も報告されている．Lakkaほかは中年男女において，血清CRP濃度と最大酸素摂取量に有意の負の相関(r＝－0.402)があることを示した[14)]．Okitaほか，Kondoほかも中年成人において，両者間に有意の負の相関があることを報告している[11,17)]．本研究においては，中年者のみでなく高齢者においても同様の関連を認め，最大運動耐容能のみならず亜最大運動耐容能とも関連があることを示した．したがって，高齢者においても亜最大運動強度で実施される運動療法が血清CRP濃度の改善に有効である可能性が示唆された．

Lakkaほか，Okitaほかの検討では本研究と同様に体格指数などの他の指標と血清CRP濃度の関連が示されているが，運動耐容能が他の指標と独立して血清CRP濃度と関連するか否かについては検討されていない[14,11)]．Rawsonほかは血清CRP濃度がBMIとは相関するが，身体活動量とは相関しないと報告している[28)]．したがって，血清CRP濃度と運動耐容能とが他の因子と独立して関連するかどうかを明らかにする必要があると考えられ，それは血清CRP濃度の改善に運動トレーニングが減量効果と独立して有効である可能性に示唆を与えると考えられる．本研究ではステップワイズ回帰分析によりこの問題を検討したが，運動耐容能指標は他の指標と独立して血清CRP濃度と関連する傾向が認められた．その機序については今後の検討が必要である．

6．研究の問題点

血清CRP濃度は多くの因子に影響される．薬剤とくに多くの抗高脂血症薬(スタチンなど)，降圧薬，抗炎症薬(アスピリンなど)が血清CRP濃度を低下させることは良く知られている[29,30)]．このことを考慮して本研究では種類の如何にかかわらず服薬者を除外した．このため，高齢者に多い高血圧罹患者の多くが除外された．

今回の検討は横断的検討であり，運動と血清CRP濃度の関連は明らかにしえたが，運動の効果は明らかでない．今後，縦断的な検討を行って検討する予定である．

結　論

以上の成績から，中高齢者において，運動耐容能は冠動脈疾患の新たな危険因子とされる炎症指標血清CRP濃度の独立した関連因子であることが示唆された．したがって，定期的運動による運動耐容能の改善は肥満の是正とは独立してCRPの改善に寄与する可能性が推定された．

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文　献

1) Pepys, M. B., Baltz, M. L.：Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. Adv. Immunol. 34：141-212, 1983.

2) Devaraj, S. et al.：C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cell. Implications for the metabolic syndrome and atherothrombosis. Circulation 107：398-404, 2003.

3) Ridker, P. M. et al.：Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N. Engl. J. Med. 336：973-979, 1997.

4) Danesh, J. et al.：C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N. Engl. J. Med. 350：1387-1397, 2004.

5) Cushman, M. C. et al.：C-reactive protein and the 10-year incidence of coronary heart disease in older men and women. The Cardiovascular Health Study. Circulation 112：25-31, 2005.

6) Greenfield, J. R. et al.：Obesity is an important determinant of baseline serum C-reactive protein concentration in monozygotic twins, independent of genetic influences. Circulation 109：3022-3028, 2004.

7) Esposito, K. et al.：Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women. JAMA 289：1799-1804, 2003.

8) Mattusch, F. et al.：Reduction of the plasma concentration of C-reactive protein following nine months of endurance training. Int. J. Sports Med. 21：21-24, 2000.

9) Hammett, C. J. K. et al. : Effect of six month's exercise training on C-reactive protein levels in healthy elderly subjects. JACC 44 : 2411-2413, 2004.

10) Obisesan, T. O. et al. : C-reactive protein genotypes affect baseline, but not exercise training-induced changes, in C-reactive protein levels. Arterioscler. Thromb. Vasc. Biol. 24 : 1874-1879, 2004.

11) Okita, K. et al. : Can exercise training with weight loss lower serum C-reactive protein levels? Arterioscler Thromb. Vasc. Biol. 24 : 1868-1873, 2004.

12) Duncan, G. E. et al. : Effects of exercise on emerging and traditional cardiovascular risk factors. Preventive Medicine 39 : 894-902, 2004.

13) You, T. et al. : Effects of hypocaloric diet and exercise training on inflammation and adipocyte lipolysis in obese postmenopausal women. J. Clin. Endocrinol. Metab. 89 : 1739-1746, 2004.

14) Lakka, T. A. et al. : Effect of exercise training on plasma levels of C-reactive protein in healthy adults : the HERITAGE Family Study. European Heart J. 26 : 2018-2025, 2005.

15) LaMonte, M. J. et al. : Cardiorespiratory fitness and C-reactive protein among a tri-ethnic sample of women. Circulation 106 : 403-406, 2002.

16) Aronson, D. et al. : The association between cardiovascular fitness and C-reactive protein in subjects with the metabolic syndrome. JACC 44 : 2003-2007, 2004.

17) Kondo, N. et al. : Association of inflammatory marker and highly sensitive C-reactive protein with aerobic exercise capacity, maximum oxygen uptake and insulin resistance in healthy middle-aged volunteers. Circ. J. 69 : 452-457, 2005.

18) Kerner, A. K. et al. : Association between elevated liver enzymes and C-reactive protein. Possible hepatic contribution to systemic inflammation in the metabolic syndrome. Arterioscler. Thromb. Vasc. Biol. 25 : 193-197, 2005.

19) Tanaka, H. et al. : Double product response is accelerated above the blood lactate threshold. Med. Sci. Sports Exerc. 29 : 503-508, 1997.

20) 北畠　顕ほか：虚血性心疾患の一次予防ガイドライン. Jpn. Circ. J. 65(Suppl. 5) : 999-1076, 2001.

21) Takano, M. et al. : Angioscopic follow-up study of coronary ruptured plaques in nonculprit lesions. JACC 45 : 652-658, 2005.

22) Nakanishi, N. et al. : C-reactive protein concentration is more strongly related to metabolic syndrome in women than in men. The Minoh Study. Circ. J. 69 : 386-391, 2005.

23) Yamada, S. et al. : Distribution of serum C-reactive protein and its association with atherosclerotic risk factors in a Japanese population. Am. J. Epidemiol. 153 : 1183-1190, 2001.

24) Saito, I. et al. : Association of body mass index, body fat, and weight gain with inflammation markers among rural residents in Japan. Circ. J. 67 : 323-329, 2003.

25) Ichihara, Y. et al. : Higher C-reactive protein concentration and white blood cell count in subjects with more coronary risk factors and/or lower physical fitness among apparently healthy Japanese. Circ. J. 66 : 677-684, 2002.

26) Ohsawa, M. et al. : CRP levels are elevated in smokers but unrelated to the number of cigarettes and are decreased by long-term smoking cessation in male smokers. Preventive Medicine 41 : 651-656, 2005.

27) Jankord, R., Jemiolo, B. : Influence of physical activity on serum IL-6 and IL-10 levels in healthy older men. Med. Sci. Sports Exerc. 36 : 960-964, 2004.

28) Rawson, E. S. et al. : Body mass index, but not physical activity, is associated with C-reactive protein. Med. Sci. Sports Exerc. 35 : 1160-1166, 2003.

29) Armani, A., Becker, R. C. : The biology, utilization, and attenuation of C-reactive protein in cardiovascular disease : Part Ⅱ. Am. Heart J. 149 : 977-983, 2005.

30) Plenge, J. K. et al. : Simvastatin lowers C-reactive protein within 14 days. An effect independent of low-density lipoprotein cholesterol reduction. Circulation 106 : 1447-1452, 2002.

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原　著

Relation between serum high-sensitive CRP concentration and exercise tolerance in middle-aged and elderly subjects -SAT project 188-

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Key words : risk factors for arteriosclerosis. exercise. C-reactive protein

〔Abstract〕 The relation between high-sensitive C-reactive protein (CRP). a new coronary risk factor and exercise tolerance has not been elucidated. The aim of this study was to show the relation between the serum CRP concentration and peak oxygen uptake in 270 middle-aged and elderly subjects (67.4±6.6 years) with no cardiorespiratory diseases. Physical characteristics and blood chemistry indices were evaluated in the fasting state and peak oxygen uptake was estimated by a symptom-limited cardiopulmonary ergometer exercise test. Results : The mean serum CRP concentration was 0.637±0.715㎎/L. The serum CRP concentration was significantly related to age ($r=0.138$, $p=0.0238$). body weight ($r=0.183$, $p=0.0027$). body mass index ($r=0.345$, $p<0.0001$). serum insulin concentration ($r=0.187$, $p=0.0021$). HOMA-R ($r=0.171$, $p=0.0052$). serum HDL-cholesterol concentration ($r=-0.254$, $p<0.0001$). serum LDL-cholesterol ($r=0.138$, $p=0.0234$). and peak oxygen uptake ($r=-0.384$, $p=.0003$). In stepwise multivariate analysis. only the peak oxygen uptake and BMI were significantly correlated with serum CRP concentration. In conclusion. these data suggest that exercise tolerance was independently associated with serum CRP concentration in middle-aged and elderly subjects.

