during the different measurement periods are shown in Fig. 2. During the first 6 months (measurement periods I and II), there was no difference between the 2 groups. During the last 6 months (measurement periods III and IV), the number of impacts at high

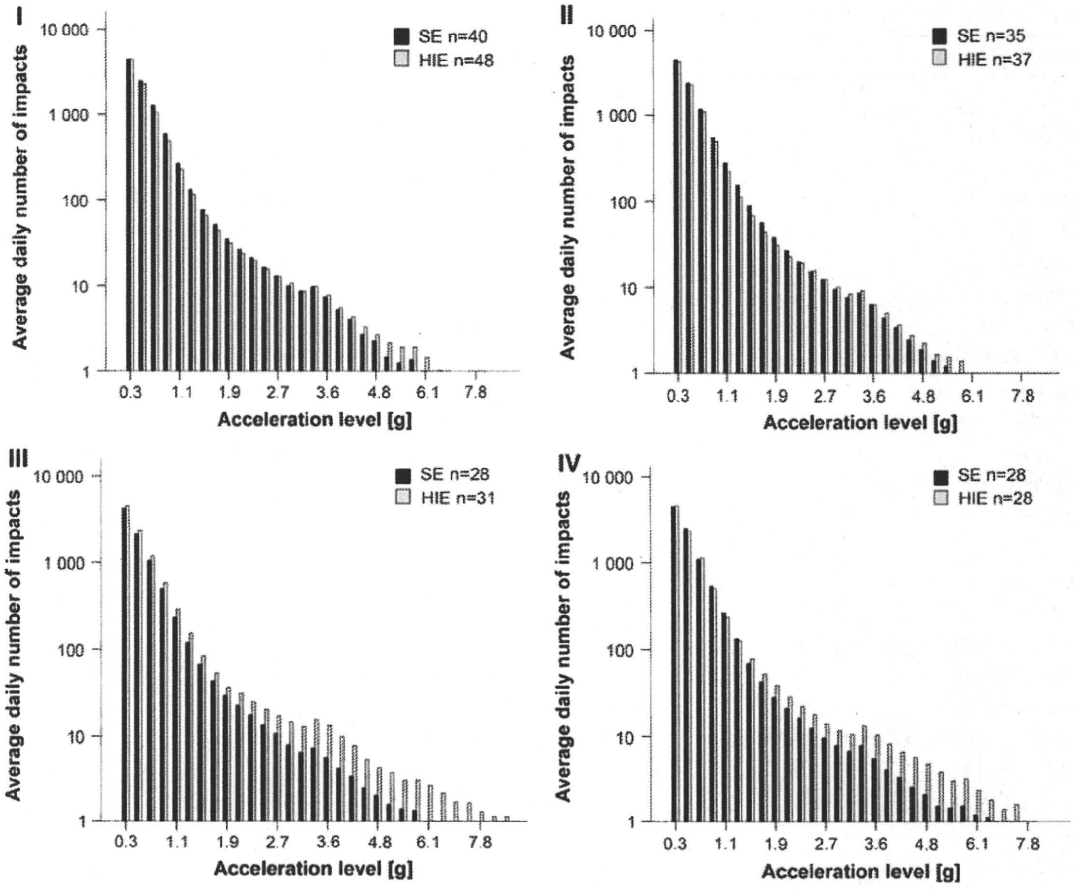

Fig. 2 Average number of impacts recorded daily at different acceleration levels during the four 1-week measurement periods (I–IV) in the 12-month study. Stretching exercise (*SE*) and high-impact exercise (*HIE*) groups

Table 2 Comparison of BMD and its changes between the groups at baseline and after 12-month follow-up

Variables	Stretching exercise ($n = 33$)		High-impact exercise ($n = 34$)		Effect size	P values[a] (time × group)	P values[b] (time × group)
	Baseline	At 12 months	Baseline	At 12 months			
DXA BMD (g/cm^2)							
Spine (L1–L4)	0.983 (0.131)	0.985 (0.129)	0.999 (0.133)	1.007 (0.131)[c]	0.316	0.20	0.10
Proximal femur							
Femoral neck	0.766 (0.090)	0.758 (0.090)	0.763 (0.136)	0.767 (0.135)	0.514	0.04	0.02
Trochanter	0.631 (0.082)	0.629 (0.080)	0.637 (0.105)	0.636 (0.107)	0.086	0.73	0.64
Intertrochanteric	1.002 (0.111)	0.999 (0.114)	0.998 (0.150)	0.997 (0.143)	0.039	0.87	0.43
Femoral total	0.856 (0.091)	0.852 (0.094)	0.858 (0.134)	0.857 (0.128)	0.150	0.54	0.26
Ward's triangle	0.658 (0.130)	0.654 (0.125)	0.642 (0.176)	0.643 (0.163)	0.127	0.61	0.26

Values are mean (standard deviation)

BMD bone mineral density, *DXA* dual-energy X-ray absorptiometry

[a] P values for differences between the low-impact exercise group and the high-impact exercise group over the 12-month study period (repeated-measure ANOVA)

[b] P values for differences between the low-impact exercise group and the high-impact exercise group over the 12-month study period (repeated-measure ANCOVA: adjusted for changes in the BMI and energy-adjusted Ca intake during the trial)

[c] $P < 0.05$, annual change within the group (paired samples t test)

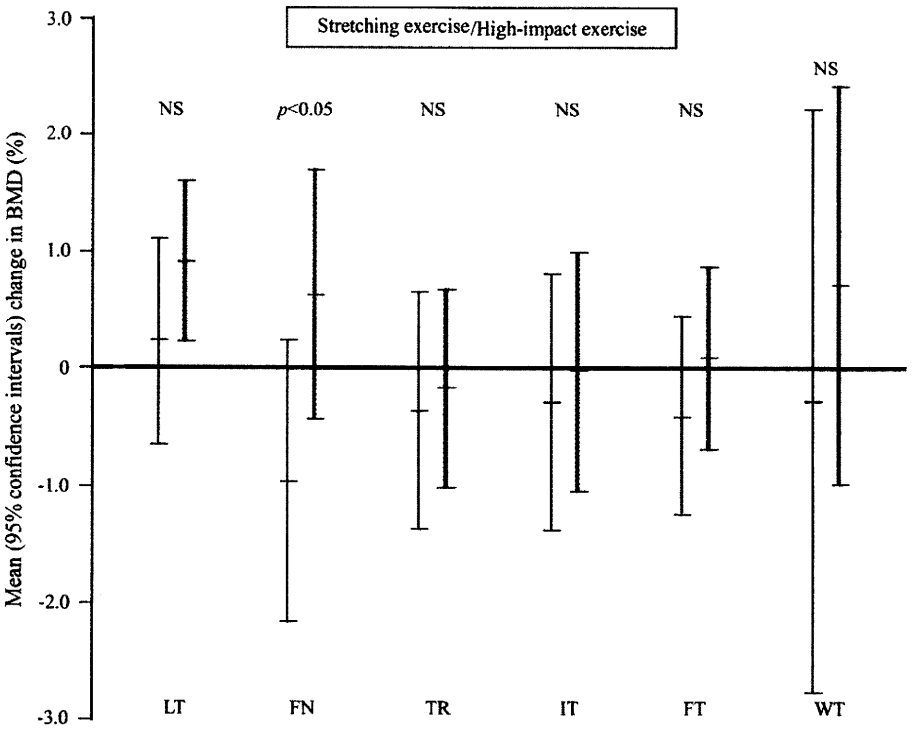

Fig. 3 Mean percent changes (95% confidence intervals) in the BMD of the whole lumbar spine and femur over the 12-month study period. *LT* total lumbar spine, *FN* femoral neck, *TR* greater trochanter, *IT* intertrochanteric region, *FT* total femur, and *WT* Ward's triangle. *P* values indicate the difference between the stretching and high-impact exercise groups over the 12-month study period (unpaired *t* test)

acceleration levels (>3.3 g) in the HIE group was more than twofold that in the SE group.

