改善効果は期待されるものと推察する。

本研究は、特定保健指導における運動実施において、健康運動指導士の指導が有効であるかどうかに着目した。健康運動指導士は、昭和63年に地域保健法に基づく厚生省令「健康づくりのための運動指導者の知識および技能の審査・証明事業の認定に関する規定」(本省令は平成17年に廃止)により発足し、現在は財団法人健康・体力づくり事業財団独自の事業として継続している[40]。保健医療関係者と連携しつつ安全で効果的な運動を実施するための運動プログラム作成及び実践指導計画の調整等を行なう役割を担っている[41]。しかしながら、筆者が調べた中高齢者を対象とした運動健康教室における生活習慣病リスクの改善効果に関する先行研究において、健康運動指導士が運動指導したと明記されている論文は3報であった[7~9]。韓ら[7]は、60歳以上の女性を対象に26週間週1回の健康運動指導士が指導する「健康づくり運動」により、等尺性膝伸展力や歩行機能が改善したと報告している。このことは6.5ヵ月の継続であれば週1回の頻度でも改善することを示唆する。河村ら[8]は、高齢者を対象に12週間週2回の健康運動指導士が指導する介護予防筋力トレーニング事業の実施により、運動機能の向上及び腹腔内脂肪やアディポカインの分泌活性に好影響を及ぼすことを報告している。このことは3ヵ月の継続であれば週2回の頻度で改善することを示唆する。本研究と同様に動脈スティフネスに着目した研究では、柿山ら[9]が、6ヵ月間週2回の頻度で健康運動指導士が中高年を対象に、個別に低強度の運動トレーニングをエアロバイクや筋力トレーニングマシンを用いて実施し、動脈硬化性疾患の無い健常者の動脈スティフネス及び血圧が減少することを報告している。本研究は、3ヵ月間週1回の集団運動プログラムの実施により、baPWV及び血圧が低下したことを示した。トレーニングマシンを用いない方法論であっても、地域の実情に合わせた個々の健康運動指導士からの運動指導が、baPWV及び血圧値を改善させる可能性を示唆する。

2005年に山下ら[42]が市町村保健センターを対象に行なったアンケート調査では、73%の保健センターで運動を通した健康づくり事業を実施していることを報告している。事業に携わっているスタッフは保健師、栄養士についで健康運動指導士・実践指導者の順に多く、実際に運動指導を行なっているスタッフの資格は健康運動指導士・実践指導者、保健師、体育系指導員・インストラクターの順に多いことも報告されている。しかしながら、地域住民を対象とした健康づくり事業の報告は多くあるが、実際にその指導を行なった者の資格と指導内容の関連を明記してあるものは少ない。2008年度から、特定健康診査・保健指導は40~74歳の国民に義務化された[3]。医療保険者は、メタボリックシンドロームに着目し、生活習慣病の予防を重視した特定健康診査とその結果により生活習慣の改善が必要な人への特定保健指導を実施しなければならず、その構成する項目に運動が含まれる。特定保健指導の基本的な考え方は、対象者の自己選択と行動変容に着目し、個々人の検診結果を読み解くとともにライフスタイルを考慮した方法で、さらに科学的根拠に基づく指導をすることである。現行の健康運動指導士の養成プログラムはこの内容を網羅しており、特定保健指導において健康運動指導士は、特定保健指導を統括する医師、保健師、管理栄養士等と協力して事業に貢献できるものと考える。特定健康診査・保健指導は登録された保健事業者へアウトソーシングが可能である。集団運動プログラムとして積極的支援を行う場合、保健指導を請け負うもしくは実施する側が、参加率が動脈スティフネスや血圧に影響を与える事実も把握して保健指導プログラムを作成する、もしくはコーディネイトすることが成果に結びつくものと予測する。

本研究から、週1回の健康教室への参加で動脈スティフネスと血圧が改善する可能性が示唆された。血清脂質の面からみると明らかな改善は認められなかった。血清脂質の運動効果に関する統一した見解を示す報告は今のところない[34,35,43,44]。今後、運動強度や運動内容の面からの検討が必要であると考える。しかしながら、本研究のような地域住民を対象とした集団運動プログラムであったとしても、健康運動指導士が指導し実施することで、メタボリックシンドロームの診断基準項目の一つである血圧が改善するという事実は、今後のメタボリックシンドローム対策に貢献するものと考える。

本研究の対象者は、任意で健康教室に参加しており、運動を肯定的にとらえている者が多かったと推察する。健康運動指導士による運動介入の効果だけでなく、健康増進に対する関心の高さが本研究の結果に付加されている可能性は否定できない。今後は、生活習慣是正の糸口をみつけられない者や運動習慣獲得に興味を示さないような者を対象に健康運動指導士と健康づくり事業に携わるその他の職種を加えた介入の有効性を検討し、地域住民に対し、特定保健指導を活用した健康づくり制度の構築が望まれる。

## V. まとめ

地域住民を対象とした週1回3ヵ月間の健康運動指導士が指導する有酸素運動を主体とする健康教室の実施が、動脈スティフネス及び血圧を改善させる可能性が示唆された。参加率が高く、健康運動指導士からの介入を多く受けることが、動脈スティフネスや血圧の

改善に有効である可能性も示唆された。

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# Habitual rowing exercise is associated with high physical fitness without affecting arterial stiffness in older men

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**Abstract**

The present study elucidated the effects of habitual rowing exercise on arterial stiffness and plasma levels of the vasoconstrictor endothelin-1 and the vasodilator nitric oxide (NO) in older men. Eleven rowers (68.0 $\pm$ 1.6 years) and 11 sedentary control older men (64.9 $\pm$ 1.1 years) were studied. Peak oxygen uptake (36.0 $\pm$ 1.7 *vs.* 27.7 $\pm$ 1.9 ml $\cdot$ kg$^{-1}$ $\cdot$ min$^{-1}$), leg press power (1346 $\pm$ 99 *vs.* 1077 $\pm$ 68 W), and HDL-cholesterol (75 $\pm$ 5 *vs.* 58 $\pm$ 3 mg $\cdot$ ml$^{-1}$) were higher and triglyceride (78 $\pm$ 9 *vs.* 120 $\pm$ 14 mg $\cdot$ ml$^{-1}$) was lower in rowers than in control participants (all $P < 0.05$). Arterial stiffness indices (carotid $\beta$-stiffness and cardio-ankle vascular index) and plasma endothelin-1 and NOx (nitrite + nitrate) levels did not differ between the two groups. These results suggest that habitual rowing exercise in older men is associated with high muscle power and aerobic capacity, and favourable blood lipid profile without affecting arterial stiffness or plasma levels of endotheline-1 and NO.

**Keywords:** *Rowing, arterial stiffness, combined training, endothelin-1, nitric oxide*

## Introduction

Arterial stiffening (Avolio et al., 1985; Tanaka, DeSouza, & Seals, 1998; Vaitkevicius et al., 1993) and muscular weakening (Janssen, Heymsfield, & Ross, 2002; Metter, Talbot, Schrager, & Conwit, 2002) develop with advancing age. Increased arterial stiffness is associated with mortality in patients with end-stage renal failure (Blacher et al., 1999) and essential hypertension (Laurent et al., 2001). Arterial stiffness is reduced with endurance training (Tanaka et al., 2000; Vaitkevicius et al., 1993), and increases with resistance training (Bertovic et al., 1999; Miyachi et al., 2003; Miyachi et al., 2004). Moreover, simultaneously performed aerobic training prevents the arterial stiffening caused by resistance training (Kawano, Tanaka, & Miyachi, 2006). Therefore, combined aerobic and resistance training may be used as protocol for maintaining vascular health.