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ORIGINAL ARTICLE

Effect of regular exercise on homocysteine concentrations: the HERITAGE Family Study

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Abstract We investigated whether regular aerobic exercise could affect plasma total homocysteine (tHcy), and whether there were sex-related or racial differences in tHcy changes. Data were available for 816 black and white men and women, aged 17–65 years, 711 of whom completed a 20 week aerobic exercise training program. The tHcy concentration was measured in frozen plasma samples by an HPLC method. In Blacks, tHcy did not change with exercise training [men −0.5 (SD 3.7) μmol/l, women 0.0 (2.2) μmol/l) but increased significantly in Whites (men +0.3 (1.7) μmol/l, women +0.2 (1.6) μmol/l). No sex-related differences were found in either racial group. Changes in tHcy correlated negatively with baseline homocysteine ($r = -0.40$, $P < 0.0001$). Homocysteine levels of the "High" (hyperhomocysteinemia) (≥15 μmol/l) group ($n = 30$) decreased significantly with regular aerobic exercise from 23.1 (12.1) to 19.6 (7.6) μmol/l. Homocysteine levels of the "Normal" group increased slightly from 8.2 ± 2.2 to 8.5 ± 2.4 μmol/l. Men exhibit racial differences for tHcy responses to exercise training. Regular aerobic exercise has favorable effects on individuals with hyperhomocysteinemia, but tHcy slightly increased in individuals within the normal range.

Keywords Aerobic exercise training · Sex · Homocysteine · Hyperhomocysteinemia · Racial differences

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Introduction

Elevated plasma total homocysteine concentrations (tHcy) are thought to contribute to atherosclerosis and thrombosis in several ways: (a) endothelial cell injury and endothelial dysfunction, (b) increased vascular smooth muscle cell growth, (c) increased platelet adhesiveness, (d) enhanced low density lipoprotein oxidation and deposition in the arterial wall, and (e) direct activation of the coagulation cascade (Fonseca et al. 1999). Epidemiological studies have shown that elevated tHcy are independently associated with an increased risk of coronary artery disease (Langman et al. 2000; Ridker et al. 1999). Further, several observational studies have found that lowering tHcy was associated with reduced incidence of heart attack and strokes (Homocysteine Studies Collaboration 2002; Boushey et al. 1995; Schnyder et al. 2001).

Little information is available on the effect of exercise on tHcy. Although a few studies on endurance trained men (Konig et al. 2003) and untrained young women (De Cree et al. 1999) have shown that a single bout of intensive exercise acutely increased tHcy, long-term (6 months) regular exercise may be associated with a reduction in tHcy in young overweight and obese women (Randeva et al. 2002). These studies were, however, too small to provide conclusive evidence for an effect of regular exercise on tHcy.

An epidemiological study (Ganji and Kafai 2003) showed that both sex and race were important predictors of tHcy. The difference in tHcy between men and women was explained by alcohol consumption and blood concentrations of folate, vitamin B_{12} (cyanocobalamin), creatinine and cotinine. On the other hand, racial differences are thought to be partly accounted for variation in allele frequency at the methylenetetrahydrofolate reductase gene (Cappuccio et al. 2002). To our knowledge, no data have been reported on race or sex differences for the changes in tHcy in response to exercise training. Therefore, the purpose of this study was to investigate the effects of relatively long-term regular exercise on tHcy in a large sample of black and white men and women.

Methods

Subjects

The HERITAGE Family Study was designed to define the role of the genotype in cardiovascular, metabolic and hormonal responses to aerobic exercise training. The aim, design and measurement protocol of the HERITAGE Family Study has been previously described in details elsewhere (Bouchard et al. 1995).

The present study is based on baseline data from 816 sedentary subjects, who were recruited and studied at four clinical centers. They came from families that included parents (aged ≤65 years) and adult offspring (aged ≥17 years). They were required to be sedentary at baseline, with a body mass index (BMI) <40 kg/m^2 (a few cases with BMI ≥40 kg/m^2 were included with sufficient clinical justification), resting systolic blood pressure <160 mm Hg, resting diastolic blood pressure <100 mm Hg, plasma total cholesterol <350 mg/dl, and fasting plasma triglycerides <500 mg/dl. Subjects who had renal, hepatic or cardiac disease, were diabetic, or had hypothyroidism, or were being treated with lipid-lowering, hypertensive or hypoglycemic drugs were excluded. The study relies also on data from the response to a 20 week aerobic exercise training program in 730 subjects (90 black men, 159 black women including 18 post-menopausal and 46 who were taking hormones, 236 white men, and 245 white women including 45 post-menopausal and 105 who were taking hormones). The study protocol was approved by the Institutional Review Board at each clinical center. The aim and design of the study were explained to all subjects before they gave written informed consent.

Measures

Body weight, height, waist and hip circumferences were measured according to standardized procedures (Wilmore et al. 1997), and BMI was calculated as body weight (kg) divided by height (m^2). Body composition (fat mass and fat free mass) was estimated using the hydrostatic weighing technique. Details of the protocol of the hydrostatic weighing technique and body composition estimation are provided elsewhere (Wilmore et al. 1997).

Resting BP was measured using Colin STBP-780 automated units before 11 AM in the post-absorptive state (Rankinen et al. 2000). Subjects were asked to abstain from caffeine-containing or tobacco products for 2 h before measurements were made. Subjects rested for 5 min before the initial measurement. Blood samples were obtained after a 12 h fast. Cholesterol was determined in plasma by enzymatic methods (Despres et al. 2000).

The tHcy (the sum of homocysteine, homocystine, and homocysteine–cysteine mixed disulfides, free and protein bound) were measured in frozen plasma samples (−70°C) by an HPLC method (Durand et al. 1996). The intra- and inter-assay coefficients of variation for tHcy are 3.0% ($n = 12$) and 3.3% ($n = 50$), respectively. Plasma folate and vitamin B_{12} concentrations were determined by means of radioimmunoassay using a commercial kit (SimulTRAC-SNB_B_{12}/Folate, ICN Diagnostics, Orangeburg, NY). The intra- and inter-assay coefficients of variation are 6.3% ($n = 20$) and 10.3% ($n = 14$), respectively, for folate, and 4.9% ($n = 20$) and 4.3% ($n = 14$), respectively, for vitamin B_{12}. Plasma vitamin B_6 (pyridoxal phosphate) concentrations were also determined by means of a radioimmunoassay using a commercial kit (Vitamin B_6 (^{3}H) REA, American Laboratory Products Company, Windham, NH). The intra- and inter-assay coefficients of variation for vitamin B_6 are 4.6% ($n = 9$) and 10.2% ($n = 8$), respectively.

Progressive maximal exercise tests to exhaustion were conducted both before and after the exercise training program on a stationary cycle ergometer (Ergo-Metrics 800S, SensorMedics, Yorba Linda, CA) connected to a SensorMedics 2900 metabolic cart.

Exercise training protocols

Each subject was trained three sessions per week for 20 weeks on stationary cycle ergometers that were computer controlled to automatically maintain the participant's target heart rates corresponding to heart rates associated with fixed percentages of the baseline VO_{2max} test (Skinner et al. 2000). The intensity and duration of the training program was adjusted each 2 weeks. Training began at a heart rate corresponding to 55% of each subject's baseline VO_{2max} for 30 min per session and progressed to an intensity of 75% for 50 min during the last 6 weeks. All training sessions were supervised on site.

Subjects were asked not to change their eating patterns (meals and supplement use) during the intervention period.

Statistical analysis

General linear model analyses were used to test for differences between men and women as well as between the two ethnic groups. Paired *t*-tests were used to assess differences between variables before and after the exercise training program. Multiple regression analyses with the forward stepwise method were performed to estimate the independent contributions of age, sex, race, menopausal status, taking hormones, BMI, waist circumference, body composition, VO_{2max}, resting blood pressure, plasma levels of cholesterol, folate, vitamin B_6 and vitamin B_{12} to the variations in tHcy at baseline and changes in tHcy in response to aerobic exercise training. The relationship between two measurements was assessed by Pearson and Spearman rank correlation coefficients. Based on a position statement of the American Heart Association, we classified subjects into two groups: subjects with tHcy in the normal range (<15 μmol/l, "Normal" group) and subjects with elevated levels or hyperhomocysteinemia (≥15 μmol/l, "High" group) (Malinow et al. 1999). Probability values below 0.05 were regarded as significant. The data were analyzed with the Statistical Analysis System (SAS), version 9.1.

Results

Physical and biochemical characteristics of subjects at baseline are presented in Table 1 ($n = 816$). Detailed values of anthropometric and body composition measurements (Wilmore et al. 1997), resting blood pressure (Rankinen et al. 2000), and plasma lipids (Despres et al. 2000) were presented in previous reports. The tHcy levels were higher in men in both ethnic groups, and were higher in Blacks in both sexes.