Table 2 shows the groupwise changes in BMD over 12 months. The HIE group maintained their femoral neck BMD, and there was a significant difference in change in BMD compared to the SE group (0.6% (95% CI: −0.4, 1.7) vs. −1.0% (95% CI: −2.2, 0.2) Fig. 3). The statistically significant difference remained after adjustment for changes in BMI and energy-adjusted Ca intake during the trial. The effect size also suggested that a HIE-intervention led to one-half of a SD increase in femoral neck BMD (Table 2). The BMD of the whole lumbar spine (L1–L4) increased within the HIE group ($P = 0.02$). There were no significant inter-group changes in other bone regions (L1–L4, trochanter, intertrochanteric region, total femur, and Ward's triangle). Because BMI is strongly associated with femoral neck BMD [27] and hip fracture risk [28], we also analyzed the relationship between BMI change and femoral neck BMD change over 12 months. There was no significant relationship between BMI change and femoral neck BMD change (correlation coefficient 0.12; *P* value = 0.50). Furthermore, no significant relationship was found between BMI change and average number of impacts, even when analyzed at different acceleration levels.

Complete cardiovascular risk factors, QOL, and leg strength data were obtained from 63 women (31 in the SE group and 32 in the HIE group) (Table 3). Although no significant intergroup differences were observed, SBP and LDL-C significantly decreased in both groups, while HDL-C, adiponectin concentration, and leg extension power significantly increased in both groups. Furthermore, the DBP and T-C significantly increased in the SE group, while BMI increased significantly in the HIE group. There were no significant inter-group differences in the changes of energy-adjusted Ca, protein, fat, and carbohydrate intake and total physical activity during the trial ($P > 0.10$).

Discussion

The present trial demonstrated that a brief office-based high impact exercise program prevents femoral neck bone loss in healthy premenopausal women. Moreover, both office-based stretching and impact exercise have a positive influence on cardiovascular risk factors and leg strength.

In a previous intervention with Finnish women aged between 35 and 40 [24], the threshold for improving femoral BMD appeared to be less than 100 impacts per day exceeding 3.9 g. The present results support the previous findings for effective bone exercise. However, there is some concern whether the improved femoral neck BMD in the HIE group is maintained after withdrawal. There is some controversy in the previous studies. A study of Winters et al. indicated that the positive benefits of impact plus strength training on the BMD in premenopausal women reverse when training was withdrawn [29], whereas

Table 3 The change in cardiovascular risk factors and leg strength over time and the difference between the groups at baseline and after 12-month follow-up

Variables	Stretching exercise ($n = 31$)		High-impact exercise ($n = 32$)		P values[a] (time × group)
	Baseline	At 12 months	Baseline	At 12 months	
BMI (kg/m^2)	22.6 ± 1.2	22.8 ± 1.2	22.0 ± 1.2	22.5 ± 1.2[b]	0.13
SBP (mmHg)	114.0 ± 11.9	105.8 ± 22.0[b]	116.3 ± 18.8	108.4 ± 25.3[b]	0.96
DBP (mmHg)	75.5 ± 11.4	86.3 ± 21.5[b]	75.8 ± 12.2	79.9 ± 15.7	0.16
Total cholesterol (mg/dl)	187.3 ± 25.5	193.0 ± 29.1[b]	189.2 ± 27.3	190.3 ± 27.5	0.30
TG (mg/dl)	76.8 ± 1.6	75.0 ± 1.8	88.7 ± 2.1	87.9 ± 1.9	0.31
LDL (mg/dl)	106.0 ± 28.8	79.3 ± 22.9[b]	105.3 ± 30.5	81.4 ± 23.8[b]	0.65
HDL (mg/dl)	65.8 ± 16.2	71.3 ± 17.5[b]	63.3 ± 17.6	68.8 ± 20.1[b]	1.00
CRP (ng/ml)	210.7 ± 2.5	225.0 ± 3.0	290.3 ± 4.3	274.8 ± 4.0	0.22
Adiponectin (μg/ml)	9.2 ± 1.9	11.7 ± 1.6[b]	6.8 ± 2.1	10.2 ± 2.0[b]	0.35
SF-36 (total scores)	85.6 ± 1.3[c]	82.7 ± 1.3[c]	83.4 ± 1.2	79.2 ± 1.4	0.97
Energy-adjusted Ca intake (mg/d × 2,000 kcal)	462.4 ± 150.0[c]	545.7 ± 177.3[b,c]	445.5 ± 172.1	559.0 ± 262.5[b]	0.47
Leg extension power (W/kg)	9.2 ± 3.6[d]	10.9 ± 3.5[b,d]	8.2 ± 3.0[d]	10.2 ± 3.5[b,d]	0.60

Convert from milligram per deciliter (mg/dl) to millimole per liter (mmol/l), divide by 38.67 (for total cholesterol, LDL, and HDL) or 88.57 (for TG)

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *TG* triglyceride, *LDL* low density lipoprotein cholesterol, *HDL* high-density lipoprotein-cholesterol, *CRP* C-reactive protein, *SF-36* MOS short-form 36-item health survey

Values are mean ± standard deviation

[a] P values for differences between the low-impact exercise group and the high-impact exercise group over the 12-month study period (repeated-measure ANOVA)

[b] $P < 0.05$, annual change within the group (paired samples t test)

[c] $n = 29$

[d] $n = 30$

another study by Kontulainen et al. [30] showed that the training-induced BMD difference between the trainees and controls was maintained even 3.5 years after the intervention. Due to the lack of longitudinal data on the effect of premenopausal exercise on osteoporotic fractures in older age, continuation of osteogenic exercise can be recommended throughout life.

After 12 months of exercise, a significant increase was observed in the BMD of the femoral neck among the women in the HIE group relative to the SE group. Furthermore, a significant change was observed in the total lumbar (L1–L4) BMD within the HIE group but not within the SE group. These results are in agreement with a previous randomized controlled HIE intervention conducted on British premenopausal women [15]. A systematic review also indicated that both high-impact and nonimpact exercises had a positive effect on the BMD of the lumbar spine, while only HIE had a positive effect on that of the femoral neck [14]. This might be due to the fact that the femoral neck is subjected to compressive and bending forces during take-off and landing. The present study suggests that even a simple, office-based program may result in bone effects in the femoral neck similar to those in more demanding programs. A previous study conducted over 18 months by Heinonen et al. [31] showed that HIE significantly increased also the BMD of the total lumbar spine. This controversy may be due to the differences in the proportion of non-impact components of the exercise programs that exerts certain force to the lumbar spine. Further long-term trials are required to clarify this issue by appropriately measuring or estimating the strain and impact on lumbar spine.

Several studies have indicated that HIE has beneficial effects in the cortical bone of the femoral neck [32, 33]. In this study we used only standard DXA, with which cortical and trabecular bone cannot be discriminated, and details of the changes in cortical bone geometry remain unclear. The mechanism by which HIE improves BMD is most probably through the dynamic strains it engenders in bone tissue. A review of studies of humans suggested that physical activities involving both gravitation and muscular loading are capable of generating impact forces and are therefore likely to have beneficial effects on bone metabolism [34]. However, recent study has indicated that HIE did not induce dose-related alterations in bone metabolism markers, but had a dose-dependent effect on serum basal

parathyroid hormone (PTH) concentration, suggesting that the osteogenic effects of loading may partly be mediated by PTH [35].

Because bone adapts to habitual loading, one important feature of an exercise program is progression [36]. Here, we were able to ensure the progression of the HIE program using the acceleration-based measurement of impact loading. The daily acceleration distribution was very similar during the first 2 measurement periods; we used this information to modify the exercise protocol. The accelerometer-based measurements indicated the changes in exercise intensity for the latter half of the study.

In addition to bone health, regular exercise has a beneficial effect also on patients with cardiovascular disease and type 2 diabetes [37]. In this study, we assessed the effects of SE and HIE on several cardiovascular risk factors, including the serum lipid profile, hsCRP, and adiponectin concentration. Adiponectin is a peptide hormone secreted exclusively by adipose tissue. The adiponectin protein is abundantly expressed in plasma (range 5–30 μg/ml); however, adiponectin levels decrease in patients with obesity-linked diseases, including coronary artery diseases and type 2 diabetes [38]. In accordance with a previous study [39], we found that HIE also improved serum lipid profiles and significantly increased adiponectin concentration within groups. This improvement was found in both exercise groups, suggesting that it was mainly a result of the low-impact component of the exercise program. Thus, both HIE and SE had beneficial effects not only on bone health, but also on cardiovascular risk factors in healthy premenopausal women.