Vascular endothelial cells play an important role in the regulation of vascular tone by producing vasoactive substances, such as endothelin and nitric oxide (NO). Endotheline-1, a peptide produced by vascular endothelial cells, is a potent vasoconstrictor (Miyauchi & Masaki, 1999), and contributes to arterial stiffness (Luscher & Barton, 2000; Miyauchi & Masaki, 1999). NO produced by vascular endothelial cells has a potent vasodilator effect, and consequently prevents and inhibits hypertension and arteriosclerosis (Moncada, Palmer, & Higgs, 1991), while the bioavailability of NO decreases with advancing age (Dohi, Kojima, Sato, & Luscher, 1995; Dohi, Thiel, Buhler, & Luscher, 1990; Taddei et al., 1997). Plasma endotheline-1 levels are reduced by aerobic training in older people (Maeda et al., 2003; Stauffer, Westby, & DeSouza, 2008; White et al., 1997), but plasma nitrite/nitrate (NOx: measured as the stable end product of NO) was elevated by aerobic exercise training in elderly women (Maeda et al., 2004).

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Rowing training is unique because it includes components of both aerobic endurance and muscular strength training. In a boat race, rowers are required to have high muscular power to accelerate the boat at the beginning and large aerobic capacity to maintain the speed. In addition, when they spurt to accelerate the boat at more than a constant speed in the final phase of the race, they row at maximum muscular strength. Indeed, rowing training is identified as a combination of resistance and aerobic training (Yoshiga, Higuchi, & Oka, 2002a, 2002b). The age-related increase in brachial-ankle pulse wave velocity, as an index of systemic arterial stiffness, is attenuated in rowing-trained older men (Sanada et al., 2009). However, it remains unclear whether endogenous endotheline-1 and NO are affected by rowing training in older humans.

We hypothesised that habitual rowing training improves arterial stiffness and endogenous endotheline-1 and NO. To test our hypothesis, the present study was performed to compare arterial stiffness and plasma endotheline-1 and NOx concentrations between rowing-trained older men and age-matched controls.

## Methods

The study population included 11 rowing-trained older men aged $68.0 \pm 1.6$ years and 11 sedentary controls aged $64.9 \pm 1.1$ years (Table I). The sedentary men were recruited through advertising and had not participated in a habitual exercise training program, such as endurance or resistance training. The rowers were recruited from rowing clubs and had rowed on the water or on an ergometer at least twice per week for 5 years or more, each session lasting 90–120 min including warm-up, 12–16 km of rowing, and recovery, but had not performed particular resistance or aerobic training. All participants were free of diabetes mellitus and overt chronic diseases based on their medical history. In addition, participants who had used anabolic steroids or other performance-enhancing drugs or who had significant carotid intima-media thickening ($\geq 1.1$ mm), plaque formation, and/or other characteristics of atherosclerosis [ankle-brachial index (ankle systolic blood pressure/brachial systolic blood pressure) $\leq 0.9$] were excluded from the study. All participants provided informed consent as approved by the Human Research Ethics Committee of the Faculty of Sport Sciences of Waseda University. The study was performed in accordance with the guidelines of the Declaration of Helsinki 2006.

Table I. Participant characteristics.

| | Control | Rowers |
|---|---|---|
| N | 11 | 11 |
| Age, years | $64.9 \pm 3.5$ | $68.0 \pm 5.1$ |
| Height, cm | $169.6 \pm 3.8$ | $174.8 \pm 5.0$* |
| Body weight, kg | $69.1 \pm 11.2$ | $72.8 \pm 9.0$ |
| Fat, % | $21.4 \pm 4.7$ | $21.3 \pm 4.1$ |
| HDL cholesterol, $mg \cdot dl^{-1}$ | $58 \pm 10.3$ | $75 \pm 16.6$* |
| LDL cholesterol, $mg \cdot dl^{-1}$ | $124 \pm 25$ | $126 \pm 16$ |
| Triglycerides, $mg \cdot dl^{-1}$ | $120 \pm 46$ | $78 \pm 28$* |
| Plasma glucose, $mg \cdot dl^{-1}$ | $99 \pm 11$ | $110 \pm 29$ |
| Resting heart rate, bpm | $65 \pm 11$ | $60 \pm 7$ |
| Maximal heart rate, bpm | $169 \pm 12$ | $171 \pm 7$ |
| $\dot{V}O_{2peak}$, l/min | $1.9 \pm 0.5$ | $2.5 \pm 0.5$* |
| Leg press power, W | $1077 \pm 226$ | $1346 \pm 329$* |

Data are Means $\pm$ *S*; N, no. of subjects; HDL, high-density lipoprotein; LDL, low-density lipoprotein; $\dot{V}O_2$peak, peak oxygen consumption. *Significant at $P < 0.05$ vs Control.

### *Measurements*

Before testing, participants abstained from caffeine and fasted for at least 12 h overnight. All measurements were performed in the laboratory in the morning. Tests for the rowers were conducted 24–28 h after their last exercise training session. Participants were not smokers except for one in the control group. This participant abstained from smoking on the test day.

### *Carotid arterial intima-media thickness*

Carotid arterial intima-media thickness was measured from images obtained using an ultrasound system (SonoSite Taitan; SonoSite Instruments, Bothell, WA) equipped with a high-resolution linear-array broad-band transducer. Ultrasound images were analysed using software (ImageJ 1.41, Bethesda, MD, USA). At least 10 intima-media thickness measurements were taken at each segment, and the mean value was used for analysis. This technique has a coefficient of variance of $3 \pm 1\%$ (Kawano et al., 2006; Kawano et al., 2008).

### *Carotid arterial compliance and β-stiffness*

After 15 min of rest, carotid arterial compliance and β-stiffness were measured. A combination of ultrasound imaging of the pulsatile common carotid artery with simultaneous applanation of tonometrically obtained arterial pressure from the contralateral carotid artery permits noninvasive determination of arterial stiffness (Kawano et al., 2008; Tanaka et al., 2000). The carotid artery diameter was measured from images obtained using a SonoSite Taitan ultrasound system equipped with a high-resolution linear-array transducer. A longitudinal image of the cephalic portion of the common carotid artery was acquired 1–2 cm proximal to the carotid bulb. All image analyses were performed by the same investigator who was blinded to participants' exercise status.

Pressure waveforms and amplitudes were obtained from the common carotid artery with a pencil-type probe incorporating a high-fidelity strain-gauge transducer (SPT-301; Millar Instruments, Houston, TX) (Kawano et al., 2008; Tanaka et al., 2000). As baseline levels of blood pressure are subjected to hold-down force, the pressure signal obtained by tonometry was calibrated by equating the carotid mean arterial and diastolic blood pressure to the brachial arterial value (Kawano et al., 2008; Tanaka et al., 2000). In addition to arterial compliance (Laurent et al., 2006), we calculated the $\beta$-stiffness index (Parati & Bernardi, 2006), which provides an expression of arterial compliance adjusted for distending pressure (Hirai, Sasayama, Kawasaki, Yagi, 1989), because arterial compliance depends on blood pressure (Van Merode, Hick, Hoeks, Rahn, & Reneman, 1988). The arterial compliance and $\beta$-stiffness indices were evaluated:

$$\text{arterial compliance} = \pi\,(D_1^2 - D_0^2)/4(P_1 - P_0) \quad (1)$$

and

$$\beta\text{-stiffness index} = \frac{ln(P_1/P_0)}{(D_1 - D_0)/D_0} \quad (2)$$

where $D_1$ and $D_0$ are the maximal and minimal diameters and $P_1$ and $P_0$ are the systolic and diastolic blood pressures, respectively. The systolic and diastolic carotid blood pressures estimated by brachial blood pressures were used for calculating carotid arterial compliance and $\beta$-stiffness index. The day-to-day coefficients of variation were $7 \pm 3\%$ and $5 \pm 2\%$ for carotid arterial compliance and $\beta$-stiffness, respectively.

*Cardio-ankle vascular index*

After repetition of rest, cardio-ankle vascular index measurement was performed using a VaSera VS-1500 (Fukuda Denshi, Tokyo, Japan) from the measurements of blood pressure and pulse wave velocity, while monitoring the electrocardiogram and heart sounds (Kubozono et al., 2007; Shirai, Utino, Otsuka, & Takata, 2006). Pulse wave velocity was calculated by dividing the distance from the aortic valve to the ankle artery by the sum of the time between the aortic valve closing sound and the notch of the brachial pulse wave, and the time between rise of the brachial pulse wave and that of the ankle pulse wave.

$$\text{Cardio-ankle vascular index} = a \cdot [(2\rho/\Delta P) \cdot ln(P_1/P_0) \cdot \text{pulse wave velocity}^2] + b \quad (3)$$

Pulse wave velocity is the pulse wave velocity between the heart and ankle, $\Delta P$ is $P_1 - P_0$, $\rho$ is blood density, and a and b are constants. The systolic and diastolic brachial blood pressure and the pulse wave velocity were used for calculating the value of the cardio-ankle vascular index. The day-to-day coefficient of variation in the cardio-ankle vascular index was $2 \pm 1\%$.