For the whole sample ($n = 711$), the mean change in tHcy was only +0.1 μmol/l from baseline (8.8 ± 4.4 μmol/l) to post-exercise training (8.9 ± 3.6 μmol/l) ($P = 0.096$). Table 2 shows that tHcy did not change in Blacks but increased significantly in Whites. Vitamin B_6 remained unchanged, whereas vitamin B12 decreased significantly in all groups. Folate increased significantly in black men only. When the changes were compared between the two ethnic groups, we found a

Table 1 Comparison of baseline data between men and women, and between Blacks and Whites

	Blacks		Whites		Race difference	
	Women ($n = 191$)	Men ($n = 111$)	Women ($n = 260$)	Men ($n = 254$)	Women	Men
Age (years)	33.0 (11.4)	32.9 (12.3)	35.1 (14.1)	36.2 (14.9)	0.089	0.028
Height (m)	1.62 (6.6)	1.76 (6.7)[a]	1.64 (6.4)	1.78 (6.3)[a]	0.041	0.01
Weight (kg)	74.4 (17.9)	84.6 (18.6)[a]	67.2 (13.6)	84.4 (16.2)[a]	<0.0001	0.907
BMI (kg/m^2)	28.2 (6.5)	27.4 (5.6)	25.1 (5.0)	26.7 (4.9)[a]	<0.0001	0.218
Homocysteine (μmol/l)	8.5 (4.2)	11.0 (7.2)[a]	7.7 (2.7)	9.4 (3.7)[a]	0.01	0.004
Folate (nmol/l)	12.0 (8.3)	12.0 (8.5)	16.6 (15.0)	14.1 (10.3)	0.001	0.034
Vitamin B_6 (nmol/l)	40.6 (38.0)	64.9 (59.4)	56.2 (63.0)	60.9 (51.9)[a]	0.0001	0.763
Vitamin B_{12} (nmol/l)	332 (153)	328 (183)	286 (144)	289 (112)	0.0004	0.099
VO_{2max} (ml/min)	1,753 (365)	2,758 (490)[a]	1,912 (347)	3,026 (582)[a]	<0.0001	<0.0001

Data are expressed as mean (SD)

BMI body mass index, VO_{2max} maximal oxygen uptake

[a] $P < 0.001$ significantly different from women

Table 2 Comparison of changes in measurements between men and women, and between Blacks and Whites

	Blacks		Whites		Race difference	
	Women ($n = 151$)	Men ($n = 87$)	Women ($n = 243$)	Men ($n = 230$)	Women *P*-value	Men *P*-value
Weight (kg)	−0.4 (2.9)[a]	−0.8 (3.1)[a]	−0.1 (2.1)	−0.3 (2.1)[a]	0.224	0.252
BMI (kg/m^2)	−0.2 (1.1)	−0.2 (0.9)[a]	−0.0 (0.8)	−0.1 (0.7)[a]	0.279	0.376
Homocysteine (μmol/l)	+0.0 (2.3)	−0.5 (3.8)	+0.2 (1.6)[a]	+0.3 (1.7)[a]	0.407	0.04
Folate (nmol/l)	+0.9 (9.6)	+2.8 (7.9)[a]	−0.7 (9.0)	−0.3 (6.9)	0.111	0.003
Vitamin B_6 (nmol/l)	+3.3 (27.6)	+12.2 (76.2)	−1.5 (62.2)	+6.6 (64.7)	0.299	0.521
Vitamin B_{12} (nmol/l)	−17 (71)[a]	−14 (79)[a]	−23 (62)[a]	−18 (68)[a]	0.404	0.306
VO_{2max} (ml/min)	+336 (151)[a]	+411 (201)[a, b]	+349 (183)[a]	+450 (236)[a, b]	0.447	0.185

Data are expressed as mean (SD)

BMI body mass index, VO_{2max} = maximal oxygen uptake

[a] Training response (post–pre) $P < 0.05$

[b] $P < 0.01$ significantly different from women

significant difference in males for folate and homocysteine. No sex difference was found for any of the variables. After exercise training, the mean (SD) values of tHcy were 8.6 (3.4) μmol/l for black women, 10.8 (5.4) μmol/l for black men, 8.0 (2.4) μmol/l for white women, and 9.6 (3.4) μmol/l for white men. Sex and racial differences were identical to those at baseline.

We quantified the independent contributions of the variables considered here to the variance in tHcy. At baseline (Table 3), folate was the best predictor of tHcy in both sexes, and both races. Age, ethnicity and vitamin B_{12} were also significant predictors of tHcy. When exercise training induced changes in tHcy were used as the dependent variable, baseline homocysteine levels, and changes in folate were the strongest predictors in the whole sample as well as in the sex and race subgroups (Table 3). Although VO_{2max} was not selected as a significant predictor, increases in VO_{2max} tended to associate with decreases in tHcy ($P = 0.06$) in the whole sample.

Figure 1 illustrates the relationship between baseline tHcy and change in tHcy in response to exercise training. Changes in tHcy were negatively correlated with baseline tHcy (Pearson correlation coefficient = −0.40, $P < 0.0001$ and Spearman rank correlation coefficient = −0.25, $P < 0.0001$).

Figure 2 displays individual tHcy data for the High group ($n = 30$) at baseline and after training. Mean values of tHcy decreased from 23.1 (SD 12.1) μmol/l to 19.6 (7.6) μmol/l ($P = 0.01$). After training, 21 (70%) individuals (3 black women, 9 black men, 3 white women, and 6 white men) decreased their tHcy. Eight of the 21 individuals decreased their tHcy to less than 15 μmol/l. On the other hand, in the Normal group ($n = 681$), only 8 (1%) individuals (2 black women, 3 black men, 1 white women, and 2 white men) increased their tHcy to more than 15 μmol/l. The Normal group had a statistically significant increase in tHcy from 8.2 (2.2) μmol/l to 8.5 (2.4) μmol/l ($P < 0.0001$).

Figure 3 compares tHcy changes between the Normal and High groups. A significant ($P < 0.0001$) difference was observed between the Normal [+0.3 (1.5) μmol/l] and High [−3.5 (7.0) μmol/l] groups for the tHcy response to exercise training. The difference was still found after adjustment for age, sex, race and baseline tHcy ($P = 0.0028$). Folate levels increased significantly (+1.1 ± 3.0 nmol/l, $P < 0.05$) during the exercise training in the High group but remained unchanged in the Normal group. Vitamin B_{12} levels did not change in the High group but decreased significantly (−16.5 ± 97.8 pmol/l, $P < 0.0001$) in the Normal group.

Discussion

We found that the 20 week aerobic exercise training program reduced significantly tHcy (−3.5 μmol/l, $P = 0.01$) in those with elevated tHcy at baseline. Moreover, a significant difference in tHcy changes remained between the Normal and High groups even after adjustment for age, sex, race and baseline tHcy ($P = 0.0028$). Boushey et al. (1995) have reported that about 10% of the population's coronary artery disease risk was attributable to tHcy, and Ueland et al. (2000) found that an increase of 5 μmol/l in tHcy could be associated with a 20% increased risk of cardiovascular disease. Schnyder et al. (2001) found that lowering tHcy from 11.1 to 7.2 μmol/l reduced significantly the rate of coronary restenosis after angioplasty and decreased the incidence of major adverse cardiac events. In a more recent study, lowering tHcy by 25% (3 μmol/l) was associated with an 11% lower ischemic heart disease and 19% lower stroke risk (Homocysteine

Table 3 Results of multiple regression analysis

Independent variable	Homocysteine at baseline					Independent variable	Homocysteine training response				
	Beta	F	P	Partial R^2	Model R^2		Beta	F	P	Partial R^2	Model R^2
[All]											
Folate	−0.18	118.1	<0.0001	14.2	14.2	B_homocysteine	−5.36	72.8	<0.0001	10.5	10.5
Sex[a]	−0.1	77.4	<0.0001	8.4	22.6	ΔFolate	−0.04	21.5	<0.0001	3.0	13.5
Age	0.002	73.8	<0.0001	7.3	29.9	Age	0.02	7.9	0.005	1.1	14.6
B_{12}	−0.17	44.9	<0.0001	4.2	34.1	B_folate	−0.78	8.9	0.003	1.2	15.8
Race[b]	−0.04	18.6	<0.0001	1.7	35.8	B_VO_{2max}	0.0004	11.4	0.0008	1.5	17.3
						ΔB12	−0.002	4.5	0.036	0.6	17.9
						B_B6	−0.004	4.4	0.036	0.6	18.5
[Male]											
Folate	−0.23	65.6	<0.0001	16.7	16.7	B_homocysteine	−5.9	44.4	<0.0001	13.4	13.4
Age	0.002	48.3	<0.0001	10.8	27.5	ΔFolate	−0.05	15.3	0.0001	4.4	17.8
B_{12}	−0.16	14.6	0.0002	3.1	30.6	ΔB12	−0.004	5.7	0.018	1.6	19.4
Race	−0.05	13.4	0.0003	2.8	33.4	Age	0.03	4.1	0.044	1.2	20.6
Fat%	0.002	8.6	0.004	1.7	35.1	B_VO_{2max}	0.0006	5.9	0.015	1.6	22.2
						B_folate	−0.9	5.1	0.025	1.4	23.6
[Female]											
Folate	−0.15	60.3	<0.0001	13.6	13.6	B_homocysteine	−4.61	32.2	<0.0001	8.9	8.9
B_{12}	−0.18	35.9	<0.0001	7.4	21.0	ΔFolate	−0.03	8.9	0.003	2.4	11.3
Age	0.002	22.1	<0.0001	4.3	25.3	B_B6	−0.84	6.2	0.013	1.6	12.9
Race	−0.03	6.8	0.009	1.3	26.6	ΔB6	−0.003	4.9	0.028	1.3	14.2
						Age	0.02	5.3	0.021	1.4	15.6
[Blacks]											
Folate	−0.26	50.9	<0.0001	17.4	17.4	B_homocysteine	−8.0	61.0	<0.0001	25.0	25.0
Sex	−0.17	34.3	0.0001	10.3	27.7	ΔFolate	−0.05	8.4	0.004	3.3	28.3
Age	0.002	21.9	0.0027	6.0	33.7	ΔB6	−0.006	5.4	0.021	2.1	30.4
B_{12}	−0.17	14.6	0.0096	3.8	37.5						
Cholesterol	0.02	4.2	0.041	1.1	38.6						
[Whites]											
Folate	−0.16	66.2	<0.0001	12.4	12.4	B_homocysteine	−0.03	42.0	<0.0001	8.8	8.8
Age	0.002	61.3	<0.0001	10.2	22.6	ΔFolate		10.2	0.0015	2.1	10.9
Sex	−0.07	45.0	<0.0001	6.8	29.4	Sex		9.2	0.0026	1.8	12.7
B_{12}	−0.17	42.2	<0.0001	5.9	35.3	B_B6		9.8	0.0019	1.9	14.6
Resting DBP	0.002	7.0	0.008	1.0	36.2	B_folate		6.8	0.009	1.3	16.0
						Age		12.1	0.0006	2.3	18.3
						ΔB6		4.4	0.036	0.8	19.1

[a] 1 = men, 2 = women

[b] 1 = blacks, 2 = whites

Studies Collaboration 2002). Therefore, our observation that tHcy can be lowered by regular exercise in those with high levels is coherent with a number of studies indicating that it may reduce significantly the risk of events in individuals with hyperhomocysteinemia.