Although we assumed that the office-based setting in this study would minimize the dropout rate, the dropout rate of 26.4% over the 12-month study duration was not as low as expected. Among the dropout cases, 6 participants were excluded from the final analysis because of naturally attained menopause. The dropout of 16 study participants was due to unexpected resignation of the participants from the company or to the job transfer within the company. There were only 2 dropout cases due to non-compliance. Considering that the dropout rates reported in previous randomized controlled HIE interventions conducted in Western premenopausal women were 14–33% [10, 15, 31], the dropout rate of this study is within an acceptable range. Therefore, we suggest that the office-based brief HIE program may be an eligible option for the prevention of bone mineral loss.

Based on the previous and current studies, we can offer a bone health concept that is suitable for quite sedentary Japanese women. In this subject group, progression of the exercise program should be gradual. With the selected short office-based exercise program, significant changes in bone mineral density and fitness may be obtained.

In conclusion, the results of this 12-month trial indicate that simple office-based high impact exercise is a safe and effective method for preventing bone loss in the femoral neck. This type of exercise can be recommended for healthy premenopausal Japanese women in order to prevent bone mineral loss.

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Conflict of interest Doctors Korpelainen, Vainionpää, and Jämsä have a patent application with Newtest Ltd. Doctor Jämsä is also a minor shareholder of Newtest Ltd.

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Association of Japanese dietary pattern with serum adiponectin concentration in Japanese adult men

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Abstract *Background and aims:* Although previous studies suggest that the traditional Japanese dietary pattern is independently associated with a low cardiovascular disease mortality risk, the mechanisms mediating or linking this association are not well understood. Adiponectin has emerged as a valuable biomarker for cardiovascular diseases. The aim of present study was to evaluate whether dietary patterns are associated with serum adiponectin concentration in Japanese adult men.

Methods and results: We designed a cross-sectional study of 702 men (median [interquartile range] age, 44.5 [37.8–54.2] years) living in Japan. Dietary consumption was assessed via a 75-item food frequency questionnaire. We used principal-components analysis to derive 3 major dietary patterns-"Japanese", "sweets-fruits" and, "Izakaya (Japanese Pub)"- from 39 food groups. Serum adiponectin concentration was measured by using a specific sandwich enzyme-linked immunosorbent assay. After adjustment for potential confounders, the geometric mean (95% confidence interval) for log-transformed adiponectin concentration associated with "Japanese" dietary pattern factor score tertiles were 5.24 (4.84–5.69) for the lowest tertile, 5.82 (5.39–6.29) for the middle tertile, and 5.95 (5.47–6.46) for the highest tertile (P for trend <0.01). In contrast, a significant inverse association was found between the "Izakaya" pattern factor score tertiles and adiponectin concentration (P for trend $= 0.03$).

Conclusions: Greater adherence to the "Japanese" dietary pattern was independently associated to a higher serum adiponectin concentration in Japanese adult men. This finding supports the hypothesis that the traditional Japanese diet may have a potentially beneficial effect on adiponectin concentrations. A long-term prospective study or randomized trials are required to clarify this causality.

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Introduction

Lifestyle-related illness remains a major cause of mortality and morbidity worldwide [1]. Considerable evidence has accumulated that dietary factors are the cornerstone for the prevention and treatment of lifestyle-related illness [2]. Moreover, because diets are composed of a wide variety of foods containing complex combinations of nutrients, surveys that analyze a single nutrient component in foods may not adequately account for the complicated interactions and cumulative effects on human health.

The traditional Japanese diet is characterized by a high consumption of soybean products, fish, seaweeds, vegetables, fruits, and green tea [3]. Many epidemiological studies have shown that the consumption of these food items or adherence to the Japanese dietary pattern in itself is inversely associated with lifestyle-related illness such as cardiovascular disease (CVD), cancer, blood pressure (BP), serum lipids, diabetes, etc [3–6]. However, the mediating factors underlying these associations have not been fully identified.

Adiponectin is a peptide hormone secreted exclusively by the adipose tissue [7]. Plasma adiponectin levels are known to be associated with cases of CVD and type 2 diabetes [8,9]. Several epidemiological and experimental studies have also shown that low adiponectin levels are associated with an increased risk of cancer [10]. Studies with experimental animal models have shown that adiponectin inhibits tumorigenesis [11]. Moreover, clinical studies have shown that low adiponectin levels was associated with hypertension [12], and hypercholesterolemia [13].

Because several components of the "Japanese" dietary pattern, such as fish [14], soybean [15], vegetable [16], and green tea [17] were associated with increased insulin sensitivity, reduced risk of CVD, hypertension, and cancer, we hypothesized that the "Japanese" dietary pattern might be associated with adiponectin. However, to the best of our knowledge, no previous study has assessed the association between the Japanese dietary pattern and adiponectin.

In the present study, we designed a cross-sectional study to investigate whether dietary pattern is associated with adiponectin in Japanese adult men.

Methods

Subjects

The current analysis used data from a prospective cohort study to investigate the risk factors of chronic diseases among adult employees. The study was based on annual health examinations [18] at the Sendai Oroshisho Center. In order to stratify for potential confounders, we have added several assessment parameters to the health examination: 1) questionnaires (please see details), 2) physical performance measurement (leg extension power and grip strength), 3) blood examination (adiponectin, etc.), and 4) daily physical activity (PA) assessment using a three-dimensional accelerometer, etc.

We used baseline data in this study. The sample selection process is described in Fig. 1. There were 1833 individuals had received health examinations (lifestyle-related illnesses and health examinations A, which include blood examinations; or health examinations B, which do not include blood examinations) [18]. We invited all subjects who received lifestyle-related illnesses and health examination A (n = 1253) to participate in the study. Of those invited, 1154 agreed to participate and provided informed consent for their data to be analyzed (response rate = 92.1%). The protocol of our study was approved by the Institutional Review Board of the Tohoku University Graduate School of Medicine. Because we found that gender differences in adiponectin levels (median value and [interquartile range] of males vs. females: 5.73 [4.14–7.68] vs. 10.65 [7.71–13.20]; P value <0.0001), and the number of female subjects (n = 273) was too small to perform factor analysis [19], females were excluded from final analysis. We also excluded subjects, who did not have any dietary information (n = 48), who had a history of CVD (n = 5), and who used anti-hypertensive (n = 91), lipid-lowering (n = 14), or anti-diabetic agents (n = 16). Owing to these exclusions, the final study population comprised 702 subjects (median [interquartile range] age: 44.5 [37.8, 54.2] years).

Assessment of dietary intake

The participants were instructed to complete a brief, self-administered diet history questionnaire (BDHQ) that included questions on 75 food items along with their specified serving sizes [20]. The participants indicated their mean frequency of consumption of the food over the past month by checking 1 of the 7 frequency categories, ranging from "almost never" to "2 or more times/day". The mean daily consumption of nutrients was calculated using an *ad hoc* computer program developed to analyze the questionnaire. The Japanese food composition tables, 5th edition were used as the nutrient database. Foods from the BDHQ were categorized into 39 food subgroups, which were used to derive dietary patterns via principal-components analysis.

Measurement of serum adiponectin concentration

Adiponectin was measured using a specific sandwich enzyme-linked immunosorbent assay (Otsuka Pharmaceutical, Tokyo, Japan). The detection limit of the assay was 23.4 pg/ml, the measurement range was 0.375–12.0 ng/ml, and the intra- and inter-assay coefficients of variation were less than 10%.

Assessment of other variables

Depressive symptoms were assessed according to the Japanese version of the Self-Rating Depression Scale (SDS) [21]. An SDS score ≥40 was taken as the cutoff point indicating relatively mild or severe depressive symptoms [22]. BP was measured twice from the upper left arm by using an automatic device (YAMASU605P; Kenzmedico, Saitama, Japan) after 5 min of rest in the sitting position. The mean of the 2 measurements was taken as the BP value.