*Blood biochemistry*

Following a 12-h overnight fast, blood was collected from an antecubital vein in the early morning. Each blood sample was placed in a chilled tube containing aprotinin (300 kallikrein inhibitor $U \cdot ml^{-1}$) and EDTA ($2\ mg \cdot ml^{-1}$) and was centrifuged at 2000 rpm for 15 min at 4°C. The plasma was stored at −80°C until assay. Plasma concentrations of endotheline-1 were determined using a sandwich-enzyme immunoassay (EIA) kit (Immuno-Biological Laboratories, Fujioka, Japan) (coefficient of variation, 11%) (Iemitsu et al., 2006). Plasma concentration of NOx was determined using a commercial NO ($NO_2/NO_3$) assay kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions (coefficient of variation, 4%). Serum concentrations of cholesterol, triglycerides and plasma concentrations of glucose were determined using enzymatic techniques.

*Brachial arterial blood pressure at rest*

Arterial blood pressure at rest was measured with a semi-automated device (VaSera VS-1500) over the brachial and dorsalis pedis arteries using the oscillometric method (Shirai et al., 2006). Recordings were made in triplicate with participants supine. The day-to-day coefficient of variation in brachial blood pressure at rest was $2 \pm 1\%$.

*Peak oxygen uptake*

We measured peak oxygen consumption ($\dot{V}O_{2peak}$) during incremental cycle ergometer exercise (Miyachi et al., 2001), as the cardiorespiratory fitness index. Oxygen consumption (coefficient of variation, $4 \pm 1\%$), heart rate, and ratings of perceived exertion were monitored throughout the protocol (Miyachi et al., 2001).

*Muscle strength*

Muscle strength was assessed by leg extension power (Kawano et al., 2008). Briefly, leg extension power (coefficient of variation, $2 \pm 1\%$) was determined using a dynamometer (Anaero Press 3500; Combi Wellness, Tokyo, Japan) in the sitting position. The participants were secured in a chair using a seatbelt. In the starting position, the feet were placed on a sliding plate with the knee angle adjusted to 90°. Five trials were performed at 15-s intervals, and the average of the two highest recorded power outputs (W) was taken as the definitive measurement.

*Body composition*

Body composition was determined using the bio-electric impedance method (coefficient of variation, 4 ± 2%) (Bolanowski & Nilsson, 2001).

*Statistics*

Statistical analyses were performed using StatView (SAS, Cary, NC) with presented means ± *s*. Mean differences between rowers and control men were examined using Student's unpaired *t* test. Statistical significance was set at $P < 0.05$.

## Results

Height and HDL-cholesterol were higher, and triglyceride was lower in rowers compared with controls (Table I; all $P < 0.05$). Rowers had greater $\dot{V}O_{2peak}$ and leg press power than controls. There were no significant differences in other parameters between the two groups.

Blood pressures of the brachial and carotid arteries were not significantly different between the two groups (Table II). There were no differences in carotid systolic or diastolic diameters or in intima-media thickness between the two groups. Also there were no significant differences in cardio-ankle vascular index, carotid arterial compliance, or β-stiffness between the two groups. Plasma endotheline-1 concentration and plasma NOx concentration did not differ between rowers and controls, although plasma endotheline-1 tended to be lower in the rowers (Table II).

Table II. Vascular indices, plasma endothelin-1 and NOx concentrations.

| | Control | Rowers |
|---|---|---|
| Brachial systolic BP, mmHg | 140 ± 18 | 142 ± 19 |
| Brachial mean BP, mmHg | 111 ± 15 | 113 ± 17 |
| Brachial diastolic BP, mmHg | 92 ± 8 | 91 ± 12 |
| Brachial PP, mmHg | 48 ± 11 | 52 ± 11 |
| Carotid systolic BP, mmHg | 138 ± 24 | 147 ± 31 |
| Carotid PP, mmHg | 47 ± 16 | 55 ± 25 |
| Carotid diastolic diameter, mm | 6.7 ± 0.8 | 7.2 ± 1.0 |
| Carotid systolic diameter, mm | 7.0 ± 0.8 | 7.6 ± 1.1 |
| ΔCarotid diameter, mm | 0.3 ± 0.1 | 0.3 ± 0.2 |
| Carotid IMT, mm | 0.7 ± 0.1 | 0.8 ± 0.1 |
| Cardio-ankle vascular index, Arbitrary unit | 8.4 ± 1.0 | 8.4 ± 1.0 |
| Carotid arterial compliance, $mm^2$/mmHg | 0.06 ± 0.02 | 0.08 ± 0.03 |
| Carotid arterial compliance, $mm^2$/kPa | 0.008 ± 0.003 | 0.010 ± 0.004 |
| Carotid β-stiffness index, Arbitrary unit | 11.2 ± 2.8 | 10.0 ± 1.5 |
| Plasma endothelin-1, pg/ml | 3.0 ± 0.7 | 3.3 ± 0.8 |
| Plasma nitrite/nitrate (NOx), μM | 45 ± 30 | 40 ± 24 |

Data are Means ± *S*; BP, blood pressure; PP, pulse pressure; IMT, intima-media thickness.

## Discussion

The results indicate that rowing-trained older men demonstrate greater cardiorespiratory fitness, muscular strength, and superior blood lipid profiles, but not differences in indices of arterial stiffness or plasma endotheline-1 and NOx concentrations.

Resistance training is associated with an increase in arterial stiffness (Bertovic et al., 1999; Miyachi et al., 2003; Miyachi et al., 2004). Although rowing training includes a component of resistance training, this study demonstrated that arterial stiffness indices were not different between older men who were rowers and sedentary controls. Considering the favourable effect of aerobic training on arterial stiffness, the findings suggest that the aerobic component of rowing training negates the higher arterial stiffness associated with the resistance training component. In addition, we observed that habitual rowing training was associated with lower triglyceride and higher HDL-cholesterol levels, and also with greater leg press power and $\dot{V}O_{2peak}$. Furthermore, this type of training is not associated with unfavourable effects on arterial stiffness. Considering these results, we suggest that rowing training should be proposed as an effective exercise model for prevention of sarcopenia or lifestyle-related diseases, such as cardiovascular diseases.

The results indicated that there were no significant differences in plasma levels of endotheline-1 and NOx between rowing-trained older men and similar sedentary controls. Aerobic training induces a decrease in endotheline-1 level and an increase in NOx level with improvement of arterial stiffness (Maeda et al., 2004; Miyaki et al., 2009). On the other hand, arterial stiffening with resistance training is associated with greater plasma levels of endotheline-1 (Otsuki et al., 2007). Regulation of arterial stiffness *via* arterial tonus is adjusted by the balance between the vasoconstrictor endotheline-1 (Miyauchi & Masaki, 1999) and the vasodilator NO (Moncada et al., 1991). Furthermore, vascular adaptations to changes in physical activity (such as training) may be regulated through the interaction between vasodilation and vasoconstriction (Thijssen, Rongen, Smits, & Hopman, 2008). Since aerobic and resistance training components in rowing training may negate changes in NO or endotheline-1, we speculate that these factors balance each other, which might have contributed to the lack of a difference in arterial stiffness between rowing-trained men and controls.

Dyslipoproteinemia is risk factor for coronary artery disease, *i.e.*, elevated concentrations of triglyceride, total cholesterol, and LDL-cholesterol, and a reduced level of HDL-cholesterol, which is improved with performing aerobic (Higuchi et al., 1984) and resistance training (Fahlman, Boardley, Lambert, & Flynn, 2002). Accordingly, these observations suggest that habitual rowing training in older men is associated with lower risk factor indices for coronary artery disease.