Sex difference

The tHcy concentrations are known to be higher in men than in women (Ganji and Kafai 2003; Carmel et al. 1999; Fukagawa et al. 2000; Lussier-Cacan et al. 1996; Morris et al. 2000; Nygard et al. 1995). Ganji et al. (2003) and Carmel et al. (1999) found that the sex-related difference could be explained by alcohol consumption, and concentrations of plasma folate, vitamin B_{12}, creatinine and cotinine. Folic acid and vitamin B_{12} are involved as co-factors in metabolic pathways catalyzed by the enzymes 16,2methylenetetrahydrofolate reductase and methionine synthase, respectively, whereas vitamin B_6 is a cofactor for cystathionine beta synthase (Kang et al. 1992). A number of studies have shown inverse relationships of tHcy with plasma/serum levels of folate, vitamin B_6 and vitamin B_{12} (Robinson et al. 1998; Selhub et al. 1993). Moreover, differences in rates of homocysteine remethylation (Fukagawa et al. 2000) and estrogen concentrations (Morris et al. 2000) may also contribute to the homocysteine sex dimorphism. Remethylation is one of the major pathways for homocysteine metabolism. In remethylation, homocysteine is salvaged by acquisition of a methyl group from N^5-methyl-tetrahydrofolate in a vitamin B_{12} dependent pathway or from betaine in a pathway occurring primarily in the liver (McKeever et al. 1991). Fukagawa et al. (2000) and

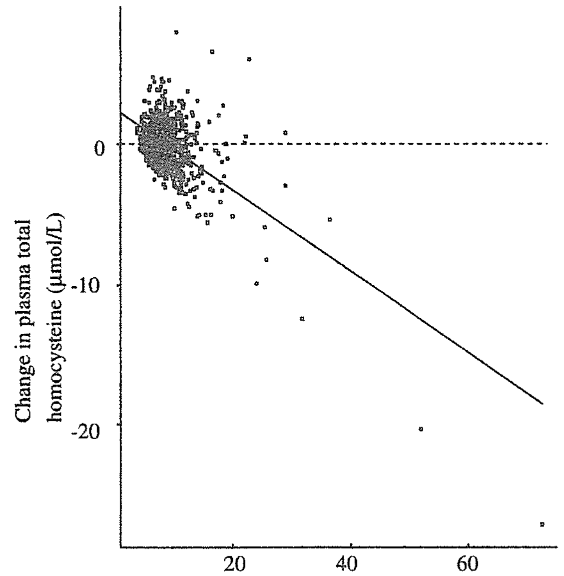

Fig. 1 Association between baseline homocysteine and regular exercise-induced changes in homocysteine. Pearson correlation coefficient = −0.40 ($P < 0.0001$), Spearman correlation coefficient = −0.25 ($P < 0.0001$)

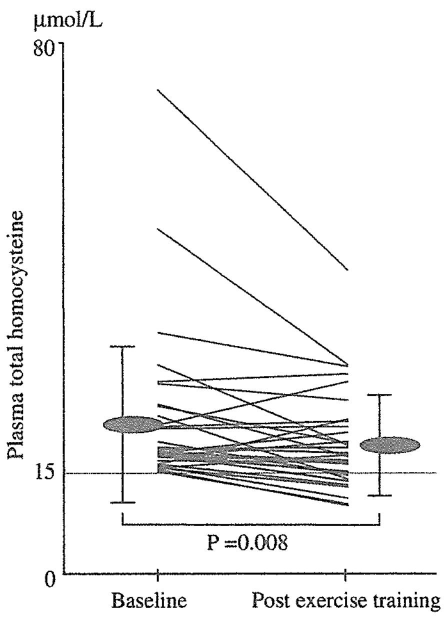

Fig. 2 Plasma total homocysteine levels in the High group at baseline and post-exercise training. *Vertical lines* indicate means (*filled circle*) and SD

McKeever et al. (1991) found that the remethylation rate was significantly higher in women than in men. They concluded that the sex-related difference is partially explained by homocysteine remethylation rates. Morris et al. (2000) found that higher estrogen concentrations

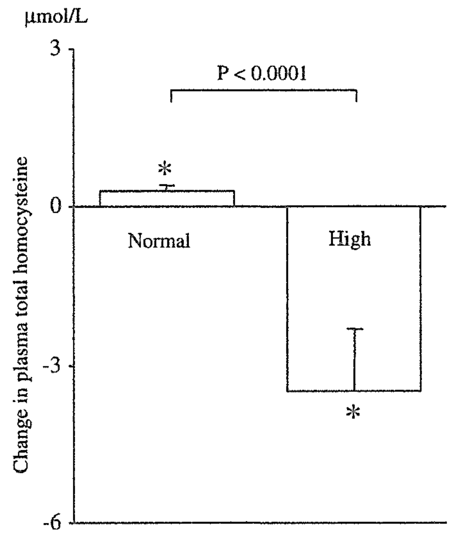

Fig. 3 Comparison of changes in plasma homocysteine levels between the "Normal" and "High" groups. Data are expressed as means ± SEM. $*P < 0.01$, within group difference (pre vs post)

are associated with lower tHcy, independent of nutritional status and muscle mass.

In the Hordaland Homocysteine Study (Nygard et al. 1995), tHcy levels were 19% higher in men (10.8 μmol/l) than in women (9.1 μmol/l) in middle age participants but only 11% (men 12.3 μmol/l and women 11.0 μmol/l) in elderly subjects. Although our data also indicated that baseline tHcy was higher in men than in women, there was no difference in exercise-induced changes in tHcy between men and women.

Racial differences

Several studies (Carmel et al. 1999; Ganji and Kafai 2003; Morris et al. 2000) have reported that Whites have 7–8% higher tHcy than Blacks. For instance, Ganji and Kafai (2003) found that tHcy was 8% higher in Whites (10.4 μmol/l) than in Blacks (9.6 μmol/l). Carmel et al. (1999) speculated that the higher tHcy in Whites might be explained by their lower vitamin B_{12} status compared with Blacks. On the other hand, the third National Health and Nutrition Survey in the United States (Jacques et al. 1999) found no racial difference in tHcy.

The present study indicates that baseline homocysteine levels were higher in Blacks than in Whites, and differences were also found for the training changes in homocysteine levels between Black and White men. Several investigators have reported that folate and vitamin B_{12} were predictors of blood homocysteine concentrations (Koehler et al. 2001; Morris et al. 2000).

Using multiple regression analyses, folate was found to be the strongest predictor of homocysteine in the present study. Our results also showed that folate levels were higher in Whites than in Blacks although vitamin B_{12} levels were higher in Blacks.

The present study has some limitations. First, since the primary goal of the HERITAGE Family Study is to study the role of genotype in the responsiveness to regular exercise, a control group was not deemed necessary. Second, subjects were asked not to change their eating patterns during the 20 week intervention period, but we have no direct assessments of folic acid, vitamin B_6 and B_{12} intakes over the duration of the exercise protocol.

In summary, we have examined the effects of exercise training on tHcy concentrations in both sexes and in Blacks and Whites. Baseline tHcy is higher in men than in women, consistent with previous studies. However, no difference between men and women was found for the changes in tHcy levels as a result of regular exercise. Baseline tHcy was higher in Blacks than in Whites, and in men, differences were also found for the changes in tHcy between the two ethnic groups. Twenty weeks of regular exercise reduced tHcy in individuals with baseline hyperhomocysteinemia, although tHcy slightly increased in individuals who were in the normal range of tHcy at baseline.

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References

Bouchard C, Leon AS, Rao DC, Skinner JS, Wilmore JH, Gagnon J (1995) The HERITAGE family study. Aims, design, and measurement protocol. Med Sci Sports Exerc 27:721–729

Boushey CJ, Beresford SA, Omenn GS, Motulsky AG (1995) A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 274:1049–1057

Cappuccio FP, Bell R, Perry IJ, Gilg J, Ueland PM, Refsum H, Sagnella GA, Jeffery S, Cook DG (2002) Homocysteine levels in men and women of different ethnic and cultural background living in England. Atherosclerosis 164:95–102

Carmel R, Green R, Jacobsen DW, Rasmussen K, Florea M, Azen C (1999) Serum cobalamin, homocysteine, and methylmalonic acid concentrations in a multiethnic elderly population: ethnic and sex differences in cobalamin and metabolite abnormalities. Am J Clin Nutr 70:904–910

De Cree C, Malinow MR, van Kranenburg GP, Geurten PG, Longford NT, Keizer HA (1999) Influence of exercise and menstrual cycle phase on plasma homocyst(e)ine levels in young women—a prospective study. Scand J Med Sci Sports 9:272–278

Despres JP, Couillard C, Gagnon J, Bergeron J, Leon AS, Rao DC, Skinner JS, Wilmore JH, Bouchard C (2000) Race, visceral adipose tissue, plasma lipids, and lipoprotein lipase activity in men and women: the health, risk factors, exercise training, and genetics (HERITAGE) family study. Arterioscler Thromb Vasc Biol 20:1932–1938

Durand P, Fortin LJ, Lussier-Cacan S, Davignon J, Blache D (1996) Hyperhomocysteinemia induced by folic acid deficiency and methionine load–applications of a modified HPLC method. Clin Chim Acta 252:83–93