Blood samples were collected in siliconized vacuum glass tubes containing sodium fluoride, for analyzing fasting

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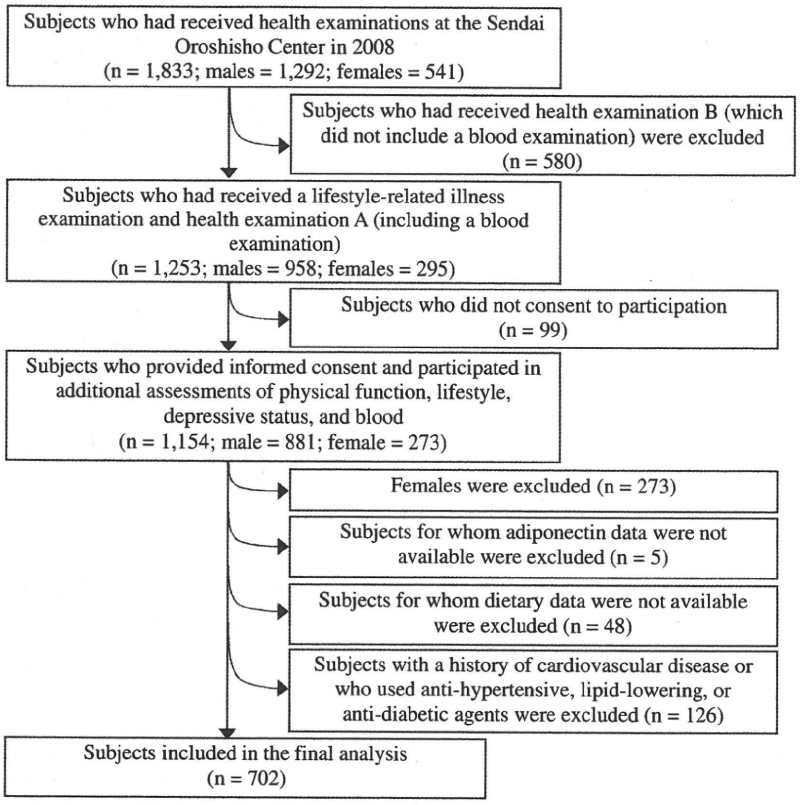

Figure 1 Flow chart of the sample selection process.

blood glucose and no additives, for analyzing lipids and adiponectin. Fasting blood glucose (FBG) was measured by using enzymatic methods (Eerotec, Tokyo, Japan). The concentrations of triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured by enzymatic methods using appropriate kits (Sekisui Medical, Tokyo, Japan).

Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). The educational level was assessed by determining the last grade level and was divided into 2 categories: <college or ≥ college. History of physical illness and current medication were noted from "yes" or "no". Information on age, sex, smoking status, sleep duration, occupation, marital status (yes/no), and living status (alone or with others) were obtained by conducting a questionnaire survey. Levels of PA were estimated using the International Physical Activity Questionnaire [23]. Total daily PA (METs × hours/week) were calculated [23]. PA was categorized into tertiles with a similar number of individuals.

Statistical analysis

All statistical analyses were performed using the Statistical Analysis System 9.1 edition (SAS Institute Inc., Cary, NC, USA). Factor analysis (principal-components analysis) was used to derive dietary patterns and to determine factor loadings. Factors were rotated with varimax rotation to maintain uncorrelated factors and enhance interpretability [24]. A combined evaluation of the eigenvalues, scree plot test, and factor interpretability was used in determining the number of retained factors. The distinctive dietary patterns were well described by the 3 factors. For each dietary pattern and each subject, we calculated a factor score by summing the consumption from each food item weighted by its factor loading as follows [24]:

$$\sum[(\text{food group}_i \text{ servings/d}) \times (\text{food group}_i \text{ factor loading})]$$

where i = food groups 1–39. For further analyses, factor scores were categorized into 3 equal groups by using tertiles cutoffs.

In this study, because the distribution of all continuous variables was non-normal, the natural logarithm was applied to normalize the data before analysis of variance (ANOVA) or analysis of covariance (ANCOVA). Descriptive data are presented as the geometric mean (95% confidence interval, CI) for continuous variables and percentages for categorical variables. Log-transformed adiponectin were used as dependent variables, and dietary pattern factor scores tertiles as independent variables. Differences among dietary pattern factor score tertiles were examined using ANOVA for continuous variables and logistic regression analysis for proportional variables. ANCOVA was used to examine relationships between dietary pattern factor score tertiles and log-transformed adiponectin. These analyses were adjusted for various variables in 3 models. In model 1, the variables adjusted for were age, BMI, PA, smoking status

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(nonsmoker, ex-smoker, or current smoker), depressive symptoms, sleep duration (6–7 h, 7–8 h, or other), educational level, occupation (desk work, or non-desk work), and energy intake. In model 2, the variables adjusted for were those of model 1 plus systolic BP, FBG, TG, LDL-C, and HDL-C. In model 3, the variables adjusted for were those of model 2 plus the score categories of the other 2 dietary patterns. Bonferroni-corrected *P* values were used for comparisons between dietary pattern factor score tertiles. All *P* values for linear trends were calculated using the median score of dietary pattern factor scores tertiles. Interactions between dietary pattern factor score tertiles and confounders of log-transformed adiponectin were tested by the addition of cross-product terms to the regression model. All tests were two-tailed and $P < 0.05$ was defined as statistically significant.

Results

Food items and factor loading scores are presented in Appendix Table 1. Factor 1, identified as a traditional "Japanese" dietary pattern was characterized by a high consumption of vegetables, seaweeds, soybean products, fish, miso soup, and green tea. This factor was positively associated with total fish, seaweeds, total vegetables, soybean products, total fruits, dairy products, green tea, total energy intake, animal protein, vegetable protein, animal fat, vegetable fat, total fiber, calcium, and eicosapentaenoic acid (EPA)+docosahexaenoic acid (DHA), and negatively associated with cola and carbohydrate (*P* for trend <0.01) (Appendix Table 2). Factor 2 was typified by a greater consumption of ice cream, cake, fruits, dairy products, cola, and lower consumption of alcohol (named the "sweets-fruits" pattern). This factor was positively associated with total fruit, dairy products, coffee, cola, total energy intake, vegetable protein, animal fat, vegetable fat, carbohydrate, total fiber, and calcium, and negatively associated with total fish, EPA + DHA, and alcohol (*P* for trend <0.05). Incidentally, although dairy product consumption is lower in the "Japanese" dietary pattern than in the "sweets-fruits" pattern, the "Japanese" dietary pattern is associated with high calcium consumption. Factor 3 was typified by a greater consumption of fish, meat, and alcohol (named the "Izakaya (Japanese Pub)" pattern). This factor was positively associated with total meat, total fish, seaweed, total fruit, green tea, black or oolong tea, total energy intake, animal protein, animal fat, EPA + DHA, alcohol, and calcium, and negatively associated with dairy products, vegetable protein, and carbohydrate (*P* for trend < 0.05). These 3 patterns explained 31.5% of the variance in dietary consumption (17.8% for factor 1, 7.3% for factor 2, and 6.4% for factor 3). These dietary patterns were similar to those reported in a previous study [3].

The participant characteristics in relation to the tertiles of each dietary pattern and factor score are presented in Table 1. Compared to subjects in the lowest "Japanese" dietary pattern tertile, the highest tertile group tended to be older (*P* for trend <0.0001) with a higher proportion of subjects having low PA ($P < 0.01$), and a lower proportion of current smokers ($P = 0.03$) and individuals with depressive symptoms ($P < 0.0001$; data not shown). Compared to subjects in the lowest "sweets-fruits" pattern tertile, the highest tertile group tended to be younger; had higher BMI and LDL; lower SBP, DBP, and HDL; and lower proportions of individuals with a sleep duration of 7–8 h (data not shown) and current smokers (*P* for all trends <0.05). Compared to subjects in the lowest "Izakaya" pattern tertile, the highest tertile group had higher SBP, TG, and lower LDL, and a higher proportion of individuals with low PA (*P* for all trends <0.05). Other than these results, no significant differences were observed between the tertiles of each dietary pattern factor score. Furthermore, we also analyzed the relationships between the "sweets-fruits" and "Izakaya" patterns and marital and living statuses (data not shown). No relationship was found between these dietary patterns and these statuses (*P* for trend, >0.08).