The reader should be aware of some study limitations associated with the present study. Firstly, study limitations include the relatively small sample size that might have led to a type 2 error. Indeed, the rowers showed modest lower levels of arterial stiffness and higher levels of arterial compliance as found by others (Cook et al., 2006; Sanada et al., 2009), but these did not reach statistical significance as opposed to the previous observational studies. Secondly, the present study focused on older men with and without habitual rowing exercise and the results should be confirmed in further large sample studies focused on rowing-trained young adults and women.

In conclusion, we showed that rowing-trained older men did not demonstrate higher arterial stiffness as determined by carotid $\beta$-stiffness and cardio-ankle vascular index, higher endogenous endotheline-1 and lower endogenous NO, but a favourable blood lipid profile, muscular strength, and cardiorespiratory fitness. These results suggest that habitual rowing exercise in older men is associated with high muscle power and aerobic capacity, and favourable blood lipid profile without affecting arterial stiffness or plasma levels of endotheline-1 and NO.

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ORIGINAL ARTICLE

# Associations among objectively measured physical activity, fasting plasma homocysteine concentration, and MTHFR C677T genotype

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**Abstract** Elevated fasting plasma homocysteine (Hcy) level is a vascular disease risk factor. Plasma Hcy is affected by 5,10-methylenetetrahydofolate reductase (MTHFR) genotype and dietary folate intake. This cross-sectional study in 434 Japanese adults examined the associations among objectively measured physical activity (PA), plasma Hcy adjusting for dietary folate intake, and MTHFR C677T genotype. Daily PA was measured by triaxial accelerometry and all subjects completed a questionnaire about their dietary habits. Plasma Hcy and MTHFR C677T genotype were determined. Plasma Hcy in subjects with the TT genotype was significantly higher than in those with CC or CT genotype ($p < 0.001$). Plasma Hcy was significantly different between ≥200 (7.6 ± 0.2 nmol/mL) and <200 μg/day (8.3 ± 0.3 nmol/mL) folate intake groups ($p = 0.003$). There were no differences in plasma Hcy adjusting for age, sex, and folate intake between groups according to PA category in all subjects. However, there were significant interactions between time spent in light PA ($p = 0.003$), vigorous PA ($p = 0.001$), or inactivity ($p = 0.004$), and MTHFR genotype. In only the TT genotype, shorter time spent in light PA was associated with higher plasma Hcy than a longer time spent in light PA (11.5 ± 3.3 nmol/mL vs. 8.5 ± 3.3 nmol/mL, $p < 0.001$), and longer time spent in vigorous PA and inactivity were associated with higher plasma Hcy (11.8 ± 3.3 nmol/mL vs. 8.4 ± 3.2 nmol/mL, 11.6 ± 3.3 nmol/mL vs. 8.4 ± 3.3 nmol/mL, respectively, $p < 0.001$). In conclusion, light and vigorous PA were associated with plasma Hcy only in the TT genotype, but there were no such associations in all genotypes.

**Keywords** Homocysteine · MTHFR genotype · Physical activity · Folate intake

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## Introduction

Elevated fasting plasma homocysteine (Hcy) is considered a risk factor for vascular disease (Homocysteine Studies Collaboration 2002; Boushey et al. 1995; Meleady et al. 2003). Hcy is a sulfur-containing amino acid derived from the metabolism of methionine, which is important for cellular methyltransferase reactions, including those of DNA, RNA, proteins, and lipids (Castro et al. 2006). Hcy can be further metabolized via two alternative pathways; it may be irreversibly degraded through the transsulfuration pathway or remethylated to methionine via the remethylation pathway. If optimal Hcy levels in cells are not maintained or reestablished through folate-dependent remethylation, Hcy will be actively exported to the extracellular compartment. In addition, the common C677T polymorphism of the 5,10-methylenetetrahydofolate reductase (MTHFR) gene, which regulates folate metabolism involved in folate-dependent remethylation of Hcy, has been established as an important genetic determinant of elevated Hcy (Bathum et al. 2007; Hustad et al. 2007; Yang et al. 2008). It has been reported that the MTHFR genotype explains ~5.1% of plasma Hcy variation in a genome-wide association study (Lange et al. 2010), and also the impact of the MTHFR gene locus was estimated to explain 24–53% of the variation using a combined association and linkage analysis in twins (Bathum et al. 2007).

Several nutritional factors, such as lower folate intake and high alcohol consumption, also contribute to higher plasma Hcy levels (Ganji and Kafai 2003; Jacques et al. 2001; Nygard et al. 1998). In addition, the larger intake of folate can reduce plasma Hcy in people with the TT genotype of the MTHFR gene with higher plasma Hcy levels; *i.e.*, plasma Hcy is affected by gene–environment interactions (Yang et al. 2008). On the other hand, although regular physical activity (PA) has been established to reduce the risk of vascular disease, there have been contradictory reports regarding its effects on plasma Hcy (Dankner et al. 2007; de Bree et al. 2001; Husemoen et al. 2004; Joubert and Manore 2008; Nygard et al. 1995; Ruiz et al. 2007a; Saw et al. 2001). Some studies have indicated that PA is inversely associated with total Hcy (Dankner et al. 2007; Nygard et al. 1995), whereas de Bree et al. (2001) reported a weak positive relation between PA and plasma total Hcy in women. Furthermore, Joubert et al. (Joubert and Manore 2008) have reported that extremely high PA of moderate and high intensities may increase Hcy level. These discrepancies may have been due to the different methods used for evaluation of PA, including self-reported questionnaires, the lack of consideration of intensity or duration of PA, limited statistical power with small sample size, and no adjustment for folate intake status.

We hypothesized that the amount of PA at certain intensity is associated with plasma Hcy level. The present cross-sectional study was performed to objectively clarify the associations among PA, plasma Hcy, and MTHFR genotype after adjusting for dietary folate intake taking intensity and duration of PA into consideration.

## Methods

### Subjects

A total of 434 Japanese adults (118 men and 316 women), 23–85 years of age, participated in this cross-sectional study. Daily PA was measured for 28 days by triaxial accelerometry, and all subjects completed a questionnaire about their dietary habits before laboratory measurements.

All subjects gave their written informed consent for participating in the present study. All procedures were reviewed and approved by the Ethical Review Board of the National Institute of Health and Nutrition.

### Anthropometry and biochemical measures

Weight and height were measured and body mass index (in kg/m$^2$) was calculated. Blood pressure was measured with form ABI/PWV (Omron Corlin, Tokyo, Japan) under quiet resting conditions in the supine position.

Venous blood samples were obtained after an overnight fast of at least 10 h. Whole blood collected into tubes without additives or with EDTA was immediately centrifuged at 3,000 rpm for 20 min to obtain serum or plasma. Hcy was analyzed in plasma by gas chromatography-mass spectrometry, whereas folate was determined in serum by microbiological methods. Plasma glucose, and total cholesterol, HDL-C, and TG in serum were also determined.