Fonseca V, Guba SC, Fink LM (1999) Hyperhomocysteinemia and the endocrine system: implications for atherosclerosis and thrombosis. Endocr Rev 20:738–759

Fukagawa NK, Martin JM, Wurthmann A, Prue AH, Ebenstein D, O'Rourke B (2000) Sex-related differences in methionine metabolism and plasma homocysteine concentrations. Am J Clin Nutr 72:22–29

Ganji V, Kafai MR (2003) Demographic, health, lifestyle, and blood vitamin determinants of serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr 77:826–833

Homocysteine Studies Collaboration (2002) Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. JAMA 288:2015–2022

Jacques PF, Rosenberg IH, Rogers G, Selhub J, Bowman BA, Gunter EW, Wright JD, Johnson CL (1999) Serum total homocysteine concentrations in adolescent and adult Americans: results from the third National Health and Nutrition Examination Survey. Am J Clin Nutr 69:482–489

Kang SS, Wong PW, Malinow MR (1992) Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. Annu Rev Nutr 12:279–298

Koehler KM, Baumgartner RN, Garry PJ, Allen RH, Stabler SP, Rimm EB (2001) Association of folate intake and serum homocysteine in elderly persons according to vitamin supplementation and alcohol use. Am J Clin Nutr 73:628–637

Konig D, Bisse E, Deibert P, Muller HM, Wieland H, Berg A (2003) Influence of training volume and acute physical exercise on the homocysteine levels in endurance-trained men: interactions with plasma folate and vitamin B12. Ann Nutr Metab 47:114–118

Langman LJ, Ray JG, Evrovski J, Yeo E, Cole DE (2000) Hyperhomocyst(e)inemia and the increased risk of venous thromboembolism: more evidence from a case-control study. Arch Intern Med 160:961–964

Lussier-Cacan S, Xhignesse M, Piolot A, Selhub J, Davignon J, Genest J Jr (1996) Plasma total homocysteine in healthy subjects: sex-specific relation with biological traits. Am J Clin Nutr 64:587–593

Malinow MR, Bostom AG, Krauss RM (1999) Homocyst(e)ine, diet, and cardiovascular diseases: a statement for healthcare professionals from the Nutrition Committee, American Heart Association. Circulation 99:178–182

McKeever MP, Weir DG, Molloy A, Scott JM (1991) Betaine-homocysteine methyltransferase: organ distribution in man, pig and rat and subcellular distribution in the rat. Clin Sci (Lond) 81:551–556

Morris MS, Jacques PF, Selhub J, Rosenberg IH (2000) Total homocysteine and estrogen status indicators in the Third National Health and Nutrition Examination Survey. Am J Epidemiol 152:140–148

Nygard O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, Ueland M, Kvale G (1995) Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. JAMA 274:1526–1533

Randeva HS, Lewandowski KC, Drzewoski J, Brooke-Wavell K, O'Callaghan C, Czupryniak L, Hillhouse EW, Prelevic GM (2002) Exercise decreases plasma total homocysteine in overweight young women with polycystic ovary syndrome. J Clin Endocrinol Metab 87:4496–4501

Rankinen T, Rice T, Perusse L, Chagnon YC, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bouchard C (2000) NOS3 Glu298Asp genotype and blood pressure response to endurance training: the HERITAGE family study. Hypertension 36:885–889

Ridker PM, Manson JE, Buring JE, Shih J, Matias M, Hennekens CH (1999) Homocysteine and risk of cardiovascular disease among postmenopausal women. JAMA 281:1817–1821

Robinson K, Arheart K, Refsum H, Brattstrom L, Boers G, Ueland P, Rubba P, Palma-Reis R, Meleady R, Daly L, Witteman J, Graham I (1998) Low circulating folate and vitamin B6 concentrations: risk factors for stroke, peripheral vascular disease, and coronary artery disease. European COMAC Group. Circulation 97:437–443

Schnyder G, Roffi M, Pin R, Flammer Y, Lange H, Eberli FR, Meier B, Turi ZG, Hess OM (2001) Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. N Engl J Med 345:1593–1600

Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH (1993) Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 270:2693–2698

Skinner JS, Wilmore KM, Krasnoff JB, Jaskolski A, Jaskolska A, Gagnon J, Province MA, Leon AS, Rao DC, Wilmore JH, Bouchard C (2000) Adaptation to a standardized training program and changes in fitness in a large, heterogeneous population: the HERITAGE Family Study. Med Sci Sports Exerc 32:157–161

Ueland PM, Refsum H, Beresford SA, Vollset SE (2000) The controversy over homocysteine and cardiovascular risk. Am J Clin Nutr 72:324–332

Wilmore JH, Stanforth PR, Domenick MA, Gagnon J, Daw EW, Leon AS, Rao DC, Skinner JS, Bouchard C (1997) Reproducibility of anthropometric and body composition measurements: the HERITAGE Family Study. Int J Obes Relat Metab Disord 21:297–303

A novel exercise for improving lower-extremity functional fitness in the elderly

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A novel exercise for improving lower-extremity functional fitness in the elderly

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ABSTRACT. ***Background and aims:*** *Many falls in the elderly are caused by tripping. After tripping, a certain level of lower-extremity functional fitness is necessary, in order to make protective responses and to avoid falling. The purpose of this study was to test whether our new exercise program (a square-stepping exercise: SSE) would improve lower-extremity functional fitness in the elderly.* ***Methods:*** *Fifty-two individuals aged 60-80 years were divided into two groups (non-randomized control design); SSE (n=26) and controls (n=26). Lower-extremity functional fitness was defined as standing up from a lying position (agility), chair-stand in ten seconds (leg power), walking round two cones (locomotion speed), sit-and-reach (flexibility) and single-leg balance with eyes closed (balance). The SSE group participated in a six-month regimen of SSE once a week. SSE was performed on a thin mat of 250 cm by 100 cm, partitioned into 40 small squares (25 cm each side). SSE included not only forward steps but also backward, lateral and oblique steps, and step patterns were progressively made more complicated. Controls maintained their usual lifestyles.* ***Results:*** *In the SSE group, significant improvements were observed in agility, leg power, locomotion speed, flexibility and balance. No significant changes were detected in any tests in the control group.* ***Conclusions:*** *The SSE program improved lower-extremity functional fitness, lack of which constitutes a risk factor for falls in the elderly. This program should be tested further to determine if it can effectively reduce the incidence of falls in the elderly.*
(Aging Clin Exp Res 2006; 18: 242-248)

INTRODUCTION

In Japan, a national survey found the incidence (per 10,000 per year) of hip fracture in 1997 was 17.3 (men) and 40.8 (women) in persons 60-69 years old, and 57.4 (men) and 147.8 (women) in those 70-79 years old (1). Falls are the most common cause of hip fractures, leading to decreased mobility, restriction to bed, and/or death; in addition, not only hip fractures but also minor injuries such as joint dislocation, lacerations and bruises are also caused by falls (2). As well as fall-associated injuries, falls may lead to an increased fear of falling which can restrict social activities, thereby decreasing independence and overall quality of life.

An interview-based community survey (3) reported that 35% of older residents had suffered one or more falls in the preceding year, and that 53% of the falls had been caused by tripping. Other causes, much less common, were dizziness (7.8%), black-out (6.4%) or accident (5.2%). For many elderly people, impact velocities at the ground after tripping were within one standard deviation of the estimate of the mean impact velocity needed to fracture a femur (4). Falling sideways, which is often observed after slipping, is also associated with the incidence of hip fractures (4, 5); the impact velocity after slipping also reaches the estimated femur fracture velocity (4). Therefore, the prevention of falls caused by elderly persons tripping or slipping is one of the most important areas for maintaining independence and quality of life in this age group.

Effective interventions for preventing falls should include quick stepping in any direction and the generation of enough force at push off to support the whole body following a trip or slip (6). Quickness (reaction speed/agility) and power in the lower extremity, which have been implicated as factors influencing falls, decrease with age (2, 7-10). In most elderly people, the ability to respond quickly and forcefully may be impaired, leading directly to falling. To the best of our knowledge, there are few studies concerning the development of an intervention program to improve speed and power in the lower extremities of the elderly. This study aimed at developing a novel exercise program for improving lower-extremity

Key words: Fall, risk, slip, square-stepping exercise, trip.
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functional fitness in the elderly, and to assess its effects on fall-related risk factors.

METHODS

Study design

An age- and gender-matched, six-month intervention and non-randomized control trial was conducted in Gifu, the capital city of Gifu Prefecture, located in the centre of Japan.

Participants

Participants in the exercise group were recruited in two ways. One was by direct mailing to pensioners who had an account in the Juroku Bank (Gifu, Japan). Approximately 38,000 letters were sent to people who lived in the area around the building in which the exercise program was conducted. Another procedure was to use leaflets, which were placed in the main office and 60 branch offices of Juroku Bank in Gifu Prefecture. The mailer or leaflet, besides our program, also contained information on several free-of-charge culture classes for the elderly, such as Chinese conversation and "shogi" (Japanese chess) classes. Persons receiving the mailer or leaflet could attend and choose any class from the list, including our program.

Twenty-six people aged 60-80 years agreed to participate in the study (6 men, 20 women). To lower the risks of accident by exercise, five consecutive low-intensity exercise sessions (60 minutes each) over a period of 10 weeks were given to participants before the study. These exercise sessions mainly consisted of stretching the lower extremities while sitting or lying on a mat, and calisthenics for the upper extremities while in a standing position. Participants then engaged in the pre-test and square stepping exercise (SSE) program (see below) for six months without interruption, but were not encouraged to engage in any other new forms of exercise. Once an exercise group was identified, age- (±3 years) and gender-matched controls were selected at random from among cohort members of another surveillance program, in which elderly individuals aged 60 or more living in the city attended as participants of another research program focused on aging and lifestyle. Controls were asked to maintain their lifestyles for six months and to refrain from engaging in the SSE program or any other new form of exercise. Individuals who were living in care facilities, who were regularly exercising twice or more weekly, had a history of illness or a condition that would affect balance (e.g., stroke or Parkinson's disease), or were unable to participate safely in the exercise program, were excluded.