Table 2 shows the adjusted relationships between tertiles of dietary pattern and the adiponectin. In the final models, the adjusted geometric mean (95% CI) of log-transformed adiponectin associated with the "Japanese" dietary pattern were 5.24(4.87–5.69) for the lowest tertile, 5.82(5.39–6.29) for the middle tertile, and 5.95 (5.47–6.46) for the highest tertile (*P* for trend <0.01). The geometric mean of log-transformed adiponectin associated with the highest "Japanese" dietary pattern tertile was 13.5% higher than that associated with the lowest tertile (Bonferroni-corrected *P* value = 0.03). In contrast, a significant inverse association was found between "Izakaya" pattern tertiles and adiponectin (*P* for trend = 0.03). No relationship was found between tertiles of "sweets-fruits" pattern score and adiponectin. We also analyzed the relationships between 8 other dietary patterns (factor loading score: >1; range: 1.01–1.62) and adiponectin, but found no relationship between them. The tests for interactions between the tertiles of "Japanese" dietary pattern factor scores and other potential confounders in the final models were also not statistically significant (interaction *P* values >0.09).

Furthermore, we analyzed how food items that contributed substantially (factor loading scores >0.40) to the "Japanese" and "Izakaya" dietary patterns (as shown in Appendix Table 1) and main nutrients were associated to adiponectin (Appendix Table 3). In the final models, significant association with adiponectin were observed between other root vegetables, carrot/pumpkin, and mushrooms (*P* for trend = 0.03, 0.049, and 0.03, respectively). Although not statistically significant, higher consumption of fish and fermented soybeans coincided with higher levels of adiponectin (trend *P* value = 0.30, and 0.08, respectively). Because the "Izakaya" dietary pattern was associated with lower LDL, we analyzed how food items that contribute to this pattern were associated with LDL. In the final models (model 3), we found that only alcohol consumption was significantly and strongly associated with lower LDL (*P* for trend <0.01).

Furthermore, we also analyzed the relationships between "Japanese" dietary pattern and several other CVD risk factors including SBP, DBP, FBG, TG, LDL, and HDL adjusted to all confounding factors. The adjusted geometric mean (95% CI) of SBP, DBP, and TG across the

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Table 1 Baseline characteristics of the participants according to the tertiles of dietary pattern score (n = 702).[a]

	Tertiles of dietary pattern factor score			P for trend[b]
	Low (n = 234)	Middle (n = 234)	High (n = 234)	
Age (years)				
"Japanese"	42.9(41.7, 44.1)[c]	45.3(44.0, 46.5)	46.8(45.5, 48.1)	<0.0001
"sweets-fruits"	46.1(44.9, 47.5)	44.6(43.4, 45.9)	44.2(42.9, 45.4)	0.03
"Izakaya"	45.1(43.8, 46.3)	45.0(43.8, 46.3)	44.8(43.5, 46.1)	0.77
BMI (kg/m^2)				
"Japanese"	23.1(22.7, 23.6)	23.4(23.0, 23.8)	23.3(22.9, 23.7)	0.60
"sweets-fruits"	23.1(22.7, 23.5)	23.1(22.7, 23.5)	23.7(23.3, 24.1)	0.04
"Izakaya"	23.0(22.6, 23.4)	23.3(22.9, 23.7)	23.6(23.2, 24.0)	0.06
SBP (mmHg)				
"Japanese"	126.5(124.5, 128.4)	126.4(124.4, 128.3)	127.0(125.1, 129.0)	0.70
"sweets-fruits"	130.3(128.3, 132.3)	125.8(124.0, 127.8)	123.8(121.9, 125.7)	<0.0001
"Izakaya"	125.5(123.6, 127.4)	126.0(124.1, 128.0)	128.3(126.4, 130.3)	0.04
DBP (mmHg)				
"Japanese"	78.7(77.3, 80.1)	78.5(77.1, 79.9)	78.9(77.5, 80.4)	0.83
"sweets-fruits"	80.8(79.4, 82.2)	78.5(77.1, 79.9)	76.9(75.6, 78.3)	0.0001
"Izakaya"	78.5(77.1, 79.9)	77.9(76.5, 79.3)	79.7(78.3, 81.2)	0.23
Fasting blood glucose (mg/dl)				
"Japanese"	92.1(90.6, 93.7)	93.4(91.8, 94.9)	94.1(92.5, 95.7)	0.08
"sweets-fruits"	94.6(93.0, 96.2)	92.3(90.8, 93.9)	92.7(91.2, 94.3)	0.10
"Izakaya"	93.4(91.9, 95.0)	92.4(90.9, 94.0)	93.8(92.2, 95.3)	0.76
TG (mg/dl)				
"Japanese"	113.3(105.3, 121.8)	117.0(108.8, 125.9)	113.4(105.5, 122)	0.98
"sweets-fruits"	118.6(110.3, 127.5)	110.3(102.5, 118.6)	115.1(107, 123.7)	0.57
"Izakaya"	109.7(102, 117.9)	109.6(101.9, 117.8)	125.2(116.4, 134.6)	0.01
LDL (mg/dl)				
"Japanese"	117.0(113.0, 121.2)	118.2(114.1, 122.4)	116.9(112.8, 121.0)	0.95
"sweets-fruits"	108.8(105.2, 112.7)	119.4(115.3, 123.6)	124.4(120.2, 128.7)	<0.0001
"Izakaya"	120.9(116.7, 125.2)	117.2(113.1, 121.4)	114.1(110.2, 118.2)	0.02
HDL (mg/dl)				
"Japanese"	51.2(49.7, 52.9)	51.8(50.2, 53.4)	52.1(50.5, 53.7)	0.47
"sweets-fruits"	54.3(52.6, 56.0)	51.3(49.8, 52.9)	49.6(48.1, 51.2)	<0.0001
"Izakaya"	51.2(49.6, 52.8)	52.2(50.6, 53.9)	51.7(50.1, 53.3)	0.64
High PA (%, median values: 58.6 METs × hours/week)				
"Japanese"	40.6	32.9	40.2	0.90
"sweets-fruits"	36.8	38.9	38.0	0.78
"Izakaya"	38.9	41.5	33.3	0.17
Low PA (%, median values: 10 METs × hours/week)				
"Japanese"	31.2	38.5	44.0	<0.01
"sweets-fruits"	38.5	37.6	37.6	0.85
"Izakaya"	32.9	38.5	42.3	0.04
Current smoker (%)				
"Japanese"	59.4	55.1	49.2	0.03
"sweets-fruits"	63.7	53.4	46.6	<0.001
"Izakaya"	52.6	56.0	55.1	0.62
Ex-smoker (%)				
"Japanese"	10.3	12.0	15.4	0.09
"sweets-fruits"	12.8	12.0	12.8	1.00
"Izakaya"	14.1	9.8	13.7	0.98

[a] "Izakaya", Japanese Pub; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; PA, physical activity; SDS, Self-rating Depression Scale.
[b] Analysis of variance or logistic regression.
[c] Geometric mean (95% confidence interval) (all such values).

"Japanese" dietary pattern tertiles were 128.6 (125.9–131.3), 126.0(123.5–128.5), and 125.8 (123.1–128.5) (P for trend, 0.07); 80.0(78.1–81.9), 77.5 (75.8–79.3), and 77.2(75.4–79.1) (P for trend, 0.01), and 113.3(102.3–125.5), 110.9(100.6–122.2), and 105.8 (95.2–117.4) (P for trend, 0.26), respectively. No trend relation was found between "Japanese" dietary pattern and FBG, LDL, and HDL (P for trend, >0.45).