### Physical activity

The duration and intensity of PA were evaluated by triaxial accelerometry (Actimarker EW4800; Panasonic Electric Works, Osaka, Japan), which has been shown to be a valid method for determining the total energy expenditure or energy expenditure associated with physical activity based on a comparison with doubly labeled water (Yamada et al. 2009). The subjects were asked to wear a belt on the lower back except during water-based activities for 28 days. The metabolic equivalent (MET) intensity levels of PA were calculated as described previously (Gando et al. 2009; Yamada et al. 2009). Briefly, acceleration in the anterior–posterior ($x$), mediolateral ($y$), and vertical ($z$) axes were calculated using a sensor with a sample rate of 20 Hz over a range from 0 to $2 \times g$. The apparatus stores the standard deviation of the vector norm of the composite acceleration ($K_m$) in three dimensions each minute as follows:

$$k_m = \sqrt{\frac{1}{n-1}\left[\left(\sum_{k=1}^{n} x_i^2 + \sum_{k=1}^{n} y_i^2 + \sum_{k=1}^{n} z_i^2\right) - \frac{1}{n}\left\{\left(\sum_{k=1}^{n} x_i\right)^2 + \left(\sum_{k=1}^{n} y_i\right)^2 + \left(\sum_{k=1}^{n} z_i\right)^2\right\}\right]}$$

where $n$ is the number of data for 1 min ($n = 1{,}200$), and $\Sigma x$, $\Sigma y$, and $\Sigma z$ are the sums of the accelerations in each axis for 1 min. The metabolic equivalent (MET) intensity levels of PA were calculated by simple linear regression of $K_m$. The average daily step counts (steps/day) and the total amount of PA (METs h) were calculated using data from at least 14 days excluding those days when subjects did not wear the accelerometer or made less than 1,000 steps/day in accordance with the method reported previously (Rowe et al. 2004). We also determined the daily time spent in PA corresponding to 1.1–2.9 METs (light), 3.0–5.9 METs (moderate), more than 6.0 METs (vigorous), and less than 1.1 METs (inactive). To assess the effects of PA on plasma Hcy, subjects were categorized into the high PA group and low PA group based on Exercise and Physical Activity Reference for Health Promotion 2006 in Japan, which proposed that the quantity of PA should be 23 METs h/week integrating the PA of more than 3 METs (equivalent to 8,000–10,000 steps per day) for health promotion. We set thresholds of categorization at 23 METs h/week integrating the PA of more than 3 METs or 10,000 steps as the total amount of PA or daily step counts. With regard to time spent in PA at each intensity, subjects were categorized on the basis of each median value.

### Assessment of dietary folate intake

The dietary habits, mainly nutrient intake, for the previous month were assessed with a validated brief-type self-administered diet history questionnaire (BDHQ) composed of 73 items developed based on the self-administered diet history questionnaire (DHQ) reported elsewhere (Okubo et al. 2008; Sasaki et al. 2000). Intake of folate was calculated in terms of energy density (per 1,000 kcal) that was used as covariate in ANOVA, and subjects were also classified into either ≥200 μg/day or <200 μg/day folate intake groups based on to the estimated average requirement (200 μg/day) for folic acid defined by Dietary Reference Intakes for Japanese, 2010.

### Genotyping of MTHFR gene

Genomic DNA was extracted from the plasma buffy coats and buccal cells using a QIAamp DNA Blood Maxi Kit (Qiagen, Tokyo, Japan). MTHFR SNP genotypes were determined by real-time PCR with TaqMan probes using an ABI Prism 7700 Sequence Detector (Perkin-Elmer Applied Biosystems, Foster, CA) as described previously with minor modifications (Iemitsu et al. 2006; Misono et al. 2009). In a preliminary study, we examined the precision of genotyping by TaqMan methods, and the concordance rate between 2 samples obtained on different days from 290 subjects was 100% (data not shown). The gene-specific primers and TaqMan probes for each SNP were synthesized using Primer Express v.1.5 software (Perkin-Elmer Applied Biosystems) according to the published DNA sequences for each SNP as follows: C677T (Ala→Val) in exon 5 of MTHFR (NCBI accession #rs1801133). The sequences of the oligonucleotides used were as follows:

MTHFR forward: 5′-GCACTTGAAGGAGAAGGTGTCT-3′

MTHFR reverse: 5′-CCTCAAAGAAAAGCTGCGTGATG-3′

MTHFR/G probe: 5′-ATGAAATCGGCTCCCGC-3′

MTHFR/A probe: 5′-ATGAAATCGACTCCCGC-3′

Ninety-six-well PCR plates were read on an ABI-7700 with end-point analysis mode of the SDS v.1.7a software package (Perkin-Elmer Applied Biosystems). Genotypes were determined automatically by the signal processing algorithms in the software.

### Statistical analyses

The $t$ test was used to compare the variables between men and women, and one-way ANOVA was used to compare the variables among genotype groups followed by Scheffé's test for multiple comparisons. Pearson's correlation coefficients ($r$) were calculated to evaluate the associations between continuous variables and plasma Hcy levels. ANOVA adjusted for age and/or sex and/or folate intake was used to test the interactions between MTHFR genotype and folate intake groups or PA groups categorized in determining plasma Hcy. When there was a significant interaction, Scheffé's test was used for multiple comparisons.

Statistical significance was set at $p < 0.05$. All statistical analyses were performed with SPSS for Macintosh, version 16.0 (SPSS Japan Inc., Tokyo, Japan).

## Results

### Characteristics of subjects

The characteristics of all subjects included in the present study are shown in Table 1. Males showed significantly higher Hcy levels than females ($p < 0.001$). There was no significant correlation between age and plasma Hcy. However, there was an interaction between age and sex in determining plasma Hcy ($p = 0.01$), with a significant positive correlation only in women ($r = 0.222, p < 0.001$). In our subjects, 36.6% (49 men and 110 women) were homozygous for the wild-type allele (CC), 49.8% (50 men and 166 women) were heterozygous (CT), and 13.6% (19 men and 40 women) were homozygous for the variant

**Table 1** The characteristics of the study population

| Characteristic | Male ($n = 118$) | Female ($n = 316$) | $p$ Value |
|---|---|---|---|
| Age (years) | 48.5 ± 13.71 | 55.4 ± 11.73 | <0.001 |
| Height (cm) | 170.0 ± 5.9 | 155.8 ± 6.0 | <0.001 |
| Weight (kg) | 69.1 ± 8.6 | 54.6 ± 8.3 | <0.001 |
| BMI (kg/m$^2$) | 23.9 ± 2.4 | 22.5 ± 3.3 | <0.001 |
| Systolic blood pressure (mmHg) | 122.3 ± 15.1 | 120.9 ± 17.1 | n.s. |
| Diastolic blood pressure (mmHg) | 75.1 ± 10.2 | 71.1 ± 10.4 | <0.001 |
| Fasting glucose (mg/dL) | 93.5 ± 11.1 | 93.0 ± 13.3 | n.s. |
| Triacylglycerol (mg/dL) | 112.0 ± 69.8 | 88.4 ± 55.3 | <0.001 |
| Total cholesterol (mg/dL) | 194.6 ± 30.3 | 215.6 ± 35.3 | <0.001 |
| HDL-cholesterol (mg/dL) | 54.3 ± 10.8 | 67.8 ± 15.0 | <0.001 |
| Serum folate (ng/mL) | 8.2 ± 3.4 | 11.1 ± 5.0 | <0.001 |
| Plasma homocysteine (nmol/mL) | 10.0 ± 5.8 | 7.1 ± 1.8 | <0.001 |
| Daily step count | 11185.4 ± 3099.2 | 11274.8 ± 3632.9 | n.s. |
| Total amount of physical activity (METs·h) | 3.9 ± 2.0 | 4.0 ± 2.2 | n.s. |
| Time spent in light physical activity (min) | 487.2 ± 103.9 | 588.5 ± 96.7 | <0.001 |
| Time spent in moderate physical activity (min) | 57.2 ± 21.9 | 62.0 ± 24.1 | n.s. |
| Time spent in vigorous physical activity (min) | 2.6 ± 6.7 | 1.8 ± 7.8 | n.s. |
| Time spent in inactive (min) | 892.9 ± 109.4 | 787.7 ± 101.4 | <0.001 |

$x \pm$ SD

Total amount of physical activity: sum of physical activity more than 3 METs

Light physical activity: less than 1.1–2.9 METs

Moderate physical activity: 3–5.9 METs

Vigorous physical activity: more than 6 METs

Inactive: less than 1.1 METs

allele (TT). The genotype distribution did not deviate from the Hardy–Weinberg equilibrium ($p > 0.05$). Mean values of plasma Hcy were significantly higher in the TT genotype compared with CC and CT genotypes (Table 2, $p < 0.001$). There were no significant differences in age, sex, dietary folate intake, or PA among genotypes (Table 2). There was an interaction between age and MTHFR genotype in determining plasma Hcy ($p < 0.001$), with a slight but not significant positive relation with age in the CT genotype ($r = 0.130$, $p = 0.056$) and a slight but not significant negative relation in the TT genotype ($r = -0.228$, $p = 0.082$). In addition, there was an interaction between sex and MTHFR genotype in determining plasma Hcy ($p < 0.001$).