The Juroku Bank and the University of Nagoya cooperated in the Revitalization of Physical and Social Activities of the Elderly Project and approved the study. All participants gave their informed consent before participating.

Outcome measures

Outcome measures were changes in functional fitness related to the risks associated with falling. Fallers are more likely to report difficulty rising from a bed or chair, and to have some form of gait abnormality (11). Lower limb weakness and poor tandem-walking ability have emerged as significant predictive factors among multiple clinical and functional falling risk factors (12). Gehlsen et al. (13) reported that functional fitness in those with a history of falls was lower than in those with no history of falls. In that study, functional fitness items included static balance (one-leg balance test), dynamic balance (backward-walking test), hip, knee and ankle joint strength, and hip and ankle flexibility tests.

From these studies, we assumed that agility, leg power, locomotion speed, flexibility and balance would be elements of functional fitness related to the risk of falls. Further, in the present study, five functional fitness tests were selected to match each element: standing up from a lying position (agility), chair-stand in 10 seconds (leg power), walking round two cones (locomotion speed), sit-and-reach (flexibility), and single-leg balance with eyes closed (balance). Details of the measurements of these fitness items have been reported elsewhere (14, 15). In standing up from a lying position on a signal, participants were asked to stand up as quickly as they could, to a stable erect position from a lying position. The process of moving to the standing position depended on each participant's customary method. Performance time was assessed in terms of time needed to reach a stable erect position. In the item of chair-stand in ten seconds, participants were asked to repeat the exercise of fully standing up and then fully sitting on a chair as many times as possible within ten seconds. During the measurement, participants crossed their arms at the wrists and held them to their chests. Walking round two cones was measured as follows: two cones were placed 1.8 m on both sides of and 1.5 m behind the chair. Participants rose from the chair, walked to the right going inside and round the back of the cone, returned to a fully seated position on the chair, stood up and walked round the left cone, and returned to a fully seated position. One trial consisted of two complete circuits (total distance about 16.8 m). Performance time was recorded in units of 0.1 seconds. In the test of sit-and-reach, participants were asked to sit on the floor and push the plastic cursor forward slowly as far as possible with their middle fingers and without bending their knees. Performance was recorded as the maximum distance between toes and middle fingers; the farther forward the fingers, the greater the performance distance. The reference position, 0 cm, was at the level of the toes. Participants performed twice and the better (further) score indicated by the cursor was noted.

Descriptive variables, such as age, height, weight and attendance rate in the exercise program, were recorded.

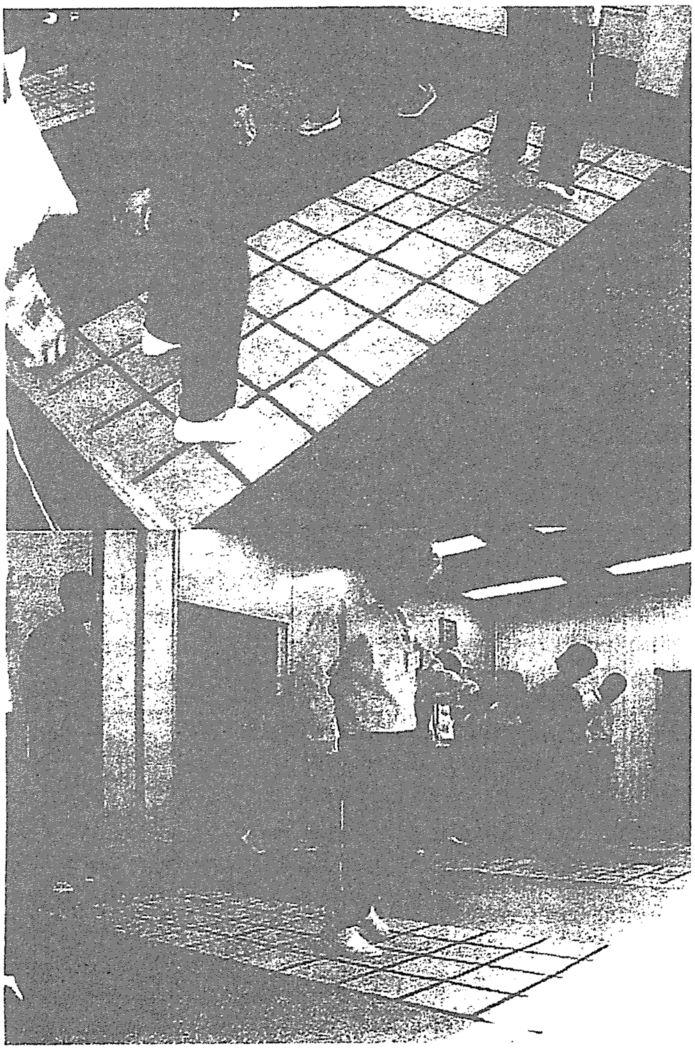

Fig. 1 - *Square stepping exercise.*

Exercise intervention

Based on the mechanism and direction of falls, and on ladder training drills designed for athletes, we developed our SSE program and used it for the intervention described here. SSE was performed on a thin mat of 250 cm by 100 cm, partitioned into 40 small squares (25 cm each side). A smaller mat, 200 x 80 cm, also partitioned with smaller squares (20 cm each side), was prepared for shorter participants (i.e., those less than 150 cm in height). Participants were required to step in the length direction (250 or 200 cm), basically without treading on the lines making up the squares. The SSE program included forward, backward, lateral and oblique steps, and step patterns were progressively made more complicated (Fig. 1).

One hundred and nineteen step patterns were developed and categorized into Junior (8 patterns), Basic (19), Semi-Regular (17), Regular (26), Senior (35) and Master (14) patterns, according to difficulty. Examples of Junior, Basic, Regular and Master patterns are shown in Fig. 2. Junior patterns consisted of easy steps, i.e., forward steps only. For Basic patterns, lateral steps were added to the Junior patterns. Further, oblique and backward steps were added in the Semi-Regular patterns; somewhat complicated oblique and backward steps were added in the Regular patterns; complicated lateral, oblique and backward steps were added in the Senior patterns; and much more complicated steps were added in the Master patterns.

There were some options, so that step patterns in each category were more complicated. Many patterns required a wide stance over one or two squares. Some patterns had bilateral symmetry, which led participants to grasp the pattern image easily. Each pattern consisted of two to 16 steps; participants were asked to repeat the step pattern until they got to the end of the mat. Once a pattern was performed, a mirror-imaged pattern was then also performed.

The exercise group participated in a six-month regimen of SSE once a week. Each SSE class consisted of 10-min warm-up activities, such as walking and mild stretching, 30-min SSE, 10-min whole-body light resistance training, and 10-min cool-down. To familiarize participants with SSE, only Junior patterns were used for the first month. From the second month, Junior patterns were performed as the warm-up. Participants were encouraged to try some new patterns in the Basic or more complicated categories. Essentially, a one-step pattern was repeated 3-5 times and then the mirror-image pattern was repeated the same number of times. If participants could not step a pattern smoothly, they repeated it until it was accomplished, and then they progressed to a new step pattern. The Basic and more complicated category patterns which included lateral, oblique and backward steps were carefully introduced, because many participants needed a long time to learn each new pattern. During the SSE program, step patterns drawn on leaflets or boards were not provided. Participants were asked to learn the pattern by watching an instructor's movements.

Participants were also asked to go forward on the mat one-way, to pass back by the mat walking normally, and then to stand in a line for the next stepping exercise: this meant that the number of steps did not differ among participants. Raising the heels was a recommended option: this made it more difficult for participants to step the patterns and ensured improvements in agility, locomotion speed and balance. Although step cadence was not determined, participants stepped slowly at first and then were able to step faster after some practice. For example, in the Basic pattern shown in Fig. 2, participants stepped at approximately 1 step/sec. They needed some time to remember where they should place their feet during the steps, and so took 20 to 30 seconds in one direction. After some practice, they could step out the patterns at approximately 2 steps/s: and took 20 or fewer seconds to complete.