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Table 2 Adjusted relationships between tertiles of dietary pattern factor score and the serum adiponectin concentration ($n = 702$).[a]

	Tertile of dietary pattern factor score			*P* for trend[b]
	Low ($n = 234$)	Middle ($n = 234$)	High ($n = 234$)	
"Japanese" dietary pattern[c]	−0.87	−0.18	0.85	–
Model 1[d]	5.26(4.82, 5.73)[g]	5.88(5.41, 6.39)[h]	6.07(5.56, 6.63)[h]	<0.01
Model 2[e]	5.21(4.81, 5.65)	5.78(5.35, 6.25)[h]	5.97(5.49, 6.48)[h]	<0.01
Model 3[f]	5.24(4.84, 5.69)	5.82(5.39, 6.29)[h]	5.95(5.47, 6.46)[h]	<0.01
"Sweets-fruits" pattern[b]	−0.87	−0.08	0.73	–
Model 1[d]	5.71(5.27, 6.20)	5.64(5.18, 6.13)	5.81(5.32, 6.34)	0.71
Model 2[e]	5.52(5.11, 5.96)	5.58(5.16, 6.03)	5.86(5.40, 6.36)	0.16
Model 3[f]	5.52(5.11, 5.97)	5.55(5.14, 6.00)	5.92(5.45, 6.42)	0.11
"Izakaya" pattern[b]	−0.85	−0.16	0.87	–
Model 1[d]	6.13(5.63, 6.67)	5.65(5.19, 6.15)	5.41(4.98, 5.88)[h]	<0.01
Model 2[e]	6.07(5.61, 6.57)	5.48(5.06, 5.92)[h]	5.38(4.97, 5.82)[h]	<0.01
Model 3[f]	6.02(5.56, 6.51)	5.52(5.10, 5.97)	5.47(5.05, 5.92)	0.03

[a] "Izakaya", Japanese Pub.
[b] Analysis of covariance (*P* values for linear trends were calculated using the median value of each dietary pattern factor score).
[c] Median score (all such values).
[d] Adjusted for age, body mass index, physical activity, smoking status, depressive symptoms, sleep duration, educational level, occupation, and total energy intake.
[e] Additionally adjusted for systolic blood pressure, blood glucose concentration, triglycerides, total cholesterol, and high-density lipoprotein cholesterol.
[f] Additionally adjusted for tertiles of other dietary pattern factor scores.
[g] Adjusted geometric mean (95% confidence interval) (all such values).
[h] Significantly different from the lowest tertile of the dietary pattern (Bonferroni correction): $P < 0.05$.

Discussion

Our results suggest that a typical "Japanese" dietary pattern was independently associated to a higher adiponectin. In contrast, a significant inverse association was found between the "Izakaya" pattern and adiponectin.

Our primary hypothesis was that the "Japanese" dietary pattern might be associated with adiponectin. The particular food items prominent in this dietary pattern may partly support our hypothesis. First, because the EPA/DHA have numerous beneficial effects on health [14], fish consumption may increase adiponectin [25]. Several experimental and clinical studies have shown that EPA/DHA increase adiponectin in mice [25,26] and obese human subjects [25]. Second, soy has been hypothesized to associate with adiponectin owing to its cholesterol-lowering, anti-obesity and anti-hypertensive effects [15,27]. One experimental study has shown that soy increased adiponectin in rats [28]. Third, fruit and vegetable consumption was found to be weakly associated with adiponectin in female twins [29]. Additionally, several studies have shown that green tea increases adiponectin in experimental animal models [30], and obese women [31]. However, these relationships associated with single food items are unlikely to explain our findings, because we did not observe significant positive relationships between adiponectin and any particular dietary factors, except some vegetables. Our findings suggest that the "Japanese" dietary pattern as a whole, or the balance of nutrients that it contains, may be a more important factor that influences adiponectin levels rather than the single components.

Although not statistically significant (excluding DBP), the "Japanese" dietary pattern was associated with lower SBP, DBP, and TG. These factors together with adiponectin, may cooperatively mediate the beneficial association between the "Japanese" dietary pattern and CVD. In contrast, no relation was found between the "Japanese" dietary pattern and FBG, LDL, and HDL. The small sample size and a rather healthy population may partly explain this result. Further large-scale study is required to clarify the association between the "Japanese" dietary pattern and these factors.

Although a significant inverse association was found between "Izakaya" dietary pattern and adiponectin, we did not observe any significant association between food items that contributed substantially to the "Izakaya" pattern and adiponectin. Thus, a low consumption of food items that contribute substantially to the "Japanese" dietary pattern or an imbalanced intake of nutrients characterizing this pattern may partly explain the result.

Cross-sectional studies have assessed the association between the Mediterranean diet pattern and adiponectin among healthy Greek adults [32] and diabetic American women [33]. These studies reported a positive association between the Mediterranean diet pattern and adiponectin. The study with diabetic women found that alcohol, nuts, and whole grain consumption had the strongest positive associations with adiponectin [33]. Moreover, another cross-sectional study with Greek women found that a dietary pattern characterized by a high consumption of whole grain cereals and low-fat dairy products was positively associated with adiponectin [34]. Because these food groups are not principal-components of the "Japanese" dietary pattern, the mechanisms mediating relationship between the dietary patterns and adiponectin reported by other studies may be different from those underlying the results of the present study. Further studies are required to clarify this hypothesis.

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In the current study, the "Japanese" dietary pattern was positively associated with low PA. Although the reason cannot be completely explained, these characteristics may reflect a traditional Japanese lifestyle. Future study is required to clarify the relationship between the "Japanese" dietary pattern and low PA, and how to associate this lifestyle with health. Furthermore, the large consumption of small fish (including bones) and vegetables may explain the association between "Japanese" dietary pattern and high calcium intake. In this study, we also found that alcohol consumption is strongly associated with lower LDL. This result agrees well with a previous prospective study [35]. Further study is required to clarify the possible mechanisms underlying the relationship between alcohol consumption and lower LDL.

The present study has several limitations. Because our study was a cross-sectional, we could not conclude whether the "Japanese" dietary pattern increased the adiponectin. Moreover, although we adjusted for a considerable number of potentially confounding factors, we cannot exclude the possibility that adiponectin are affected by other dietary habits or lifestyle variables, intrinsically associated with the "Japanese" dietary pattern. Therefore, a prospective study or an intervention trial should be undertaken to confirm the existence of a relationship between the "Japanese" dietary pattern and adiponectin.

In the present study, a higher score of the "Japanese" dietary pattern was significantly associated with a higher serum adiponectin concentration in Japanese adult men. Single food items or nutrient consumption was less likely to explain our findings. A long-term prospective study or randomized trials are required to clarify this causality.

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Appendix. Supplementary material

The supplementary data associated with this article can be found in the on-line version at doi:10.1016/j.numecd.2010.06.006.

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Presenilin-1 acts via Id1 to regulate the function of muscle satellite cells in a γ-secretase-independent manner

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Summary

Muscle satellite cells are the resident stem cells of adult skeletal muscle. Here, we have examined the role of the multifunctional protein presenilin-1 (PS1) in satellite cell function. PS1 acts as a crucial component of the γ-secretase complex, which is required to cleave single-pass transmembrane proteins such as Notch and amyloid-β precursor protein. PS1, however, also functions through γ-secretase-independent pathways. Activation of satellite cells was accompanied by induction of PS1, with PS1 knockdown enhancing their myogenic differentiation, but reducing their self-renewal. Transfection with siRNA against PS1 led to accelerated myogenic differentiation during muscle regeneration in vivo. Conversely, constitutive expression of PS1 resulted in the suppression of myogenic differentiation and promotion of the self-renewal phenotype. Importantly, we found that PS1 also acts independently of its role in γ-secretase activity in controlling myogenesis, which is mediated in part by Id1 (inhibitor of DNA binding 1), a negative regulator of the myogenic regulatory factor MyoD. PS1 can control Id1, which affects satellite cell fate by regulating the transcriptional activity of MyoD. Taken together, our observations show that PS1 is a key player in the choice of satellite cell fate, acting through both γ-secretase-dependent and γ-secretase-independent mechanisms.

Key words: Satellite cell, Myoblast, Presenilin-1, Id1, Pax7, MyoD, γ-secretase, Self-renewal, Skeletal muscle, Myogenic differentiation, Stem cell, Cell fate choice

Introduction

Muscle satellite cells are myogenic stem cells that are located between the basal lamina and the plasmalemma of myofibers (Mauro, 1961). They are necessary for postnatal muscle growth, and are responsible for maintenance, hypertrophy and repair of adult skeletal muscle. It has been shown that satellite cells are able to self-renew to maintain their population (Collins et al., 2005) and much work is currently directed at understanding how self-renewal is regulated (reviewed by Zammit, 2008).