Folate intake and plasma Hcy

The average values of folate intake were 185.8 ± 57.5 μg/day in men and 226.2 ± 69.0 μg/day in women ($p < 0.001$). There were significant positive correlations between age and folate intake in men ($r = 0.312$, $p = 0.001$) and women ($r = 0.401$, $p < 0.001$). ANCOVA performed with age and sex as covariates revealed a significant difference in plasma Hcy between the ≥200 μg/day group (7.6 ± 0.2 nmol/mL) and <200 μg/day group (8.3 ± 0.3 nmol/mL) ($p = 0.003$).

There was a significant interaction between dietary folate intake and MTHFR genotype in determining plasma Hcy ($p < 0.001$). Only the TT genotype showed a significant difference in plasma Hcy between folate intake groups; the <200 μg/day group had significantly higher plasma Hcy than the ≥200 μg/day group after adjusting for age and sex (12.36 ± 3.23 nmol/mL vs. 7.88 ± 3.19 nmol/mL, $p < 0.001$, Table 3). However, there were no such differences in the CC or CT genotypes.

Objectively measured PA and plasma Hcy

There were no gender-related differences in daily step counts or total amount of PA (METs h/week) (Table 1). However, women spent significantly longer times in light PA and shorter times in inactivity than men ($p < 0.001$). Both daily step counts and total amounts of PA were significantly negatively correlated with age ($r = -0.142$, $p = 0.003$ and $r = -0.206$, $p < 0.001$). There was a significant positive correlation between time spent in light PA

**Table 2** The characteristics by MTHFR genotype

| Characteristic | CC genotype ($n = 159$) | CT genotype ($n = 216$) | TT genotype ($n = 59$) | $p$ Value |
|---|---|---|---|---|
| Age (years) | 52.6 ± 12.3 | 54.7 ± 12.6 | 51.7 ± 13.6 | n.s. |
| Sex (men/women) | 49/110 | 50/166 | 19/40 | n.s. |
| Height (cm) | 160.6 ± 9.0 | 158.9 ± 8.6 | 159.8 ± 7.9 | n.s. |
| Weight (kg) | 59.7 ± 11.0 | 58.0 ± 10.1 | 57.6 ± 10.7 | n.s. |
| BMI (kg/m$^2$) | 23.1 ± 3.2 | 22.9 ± 3.1 | 22.4 ± 2.8 | n.s. |
| Plasma homocysteine (nmol/mL) | 7.5 ± 2.1 | 7.6 ± 2.1 | 10.3 ± 7.9 | <0.001 |
| Daily folate intake (μg/d) | 208.2 ± 68.4 | 222.4 ± 67.6 | 208.1 ± 69.9 | n.s. |
| Daily step count | 11195.3 ± 3590.3 | 11089.8 ± 3004.4 | 11987.6 ± 4672.7 | n.s. |
| Total amount of physical activity (METsịh) | 4.0 ± 2.4 | 3.8 ± 1.8 | 4.4 ± 2.6 | n.s. |
| Time spent in light physical activity (min) | 562.7 ± 111.0 | 566.3 ± 106.7 | 539.3 ± 106.3 | n.s. |
| Time spent in moderate physical activity (min) | 61.2 ± 23.5 | 59.1 ± 23.1 | 65.0 ± 25.3 | n.s. |
| Time spent in vigorous physical activity (min) | 2.2 ± 9.9 | 1.6 ± 4.5 | 3.2 ± 8.8 | n.s. |
| Time spent inactive (min) | 813.9 ± 120.6 | 813.0 ± 109.7 | 832.5 ± 108.4 | n.s. |

$x \pm$ SD

Total amount of physical activity: sum of physical activity more than 3 METs

Light physical activity: less than 1.1-2.9 METs

Moderate physical activity: 3-5.9 METs

Vigorous physical activity: more than 6 METs

Inactive: less than 1.1 METs

**Table 3** Mean plasma homocysteine concentrations of groups according to folate intake category and MTHFR genotype*

| | CC genotype | | CT genotype | | TT genotype | |
|---|---|---|---|---|---|---|
| | $n$ | Hcy (nmol/dL) | $n$ | Hcy (nmol/dL) | $n$ | Hcy (nmol/dL) |
| Daily folate intake | | | | | | |
| Less than criteria | 77 | 7.35 ± 3.26 | 88 | 7.76 ± 3.20 | 30 | 12.36 ± 3.23** |
| More than criteria | 82 | 7.43 ± 3.21 | 128 | 7.58 ± 3.25 | 29 | 7.88 ± 3.19 |

$x \pm$ SD

ANOVA adjusted for age and sex

Subjects were divided according to folate intake with the dividing point set defined by Dietary Reference Intake for Japanesem 2010

* Significant interaction between folate intake category and MTHFR genotype on plasma Hcy concentrations, $p < 0.001$

** Significantly different between the categorized groups by Bonfferoni test, $p < 0.001$

($r = 0.180$, $p < 0.001$) and age, whereas there were significant negatively correlations between time spent in moderate PA ($r = -0.128$, $p = 0.008$), vigorous PA ($r = -0.196$, $p < 0.001$), or inactivity ($r = -0.132$, $p = 0.006$) and age. The median values used for categorization were 567.7 min for light PA, 57.9 min for moderate PA, 0.2 min for vigorous PA, and 808.0 min for inactivity. A total of 27.4% of all subjects did not engage in vigorous PA at all. The characteristics of subjects divided into these categories are shown in Table 4. There were no differences in plasma Hcy between groups according to PA category. There were also no interactions between total amount of PA and age, sex, or folate intake on plasma Hcy, as well as daily step counts.

Interaction between PA and MTHFR genotype in determining plasma Hcy

There were no significant interactions between total amount of PA, daily step count, or time spent in moderate PA and MTHFR genotype in determining plasma Hcy, whereas there were significant interactions of time spent in light PA ($p = 0.003$), vigorous PA ($p = 0.001$), or inactivity ($p = 0.004$), and MTHFR genotype. Shorter time spent in light PA was associated with higher plasma Hcy only in the TT genotype (11.5 ± 3.3 nmol/mL vs. 8.5 ± 3.3 nmol/mL, $p < 0.001$, Fig. 1). Longer time spent in vigorous PA and inactivity were associated with higher plasma Hcy only in the TT genotype (11.8 ±

**Table 4** The characteristics of groups according to PA category

| Characteristic | $n$ | Age (years) | $p$ Value | Sex (men/women) | $p$ Value | BMI | $p$ Value | Hcy (nmol/dL)[a] | $p$ Value |
|---|---|---|---|---|---|---|---|---|---|
| Daily step count | | | | | | | | | |
| Less than criteria | 166 | 55.0 ± 13.3 | n.s. | 46/120 | n.s. | 23.2 ± 3.1 | n.s. | 7.9 ± 3.4 | n.s. |
| More than criteria | 268 | 52.6 ± 12.2 | | 72/196 | | 22.7 ± 3.1 | | 7.9 ± 3.4 | |
| Total amount of physical activity (METs¡h) | | | | | | | | | |
| Less than criteria | 180 | 56.0 ± 12.6 | 0.001 | 51/129 | n.s. | 23.3 ± 3.2* | 0.019 | 8.2 ± 3.4 | n.s. |
| More than criteria | 254 | 51.7 ± 12.4 | | 67 187 | | 22.6 ± 3.0 | | 7.7 ± 3.4 | |
| Time spent in light physical activity | | | | | | | | | |
| Less than median | 214 | 51.3 ± 13.6 | <0.001 | 89/125* | $p < 0.001$ | 23.2 ± 3.2* | 0.049 | 8.0 ± 3.5 | n.s. |
| More than median | 213 | 55.8 ± 11.1 | | 26/187 | | 22.6 ± 2.9 | | 7.7 ± 0.2 | |
| Time spent in moderate physical activity | | | | | | | | | |
| Less than median | 214 | 54.4 ± 13.0 | n.s. | 65/149 | n.s. | 23.3 ± 3.2* | 0.005 | 8.1 ± 3.4 | n.s. |
| More than median | 213 | 52.6 ± 12.2 | | 50/163 | | 22.4 ± 3.0 | | 7.7 ± 3.4 | |
| Time spent in vigorous physical activity | | | | | | | | | |
| Less than median | 221 | 57.7 ± 11.4 | <0.001 | 60/161 | n.s. | 23.2 ± 3.4* | 0.011 | 7.6 ± 3.5 | n.s. |
| More than median | 206 | 49.0 ± 12.4 | | 55/151 | | 22.5 ± 2.7 | | 8.2 ± 3.5 | |
| Time spent inactive | | | | | | | | | |
| Less than median | 214 | 55.2 ± 11.0 | 0.008 | 27/187* | $p < 0.001$ | 22.5 ± 2.9* | 0.008 | 7.6 ± 3.5 | n.s. |
| More than median | 213 | 51.9 ± 13.9 | | 88/125 | | 23.3 ± 3.3 | | 8.2 ± 3.5 | |