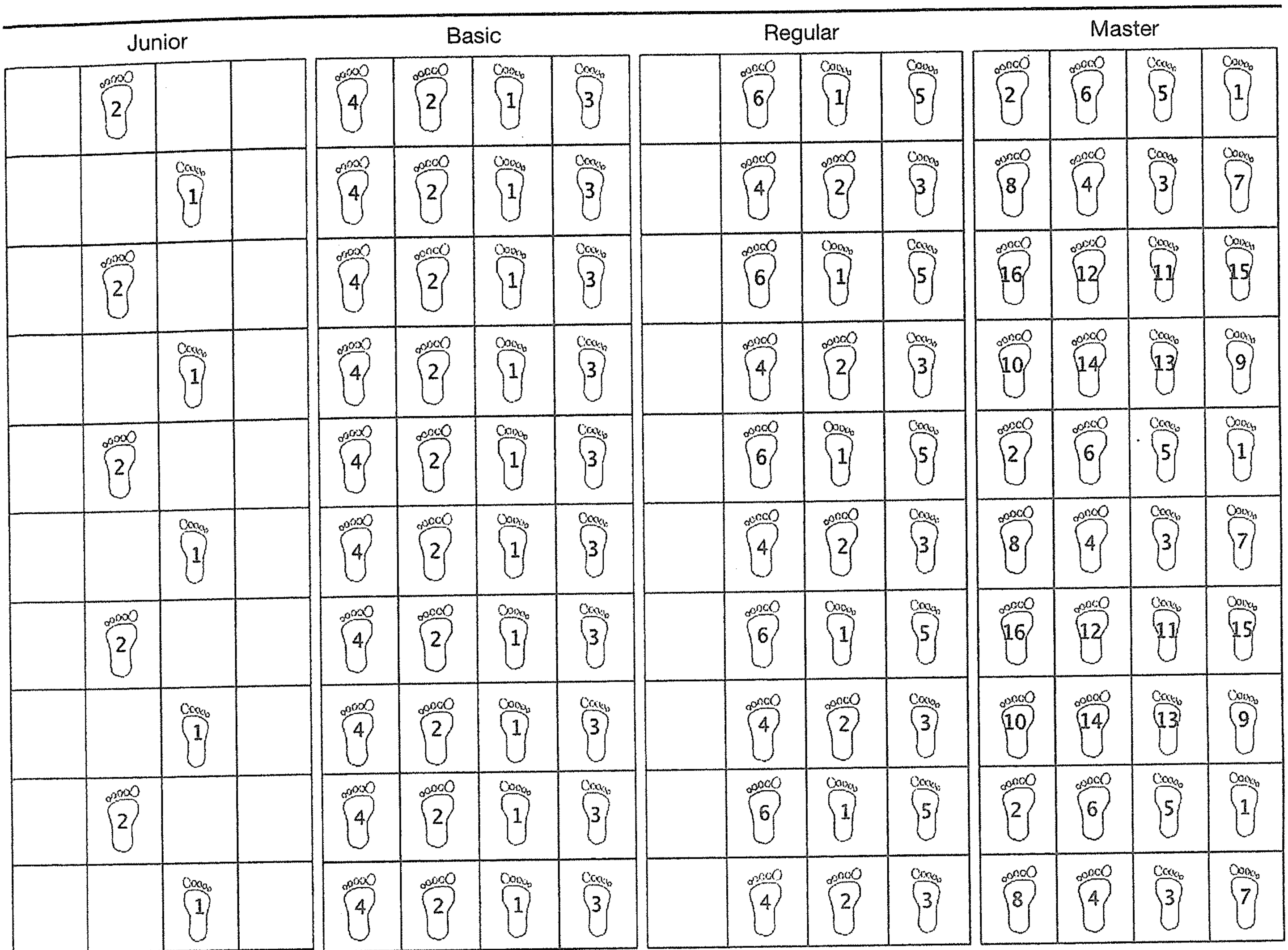

Fig. 2 - *Examples of Junior, Basic, Regular and Master patterns of square stepping exercise. Steps were performed according to numbers, from lower end of each figure to upper end. Odd and even numbers indicate location of right and left feet, respectively.*

In each SSE class, participants gathered in a room in a Juroku Bank branch building; no transportation was provided. The floor of the venue in which classes was held was fitted with a thin carpet. Two instructors concurrently taught all SSE participants.

Statistical analysis

Data are presented as means ±SD. The overall design employed a 2 (group) by 2 (time) repeated-measures format. Repeated-measures techniques were conducted to determine time-by-group interactions. Before this, the Kolmogorov-Smirnov test was applied to determine if data on each test significantly differed from normal distribution. The Levene test was then used to verify the assumption that variances were equal across groups. When significances in these tests were found, the Wilcoxon signed-rank test for paired data was used. Two kinds of correlation coefficients in the SSE group were also calculated: the first between baseline data and the pre-post change for each test; the second between the change in one test and the change in another test. These coefficients were calculated by: (i) Pearson's correlation analysis, when data were similar to normal distribution, and (ii) Spearman's correlation analysis when data were significantly different from normal distribution. All statistics were computed using SPSS version 11.5.1 J. The level of significance was set at $\alpha = 0.05$.

RESULTS

The baseline characteristics of the two groups were very similar (Table 1). Data of the SSE group in agility, leg power, locomotion speed, flexibility and balance measures were comparable with those of the control group at the beginning of the study (Table 2). Average attendance in the SSE program was 92% (±7.9, range= 75-100%). In the exercise group, no participants discontinued involvement or claimed knee or back pains that had not already existed at the start of the study. No falls or injuries

Table 1 - *Baseline characteristics of participants.*

Measure	Control (n=26)	Exercise (n=26)
Age (yrs)	68.3±6.3	67.5±4.9
Height (cm)	151.4±6.3	148.5±4.3
Weight (kg)	52.5±9.3	53.2±7.0

resulted directly from the exercises. Of all the participants in the exercise group (n=26), one achieved only the Basic pattern, ranked as the second easiest category, by the time the six-month intervention was over. He could step Basic patterns easily and safely, but had difficulty in stepping the more complicated ones. Two other participants achieved Semi-regular patterns. Of the remaining participants, 14, 4 and 5 could step Regular, Senior and Master patterns, respectively, at the end of the six-month regimen. Compliance among controls was 100%, i.e., no controls engaged in any new form of exercise, they all maintained their lifestyles, and attended pre- and post-measurement sessions.

A repeated-measure ANOVA revealed a significant group-by-time interaction for standing up from a lying position ($p<0.01$). Univariate follow-up analysis revealed that the significant agility increases were attributable to the SSE program (Table 2). The exercise group experienced a decrease of 0.39 seconds in this measurement. Walking round two cones and sit-and-reach had significant group-by-time interactions ($p<0.05$). Participants in the exercise group had a significant decrease in walking time (–1.4 s) and a significant increase in sit-and-reach (+5.8 cm). Kolmogorov-Smirnov tests revealed that data for chair-stand in 10 seconds and single-leg balance with eyes closed could not be assumed to be normally distributed. Therefore, Wilcoxon signed-rank tests were applied to these data, in which scores for chair stand (+1.8 n/10 s) and single-leg balance (+4.6 s) increased significantly in the exercise group. The control group exhibited no significant improvement in any of the five measures.

Correlation coefficients between baseline data and changes in each test are shown in Table 3a. They range from –0.45 to 0.27, accounting for 1.4-20.3% of variance; none achieved significance level ($p<0.05$) except for sit-and-reach. Correlation coefficients between the change in one test and the change in another test were not statistically significant. All coefficients were 0.37 or less in absolute value (Table 3b), which accounted for approximately 13.7% or less of each variance.

DISCUSSION

These results demonstrate improvements in agility, leg power, locomotion speed, flexibility and balance resulting from the six-month regimen of our square-stepping

Table 2 - *Functional fitness at pre- and post-treatment and comparison of changes in variables.*

Functional fitness related to risk of falls	Measurement item	Control group Pre	Control group Post	Exercise group Pre	Exercise group Post
Agility	Standing up from a lying position (s)	3.26±0.93	3.70±1.42	3.39±1.20	3.00±0.90*a
Leg power	Chair-stand in 10 seconds (n)	8.8±2.6	7.6±4.3	7.5±2.2	9.3±2.6*b
Locomotion speed	Walking round two cones (s)	22.6±3.4	22.9±4.8	21.2±3.5	19.8±3.6*a
Flexibility	Sit-and-reach (cm)	3.2±8.8	4.2±11.0	3.7±13.9	9.5±11.0*a
Balance	Single-leg balance with eyes closed (s)	11.0±15.5	6.7±9.1	9.3±11.7	13.9±15.6*b

Significances by Kolmogorov-Smirnov test were found in leg power and balance; Group-by-time interactions were found in agility, locomotion speed and flex ibility by ANOVA; *aSignificant main effect of time; *bSignificant difference by Wilcoxon signed-rank test.

Table 3 - *Correlation coefficients: (a) between baseline data and change in each test and (b) between change in one test and change ir other test.*

	(a)	(b) 1	(b) 2	(b) 3	(b) 4	(b) 5
1. Standing up from a lying position	0.27	1	0.27	0.21	0.37	0.16
2. Chair-stand in 10 seconds	–0.12		1	0.17	0.30	–0.14
3. Walking round two cones	0.24			1	0.17	0.15
4. Sit-and-reach	–0.45*				1	0.06
5. Single-leg balance with eyes closed	0.12					1

Spearman's correlation coefficients were calculated when sit-and-reach or single-leg balance with eyes closed were included as independent variables; Othe coefficients calculated by Pearson's analysis; *$p<0.05$.

exercise (SSE) program. In addition, the attendance rate was relatively high and there were no accidents during the SSE program. Therefore, we believe that it is an effective, practical means of decreasing the risk factors for falling in apparently healthy elderly people.

Standing up from a lying position, which we defined as agility, was significantly improved. This task requires a combination of quick response and whole-body movement. Whole-body movement during the test needs control over the center of gravity of the body, or dynamic balance. There are no published reports of changes in time or dynamic balance in standing up from a lying position as a result of an exercise intervention in the elderly, but it has been hypothesized that reaction time (2, 7) and dynamic balance (6, 16, 17) are major determinants of this test item. In previous studies, increases in reaction time are associated with high risk factors for falls (2, 7). The twelve-month prospective study of Nevitt et al. (2) found that those who had an increase in 0.75 or more seconds in hand-reaction time showed 1.8 times the relative risk of falls than those who had an increase of 0.74 seconds or less. In addition, anterior body mass carriage following a trip needs dynamic balance to control the whole body to avoid falling (6, 16, 17). Therefore, improving agility (standing up from a lying position) probably provided some reduction in risk factors for falls.