The paired-box transcription factor Pax7 is expressed by quiescent satellite cells and is implicated in the generation of committed myogenic progenitors, but its role in the regulation of satellite cell self-renewal is in debate (Lepper et al., 2009; McKinnell et al., 2008; Olguin et al., 2007; Seale et al., 2000; Zammit et al., 2006). Following activation, satellite cells co-express Pax7 with MyoD [a member of the myogenic regulatory factor family, together with Myf5, Mrf4 and Myog (myogenin)] and proliferate. Later, satellite-cell-derived myoblasts either downregulate Pax7, maintain MyoD and induce Myog as they undergo myogenic differentiation, or they downregulate MyoD and maintain Pax7, returning to a quiescent-like state (Halevy et al., 2004; Zammit et al., 2004). Interestingly, the total number of satellite cells in adult muscles remains relatively constant after repeated muscle injury and regeneration, indicating that the self-renewal system of satellite cells is carefully coordinated (Collins et al., 2005; Yoshida et al., 1998; Zammit et al., 2004). However, the molecular mechanism of satellite cell self-renewal remains poorly understood, although recent advances have shown that Notch and canonical Wnt signalling play a role (Brack et al., 2008; Conboy and Rando, 2002; Kitzmann et al., 2006; Kuang et al., 2007; Perez-Ruiz et al., 2008).

Notch signalling controls many events, including differentiation, proliferation and apoptosis in various tissues (Hansson et al., 2004). In skeletal muscle, the Notch-signalling pathway is involved in activation, and proliferation of muscle satellite cells, and has been implicated in their self-renewal (Conboy et al., 2003; Conboy and Rando, 2002; Kitzmann et al., 2006; Kopan et al., 1994; Kuang et al., 2007; Nofziger et al., 1999; Ono et al., 2007). For example, inhibition of Notch activity enhances myogenic differentiation of murine and human myoblasts (Kitzmann et al., 2006; Kuang et al., 2007). Notch is activated by binding of members of the delta-like and Jagged families (in mammals) to its extracellular domain, which results in γ-secretase-mediated cleavage to release the Notch intracellular domain (Notch ICD) (Herreman et al., 2000; Struhl and Greenwald, 1999; Zhang et al., 2000). The Notch ICD translocates into the nucleus, where it interacts with the DNA-binding protein CSL/RBP-J (RBP-J is a member of CSL family of proteins) to regulate the transcription of target genes such as *Hes1* (Jarriault et al., 1995).

Together with nicastrin, Pen-2 and Aph-1, the other crucial component of the γ-secretase complex is presenilin (reviewed by De Strooper, 2003). Presenilin-1 (PS1) and presenilin-2 (PS2) are membrane proteins that function as the catalytic subunit of the γ-secretase complex, an intramembrane protease with a number of substrates of the type I membrane protein family (De Strooper et al., 1999) (reviewed by Parks and Curtis, 2007; Vetrivel et al., 2006). For example, in addition to cleavage of activated Notch, γ-secretase targets also include, but are not limited to, amyloid precursor protein

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(APP), Delta, Jagged, CD44, CD43, Erb4, E-cadherin, N-cadherin and syndecan (De Strooper et al., 1999) (reviewed by Parks and Curtis, 2007; Vetrivel et al., 2006). Importantly, PS1 also functions via γ-secretase-independent pathways (Akbari et al., 2004; Esselens et al., 2004; Huppert et al., 2005; Meredith et al., 2002; Repetto et al., 2007; Tu et al., 2006; Wilson et al., 2004). For example, somitogenesis is abrogated in *PS1*-null mice, yet embryos lacking other essential components of the γ-secretase complex, such as nicastrin, Pen-2 and Aph-1, or null for the Notch pathway component CSL/RBP-J, still develop anterior somites in the complete absence of Notch signalling (Huppert et al., 2005). Furthermore, the roles of PS1 in Ca^{2+} homeostasis (Akbari et al., 2004; Tu et al., 2006); autophagy and protein degradation (Esselens et al., 2004; Repetto et al., 2007; Wilson et al., 2004); and Wnt–β-catenin signalling (Meredith et al., 2002) have all also been shown to be via γ-secretase-independent mechanisms.

Here, we sought to explore the role of PS1 in satellite cell function. We found that PS1 is strongly expressed in activated and proliferating satellite cells, where PS1 knockdown accelerates myogenic differentiation and reduces the number of cells undergoing self-renewal. By contrast, constitutive PS1 expression led to a downregulation of MyoD expression and decreased differentiation. PS1 acts via Id1 (inhibitor of DNA binding 1), a potent negative regulator of the ability of MyoD to activate its transcriptional targets. Because *PS1*-null mice die during embryogenesis, we used *PS1*$^{-/-}$ and/or *PS2*$^{-/-}$ murine embryonic fibroblasts (MEFs) and found that Id1 levels were low, but significantly upregulated by addition of PS1 or a mutant PS1 that lacks γ-secretase activity, but not by PS2. Taken together, these observations show that PS1 has an essential role in satellite cell function, and can act independently of γ-secretase activity by regulating Id proteins to control MyoD transcriptional activity.

Results

PS1 is induced during activation of satellite cells

To investigate the expression dynamics of PS1 during myogenic progression, immunostaining was performed on satellite cells retained in their niche on isolated myofibres. PS1 was not detectable in Pax7^{+} quiescent satellite cells, analysed immediately after isolation (T0). However, culture of myofibres in plating medium for 24 hours (T24) showed that activated satellite cells induced robust PS1 expression, located in the membrane compartment (Fig. 1A,E), which remained high in proliferating Ki67^{+} or undifferentiated Pax7^{+} satellite cells at T48 (Fig. 1A,B). PS1 was then downregulated in satellite cell progeny committed to myogenic differentiation after 72 hours (T72), as shown by the presence of Myog (Fig. 1C). PS1 was clearly expressed in Ki67^{+} proliferating plated satellite-cell-derived myoblasts, but not in differentiating Myog^{+} cells, 5 days after isolation (Fig. 1D). Using western blotting, we also confirmed that PS1 is highly expressed in plated proliferating satellite-cell-derived myoblasts, and decreased in cells induced to differentiate by switching to low-serum medium (Fig. 1F).

PS1 knockdown inhibits satellite cell self-renewal

Having shown that PS1 is expressed in activated and proliferating satellite cells, we next examined its function. In order to determine the effect of knockdown of PS1 on lineage progression in satellite-cell-derived myoblasts, cells were transfected with either of two siRNA duplexes against PS1 (PS1si-1 or PS1si-2) and control siRNA. Both siRNA species targeting PS1 effectively reduced PS1 protein levels in plated satellite-cell-derived myoblasts, when assayed 48 hours after transfection using western blot (Fig. 2A). At 72 hours after transfection, reduced PS1 levels resulted in a marked promotion of differentiation, as indicated by the higher fusion index for cells transfected with *PS1* siRNA than for cells