$x \pm$ SD

Subjects were divided on the basis of each criteria or median value

[a] ANOVA adjusted for age, sex, and folate intake

3.3 nmol/mL vs. 8.4 ± 3.2 nmol/mL, 11.6 ± 3.3 nmol/mL vs. 8.4 ± 3.3 nmol/mL, respectively, $p < 0.001$, Fig. 1). According to the classification of Kang et al., normal range of plasma Hcy is 5–15 nmol/mL, and 15–30 nmol/mL represents moderate hyperhomocysteinemia. In the present study, 7 subjects had plasma Hcy levels of more than 15 nmol/mL, consisting of 3 with the CT genotype and 4 with the TT genotype. All 4 subjects with the TT genotype were grouped into the shorter light PA group, longer vigorous PA group, and longer inactive group. Mean values of time spent in vigorous PA were 4.7 ± 14.1 min in the "more than" criteria group and 0.1 ± 0.1 min in the "less than" criteria group, respectively ($p < 0.001$).

## Discussion

In the present study, we investigated the associations between objectively measured PA and plasma Hcy in consideration of MTHFR C677T genotype. There were no significant differences in plasma Hcy adjusting for age, sex, and folate intake between groups categorized according to the daily step count, total amount of PA, or time spent in PA at each intensity in all subjects. However, when MTHFR genotype was included in the analysis, significant interactions were identified between MTHFR genotype and time spent in PA of certain intensities to determine plasma Hcy. The subjects who spent shorter time in light PA had significantly higher plasma Hcy than those who spent longer time in light PA in the TT genotype. Moreover, the subjects who spent longer time spent in vigorous PA or inactivity had significantly higher plasma Hcy than those who spent shorter time in the TT genotype. The differences in plasma Hcy between PA groups in the TT genotype were 3.0–3.4 nmol/mL, and this difference was in agreement with the values that have been reported to be associated with an 11% lower ischemic heart disease and 19% lower stroke risk (Homocysteine Studies Collaboration 2002). These observations represent the first evidence related to the interactions among PA according to intensity, plasma Hcy, and MTHFR genotype.

Nygard et al. (1995) and Dankner et al. (2007) reported that PA was inversely associated with total Hcy, whereas de Bree et al. (2001) reported a weak positive relation between PA and plasma total Hcy in women. Furthermore, several studies indicated no relationship between PA and plasma Hcy (Husemoen et al. 2004; Joubert and Manore 2008; Ruiz et al. 2007a; Saw et al. 2001). These discrepancies may have been due to the different methods used for evaluation of PA or lack of classification by MTHFTR genotype. The self-reported questionnaire has often been

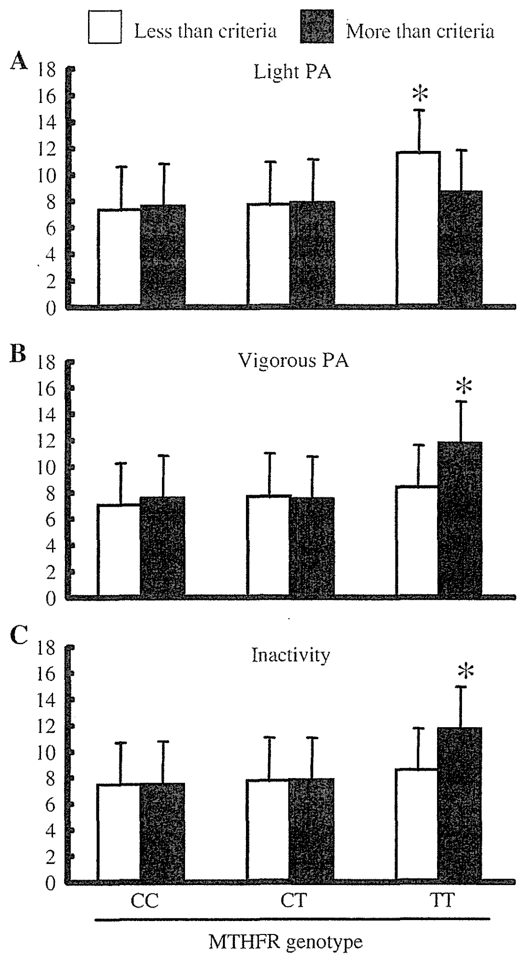


**Fig. 1** Mean plasma homocysteine concentrations of groups according to PA category (amount of light PA, vigorous PA, inactivity) and MTHFR genotype. Subjects were divided into "less than" criteria and "more than" criteria groups, with the dividing line set at the median value of time spent in PA at each intensity. $*p < 0.001$, significant difference compared with other groups. Data are expressed as mean ± SD

used for assessment of PA, but it is not suitable for precise assessment of PA. The first strength of the present study was that daily PA of subjects was evaluated by triaxial accelerometry because self-reported PA may be subject to bias and misclassification (Freedson et al. 1998). Moreover, this study analyzed the interaction between amount of PA and MTHFR C677T genotype. Thus, although we failed to identify differences according to daily step count or total amount of PA, the detailed PA indexes, such as time spent in light PA, vigorous PA, or inactivity, showed significant interactions with MTHFR genotypes on plasma Hcy. In addition, several studies indicated that cardiovascular fitness is associated with plasma Hcy (Kuo et al. 2005; Ruiz et al. 2007b), and the association between UCP3 polymorphism and plasma Hcy was modified by cardiorespiratory fitness (Labayen et al. 2010). These studies suggested that the concepts of gene–gene interactions and gene–environment interactions are critical to provide personalized prescriptions to prevent hyperhomocysteinemia and cardiovascular diseases.

We speculate that there may be two pathways by which PA influences plasma Hcy. The first is the pathway associated with creatine. Phosphocreatine (PCr) is a high-energy phosphate for muscle contraction, and Cr is endogenously synthesized in the liver and kidney from arginine and glycine via methylation by *S*-adenosyl-L-methionine:guanidinoacetate *N*-methyltransferase (GAMT) closely linked to Hcy metabolism (Wyss and Kaddurah-Daouk 2000). In humans, Cr synthesis has been reported to account for 70% of Hcy formation (Mudd et al. 1980; Mudd and Poole 1975). Therefore, the increase in Cr synthesis in the liver corresponding to high-intensity PA may underlie the higher plasma Hcy level in TT genotype with longer time spent in vigorous PA in the present study. However, we could not reach definitive conclusions because we did not examine Cr concentration in skeletal muscle. The second possibility is the pathway via betaine, which is one of the remethylation pathways of Hcy. Betaine supplementation decreases plasma Hcy (Olthof and Verhoef 2005), and plasma betaine was reported to be inversely related to plasma Hcy (Holm et al. 2004). In addition, it has been reported that betaine was positively associated with PA (Konstantinova et al. 2008). Betaine is formed in the kidney and liver from choline catalyzed by the mitochondrial enzyme choline dehydrogenase, the level of which may increase accompanying mitochondrial biogenesis by light PA. Therefore, the inverse correlation between time spent in light PA and plasma Hcy in the TT genotype may be mediated through the betaine pathway. However, the physiological mechanisms underlying the associations between time spent in light PA and plasma Hcy warrant further investigation.