The increase in the test of chair-stand in 10 seconds probably reflected the improvement in participants' leg power (14) even though it was not assessed directly. A number of studies have identified a lack of muscular strength or power as risk factors for falling (18, 19), especially trip-induced falls (20). For example, being unable, or taking more than 2 seconds, to rise from a chair without using one's arms was associated with an increased risk of injury (2); fallers show a significant decrease in the strength of knees and ankles compared with non-fallers (21). Hip flexion joint torque from a sitting to standing position, which is related with leg power, may also be an important factor for quick chair-stand movement (22).

A low walking speed is also considered as a risk factor for falling (23-25). In the prospective study of Taylor et al. (25), a decrease of 0.22 m/sec in maximal walking speed over ten years was associated with an increase in hip fracture risk (RR 1.25, 95% CI 1.17-1.33). In the present study, the speed of walking round two cones changed from 0.79 m/sec before the program to 0.85 m/sec at the end of the program. Although a decrease in falls in itself cannot be attributed to a change of only 0.06 m/sec, the improvement in this test probably provided some improvement in risk factors for falls. Also, this improvement may play a distinct role in falls, by improving neuromuscular function, so that the SSE program influences an individual's speed, coordination and protective responses during a fall (23).

Increases in hip joint flexibility, which was measured by the sit-and-reach test in this study, should result in decreasing the risk factors for falls (13). The increase in the present study (about 6 cm) was slightly higher than our expectations, because some recent reports have found that exercise intervention that did not focus on flexibility showed an increase of about 3 cm; in these, exercise frequency or duration were not very different from those of our study (26, 27). This improvement may be due to the leg and hip stretching exercise that was provided in every warm-up period. We also told participants that doing sufficient stretching could prevent falls and injuries from occurring in the SSE program, which made them concentrate on stretching and thus improving sit-and-reach scores. A previous study (13) showed that those who had fallen three or more times in the previous year had significantly lower hip-joint flexibility than those who had had no falls. It is probable that hip-joint flexibility is related to stability and mobility in the elderly. Those with lower hip joint flexibility could not walk smoothly with a certain stance, and had great difficulty in maintaining their stability and placing the foot as a support for their bodies when a trip occurred.

Postural instability has also been cited as a risk factor for falls in the elderly (2, 12, 13, 16, 25, 28). It was hypothesized that single-leg balance with eyes closed, defined as balance, would be improved due to the stepping imposed by the SSE program with raised heels. Raised heels, which for safety reasons was not added until participants were familiar with a step pattern, probably afforded neuromuscular stimulation to the exercisers. Shigematsu et al. (29) demonstrated increases in single-leg balance with eyes closed in women aged 72-87 years who participated in a three-month dance-based aerobic exercise. They performed movements that extended, flexed, abducted, adducted and rotated the leg and foot, such as side-stepping, fast walking, forward-backward stepping and heel rises. Participants in the present study performed steps similar to those of the dance movements of Shigematsu et al.

These findings indicate that individuals in the exercise group were able to move their whole body more quickly and accurately after the exercise program. This may have clinical meaning, given that the leg movement in the front, side or oblique directions after tripping or slipping is appropriately carried out, and the center of gravity of the body is controlled to avoid falls. Since only 1.4-20.3% of the variance of the changes in each test could be explained by the initial levels, it is suggested that all persons, independently of their initial performance on any test, could benefit from our SSE program in at least one fitness element.

The present study was conducted with non-randomized allocation of participants. However, it may be difficult for persons who are transitioning to frailty or who are already frail to attend an SSE program. Therefore, our results may not be generalizable to a wider population, although they do appear to demonstrate that the SSE program is applicable as a primary prevention strategy. Only data concerning some functional fitness elements according to risk factors were used, so that other functional fitness elements, such as maximum step length or rapid stepping ability (30) and the effect of the SSE program on fall occurrence were not determined. However, these results clearly indicate that apparently healthy individuals aged 60-80 with no regular exercise habits can reduce their risk factors for falling by undertaking a square stepping exercise program once a week for six months.

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REFERENCES

1. Orimo H, Hashimoto T, Sakata K, Yoshimura N, Suzuki T, Hosoi T. Trends in the incidence of hip fracture in Japan, 1987-1997: The third nationwide survey. J Bone Miner Metab 2000; 18: 126-31.
2. Nevitt MC, Cummings SR, Hudes ES. Risk factors for injurious falls: A prospective study. J Gerontol 1991; 46: M164-70.
3. Blake AJ, Morgan K, Bendall MJ, et al. Falls by elderly people at home: Prevalence and associated factors. Age Ageing 1998; 17: 365-72.
4. Smeesters C, Hayes WC, McMahon TA. Disturbance type and gait speed affect fall direction and impact location. J Biomech 2001; 34: 309-17.
5. Greenspan SL, Myers ER, Kiel DP, Parker RA, Hayes WC, Resnick NM. Fall direction, bone mineral density, and function: Risk factors for hip fracture in frail nursing home elderly. Am J Med 1998; 104: 539-45.
6. Pavol MJ, Owings TM, Foley KT, Grabiner MD. Mechanisms leading to a fall from an induced trip in healthy older adults. J Gerontol 2001; 56A: M428-37.
7. Bogert AJ, Pavol MJ, Grabiner MD. Response time is more important than walking speed for the ability of older adults to avoid a fall after a trip. J Biomech 2002; 35: 199-205.
8. Herala M, Kivela SL, Honkanen R, Koski K, Laippala P, Luukinen H. Recent decline in heavy outdoor work activity predicts occurrence of fractures among the home-dwelling elderly. Osteoporos Int 2002; 13: 42-4.
9. Luukinen H, Koski K, Laippala P, Kivela SL. Factors predicting fractures during falling impacts among home-dwelling older adults. J Am Geriatr Soc 1997; 45: 1302-9.
10. Sherrington C, Lord SR. Increased prevalence of fall risk factors in older people following hip fracture. Gerontology 1998; 44: 340-4.
11. Koepell TD, Wolf ME, Buchner DM, et al. Footwear style and risk of falls in older adults. J Am Geriatr Soc 2004; 52: 1495-501.
12. Chu LW, Pei CK, Chiu A, et al. Risk factors for falls in hospitalized older medical patients. J Gerontol 1999; 54: M38-43.
13. Gehlsen GM, Whale MH. Falls in the elderly: Part II: Balance, strength, and flexibility. Arch Phys Med Rehabil 1990; 71: 739-41.
14. Rikli RE, Jones CJ. Development and validation of a functional fitness test for community-residing older adults. J Aging Phys Act 1999; 7: 129-61.
15. Shigematsu R, Tanaka K. Age scale for assessing functional fitness in older Japanese ambulatory women. Aging Clin Exp Res 2000; 12: 256-63.
16. Rogers ME, Rogers NL, Takeshima N, Islam MM. Methods to assess and improve the physical parameters associated with fall risk in older adults. Prev Med 2003; 36: 255-64.
17. Wolfson LI, Whipple R, Amerman P, Kleinberg A. Stressing the postural response. A quantitative method for testing balance. J Am Geriatr Soc 1986; 34: 845-50.
18. American Geriatrics Society, British Geriatric Society, and American Academy of Orthopaedic Surgeons Panel on Falls Prevention. Guideline for the prevention of falls in older persons. J Am Geriatr Soc 2001; 49: 664-72.
19. Moreland JD, Rchardson JA, Goldsmith CH, Clase CM. Muscle weakness and falls in older adults: A systematic review and meta-analysis. J Am Geriatr Soc 2004; 52: 1121-9.
20. Pavol MJ, Owings TM, Foley KT, Grabiner MD. Influence of lower extremity strength of healthy older adults on the outcome of an induced trip. J Am Geriatr Soc 2002; 50: 256-62.
21. Whipple RH, Wolfson LI, Amerman PM. The relationship of knee and ankle weakness to falls in nursing home residents: An isokinetic study. J Am Geriatr Soc 1987; 35: 13-20.
22. Mak MK, Levin O, Mizrahi J, Hui-Chan CW. Joint torques during sit-to-stand in healthy subjects and people with Parkinson's disease. Clin Biomech 2003; 18: 197-206.
23. Dargent-Molina P, Favier F, Grandjean H, et al. Fall-related factors and risk of hip fracture: the EPIDOS prospective study. Lancet 1996; 348: 145-9.
24. Rekeneire N, Visser M, Peila R, et al. Is a fall just a fall: correlates of falling in healthy older persons. The Health, Aging and Body Composition Study. J Am Geriatr Soc 2003; 51: 841-6.
25. Taylor BC, Schreiner PJ, Stone KL, et al. Long-term prediction of incident hip fracture risk in elderly white women: Study of Osteoporotic Fractures. J Am Geriatr Soc 2004; 52: 1479-86.
26. Adams KJ, Swank AM, Berning JM, Sevene-Adams PG, Barnard KL, Shimp-Bowerman J. Progressive strength training in sedentary, older African American women. Med Sci Sports Exerc 2001; 33: 1567-76.
27. Ransdell LB, Taylor A, Oakland D, Schmidt J, Moyer-Mileur L, Shultz B. Daughters and mothers exercising together: effects of home- and community-based programs. Med Sci Sports Exerc 2003; 35: 286-96.
28. Lord SR, Ward JA, Williams P, Anstey KJ. Physiological factors associated with falls in older community-dwelling women. J Am Geriatr Soc 1994; 42: 1110-7.
29. Shigematsu R, Chang M, Yabushita N, et al. Dance-based aerobic exercise may improve indices of falling risk in older women. Age Ageing 2002; 31: 261-6.
30. Mendell JL, Alexander NB. A clinical measure of maximal and rapid stepping in older women. J Gerontol 2000; 55A: M429-33.