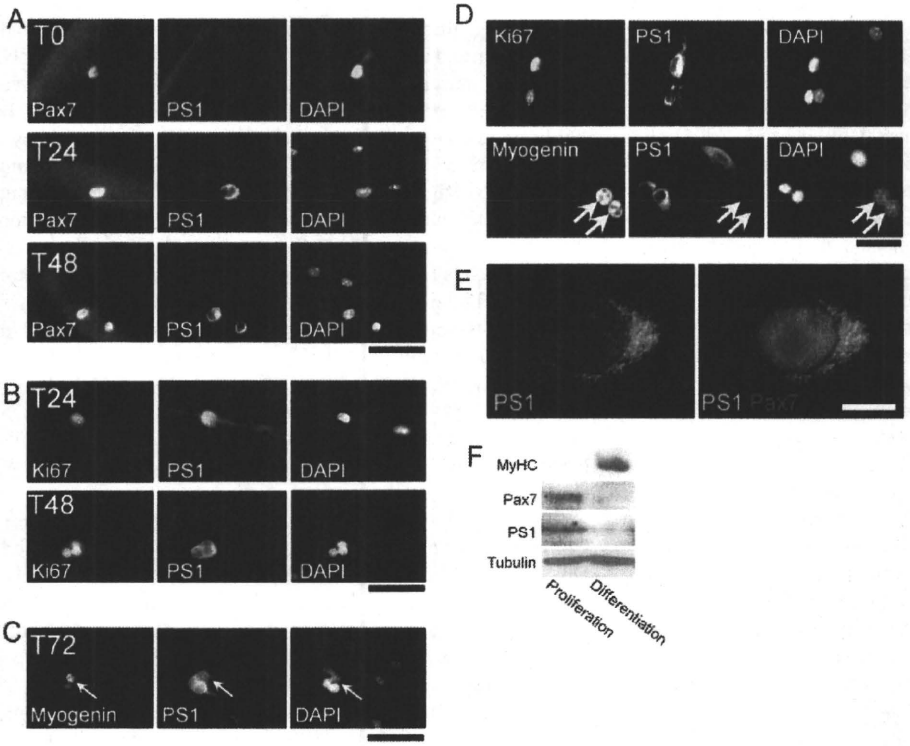

Fig. 1. PS1 is expressed by activated and proliferating satellite cells. Myofibres and their associated satellite cells were isolated and either immediately fixed (T0) or cultured in activation medium for either 24 hours (T24), 48 hours (T48) or 72 hours (T72) before fixation. (A) Immunostaining showed that Pax7^{+} satellite cells on freshly isolated myofibres (T0) did not express PS1. (B) PS1 could be detected after activation and during proliferation, as shown by the co-expression of PS1 and Ki67. (C) PS1 was downregulated as satellite-cell-derived myoblasts committed to myogenic differentiation, as demonstrated by the presence of Myog (arrows). (D) Immunocytochemistry on plated satellite-cell-derived myoblasts confirmed that expression of PS1 was associated with proliferating Ki67^{+} cells but not differentiating Myog^{+} cells (arrows). (E) High magnification immunofluorecent image to show the localisation of PS1 in plated satellite-cell-derived cells. (F) Western blot to illustrate that PS1 expression decreases after myogenic differentiation in plated satellite-cell-derived cells. Representative data of at least three independent experiments are shown. Scale bars: 60 μm (A-C), 30 μm (D) and 5 μm (E).

transfected with control siRNA (fold change in the number of DAPI-stained nuclei of differentiated cells [as shown by the presence of myosin heavy chain (MyHC)] divided by the total number of DAPI-stained nuclei; Fig. 2B and quantified in 2C). Importantly, siRNA-mediated knockdown of PS1 drastically reduced both the number of $Pax7^+$ cells and the percentage of cells with the $Pax7^+MyoD^-$ self-renewal phenotype (Zammit et al., 2004), compared with controls (Fig. 2D and quantified in 2E,F).

Transfection of *PS1* siRNA was also performed to assay the effects of PS1 knockdown on the fate of satellite cells retained in their niche on the myofibre. First, we determined the transfection efficiency of siRNA into satellite cells on a myofibre by using AlexaFluor488-conjugated siRNA (Fig. 2G) and found that levels of >95% could be obtained, and that PS1 was successfully knocked down (Fig. 2G). The percentage of $Pax7^+MyoD^-$ self-renewed cells was significantly decreased 72 hours after transfection with *PS1* siRNA, with an increased amount of differentiation-committed $Pax7^-MyoD^+$ and $Pax7^-Myog^+$ cells present, in comparison with cells transfected with control siRNA (Fig. 2H and quantified in 2I,J). Thus, PS1 is required for satellite cell self-renewal and for inhibition of myogenic differentiation.

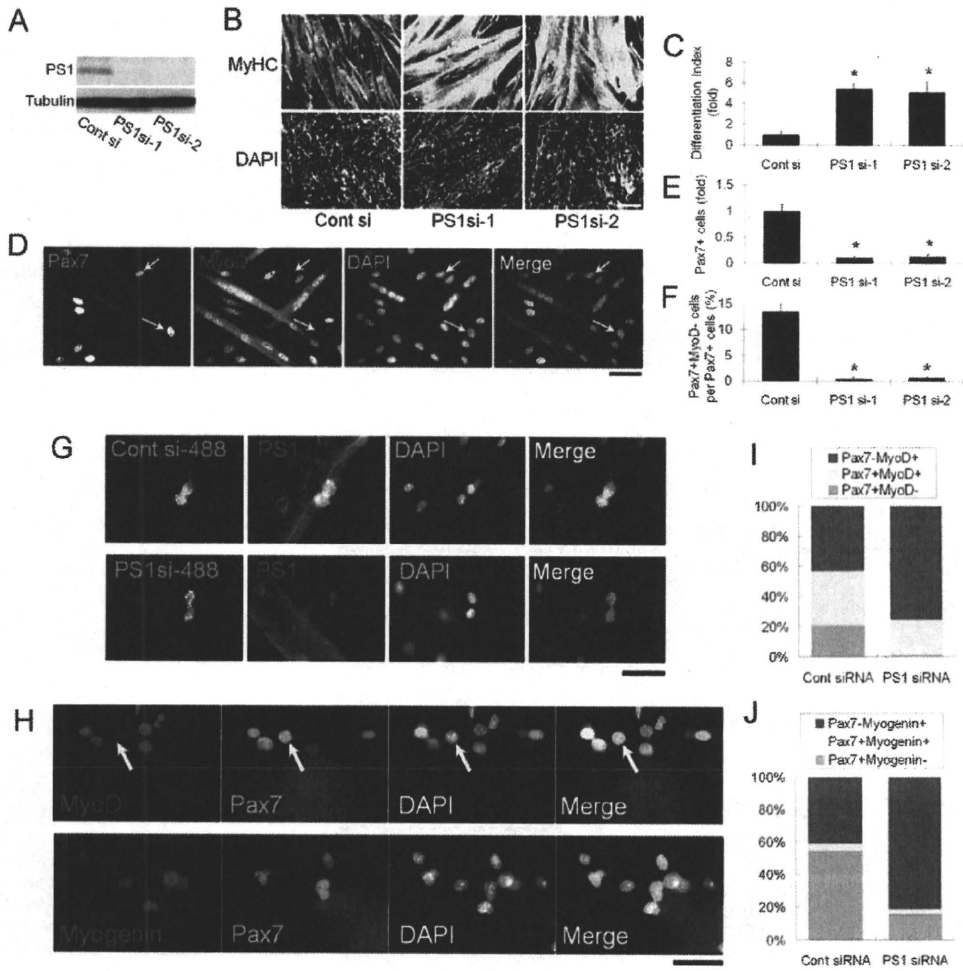

Fig. 2. PS1 is required for satellite cell self-renewal and maintenance of progenitor cells. To examine the effects of PS1 on satellite cell myogenic progression, we first knocked-down PS1 protein levels using siRNA. (A) Both *PS1* siRNAs (PS1si-1 and PS1si-2) efficiently knockdown PS1 protein in plated primary satellite-cell-derived myoblasts as shown by western blotting. (B) Satellite-cell-derived myoblasts were cultured for 3 days after siRNA transfection and then immunostained for MyHC. A dramatic increase in the extent of myogenic differentiation and formation of myotubes was observed with both siRNA species directed against PS1 as compared to controls (Cont si). (C) Differentiation index quantifying the increased myogenic differentiation caused by PS1 knockdown, calculated as fold change in the number of DAPI-stained nuclei in $MyHC^+$ cells, divided by the total number of DAPI-stained nuclei. (D) Immunocytochemistry of plated primary myoblasts was also used to investigate the presence of cells with a self-renewal phenotype ($Pax7^+MyoD^-$) 3 days after siRNA transfection (arrows indicate $Pax7^+MyoD^-$ cells), and clearly demonstrate that reduced PS1 levels resulted in less $Pax7^+$ cells (quantified in E) and self-renewal (quantified in F). (G) AlexaFluor488-conjugated control siRNA and *PS1* siRNA duplexes were transfected into satellite cells retained in their niche on isolated myofibres, and knockdown of PS1 protein confirmed by immunocytochemistry 48 hours later. (H) The effects of PS1 knockdown on satellite cell fate were examined by immunostaining for Pax7, MyoD (arrows indicates $Pax7^+MyoD^-$ cell) and Myog 72 hours after PS1si-1 transfection. (I,J) PS1 knockdown reduced the percentage of satellite-cell-derived myoblasts exhibiting the self-renewal phenotype ($Pax7^+MyoD^-$), while increasing the percentage of cells committed to differentiation ($Pax7^-MyoD^+$ and $Pax7^-Myog^+$). Data from at least three independent experiments is shown ± s.d. Asterisks in C, E and F indicate that data are significantly different from control values ($P<0.05$). Scale bars: 100 μm (B), 30 μm (D,G,H).

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