This study had some limitations. First, we analyzed the effects of PA on plasma Hcy in considering MTHFR genotype with men and women together to achieve statistical power. However, there was a gender difference in plasma Hcy, and it will be necessary to analyze men and women separately in a larger study population. Second, we could not eliminate other predictors of Hcy, such as smoking status or alcohol consumption, in the present study. These factors may obscure the effect of PA on plasma Hcy. Finally, due to the cross-sectional design of this study, causality could not be established. It will be necessary to confirm the effects of PA on plasma Hcy in a future longitudinal study, and replication of the results in larger study populations is necessary before firm conclusions can be drawn.

## Conclusion

There were no significant differences in plasma Hcy adjusting for age, sex, and folate intake between groups categorized according to PA among all subjects. However, in the TT genotype alone, shorter time spent in light PA was associated with higher plasma Hcy than a longer time spent in light PA, and longer time spent in vigorous PA and inactivity was associated with higher plasma Hcy. Further investigations with a larger sample size or a longitudinal design are required.

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調査・研究

# 日本人の閉眼片足立ちの評価と運動習慣との関連

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## はじめに

平衡機能は，全身持久力，筋力，柔軟性とともに体力の要素である．全身持久力[1)]，筋力[2)]が生命予後と関連していることが報告されているものの，平衡機能については明らかではない．また，2006年，厚生労働省から日本人のための運動基準[3)]が示されたが，その中でも平衡機能に関する基準値は示されなかった．一方，文部科学省では，新体力テスト実施要項を発表しているが，65～79歳では平衡機能の指標として開眼片足立ちが推奨されているものの，20～64歳では平衡機能の項目はない[4,5)]．

今回われわれは，今後の日本人の適切な運動処方の基礎資料とするために，岡山県南部健康づくりセンターでメディカルチェック（尿，血液検査），ヘルスチェック（健康度測定）を受け，治療を受けていない人を対象に平衡機能の指標として閉眼片足立ちを測定し，性，年代別の平均値を算出して，運動習慣との関連を検討した．

## 1．対象と方法

対象は1997年6月～2008年3月までに岡山県南部健康づくりセンターで，メディカルチェック，ヘルスチェックを受け，糖尿病，高血圧，整形外科的疾患などで治療を受けていない20歳以上69歳未満の男性2,472人，女性5,780人，合計8,252人であった（表1）．メディカルチェック，ヘルスチェックは同センターで健康づくり実践のために年1回受けてもらうことになっており，複数回受診の場合は1回目の測定値を採用した．

測定項目は，閉眼片足立ちのほか，身長，体重，腹囲，ヒップ囲，運動習慣の有無であった．閉眼片足立ちは，次のように測定した．①両手を腰にあて，どちらの足が立ちやすいかを確かめるため，片足立ちを左右について行なう．②支持脚が決まったら，両手を腰に当て，閉眼し，「片足をあげて」の指示で片足立ちの姿勢をとる（片足を前方にあげる）．③片足立ちの持続時間を計測し，最長120秒で打ち切る．④記録は秒単位とし，終了の条件はあげた足が支持脚や床に触れた場合，

表1　対象

| | 男性 | 女性 |
|---|---|---|
| 症例数 | 2,472 | 5,780 |
| 年齢 | 39.1 ± 12.0 | 39.6 ± 12.6 |
| 身長（cm） | 169.8 ± 6.0 | 156.9 ± 5.5 |
| 体重（kg） | 70.3 ± 11.5 | 54.6 ± 8.7 |
| BMI（kg/m²） | 24.3 ± 3.6 | 22.2 ± 3.4 |
| 腹囲（cm） | 83.1 ± 9.9 | 70.8 ± 8.9 |
| ヒップ囲（cm） | 94.3 ± 6.1 | 90.8 ± 5.8 |
| 閉眼片足立ち（秒） | 37.2 ± 34.7 | 36.7 ± 34.9 |

平均値±標準偏差

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表2 性，年代別の閉眼片足立ち（秒）の変化

男性

| 年代 | 症例数 | 平均値±標準偏差 | 120秒達成者数 | |
|---|---|---|---|---|
| 20～29 | 657 | 51.6 ± 40.0 | 103 (18.3%) | |
| 30～39 | 738 | 40.6 ± 35.0 | 54 (7.3%) | a |
| 40～49 | 530 | 32.8 ± 29.6 | 15 (2.8%) | ab |
| 50～59 | 369 | 22.5 ± 23.1 | 4 (1.1%) | abc |
| 60～69 | 178 | 13.1 ± 12.8 | 0 (0%) | abcd |

女性

| 年代 | 症例数 | 平均値±標準偏差 | 120秒達成者数 | |
|---|---|---|---|---|
| 20～29 | 1,635 | 49.2 ± 39.2 | 223 (13.6%) | |
| 30～39 | 1,469 | 44.2 ± 36.7 | 121 (8.2%) | a |
| 40～49 | 1,229 | 32.3 ± 29.4 | 43 (3.5%) | ab |
| 50～59 | 967 | 22.6 ± 23.6 | 18 (1.9%) | abc |
| 60～69 | 480 | 11.5 ± 14.0 | 3 (0.6%) | abcd |

a: p<0.05 vs 20～29，b: p<0.05 vs 30～39，
c: p<0.05 vs 40～49，d: p<0.05 vs 50～59

表3 性，年代別の運動習慣ありの者

| 年代 | 男性 | | 女性 | |
|---|---|---|---|---|
| | 人数 | % | 人数 | % |
| 20～29 | 189 | 28.8 | 274 | 16.8 |
| 30～39 | 199 | 27.0 | 256 | 17.4 |
| 40～49 | 190 | 35.8 | 329 | 26.8 |
| 50～59 | 139 | 37.7 | 347 | 35.9 |
| 60～69 | 86 | 48.3 | 240 | 50.0 |
| 合計 | 803 | 32.5 | 1,446 | 25.0 |

支持脚の位置がずれた場合，腰にあてた両手もしくは片手が腰から離れた場合とした．①～④を2回繰り返し，よい方の値を閉眼片足立ちの値として採用した．

腹囲は，立位呼気時に臍部で測定[6]し，運動習慣の有無は国民健康・栄養調査の運動習慣の定義にもとづいて，1回30分，週2回以上，3カ月以上継続している場合を運動習慣ありとした．

結果は平均値±標準偏差で表し，有意差検定は，対応のないt検定，一元配置分散分析法，Scheffe法，共分散分析法を用い，有意水準5%未満を有意とした．

なお，本調査に関しては岡山県健康づくり財団倫理委員会の承認を得た．

## 2. 結 果

性，年代別に閉眼片足立ちの値を比較したものを表2に示す．120秒達成者を120秒として，性，年代別に比較すると，男女とも加齢に伴って有意な低下を認めた．また，120秒達成者数，割合ともに加齢に伴って低下していた．

運動習慣の有無を性，年代別に検討すると（表3），男性の運動習慣者は803人（32.5%）で，加齢に伴い運動習慣者の割合が増加し，60歳代での運動習慣者は86人（48.3%）ともっとも高かった．女性の運動習慣者は1,446人（25.0%）で男性より運動習慣者の割合は低かったが，年代別の検討では男性と同様に加齢に伴い運動習慣者の割合が増加し，60歳代では運動習慣者は240人（50.0%）となっていた．

性，年代別に運動習慣の有無による閉眼片足立ちの値を比較した（表4，図1）．男性の30歳代，40歳代，女性の20歳代で，運動習慣のある者では運動習慣のない者に比較すると閉眼片足立ちの値が有意に高値を示した．その他の性，年代では運動習慣の有無による閉眼片足立ちの差は認めなかった．

## 3. 考 察

今回，われわれは岡山県南部健康づくりセンターのメディカルチェック，ヘルスチェック受診者で，閉眼片足立ちを測定し，運動習慣との関連を検討した．

今回の調査の特徴は治療を受けていない，いわゆる健常と思われる人での閉眼片足立ちの性，年代別の平均値を算出したことである．文部科学省の新体力テストでは前述のように65～75歳で閉眼片足立ちが推奨されているものの，20～64歳の項目では採用されていない．一方，松原ら[7]は，当センターの類似施設で，20～70歳代男女6,287人の閉眼片足立ちの測定を行ない，男女とも20