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ORIGINAL ARTICLE

Higher cardiorespiratory fitness attenuates the risk of atherosclerosis associated with *ADRB3* Trp64Arg polymorphism

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Abstract
Purpose β3-Adrenergic receptor (*ADRB3*) Trp64Arg polymorphism is associated with atherogenic risk factors that include weight gain, insulin resistance, and diabetes. Habitual exercise brings higher cardiorespiratory fitness and results in the amelioration of atherosclerotic risk factors. However, the effects of cardiorespiratory fitness level and *ADRB3* Trp64Arg polymorphism on the risk of cardiovascular disease remain unclear. A cross-sectional investigation of 877 Japanese men and women (18–75 years old) was performed to clarify the effects of cardiorespiratory fitness on the relationship between *ADRB3* Trp64Arg polymorphism and risk of cardiovascular disease.
Method Common carotid intima-media thickness (ccIMT) and blood lipid profiles were assessed as surrogate markers of atherosclerosis. We measured peak oxygen uptake ($\dot{V}O_{2peak}$) during incremental cycle ergometer exercise testing. Subjects were divided into groups with high (High-Fit) and low (Low-Fit) levels of cardiorespiratory fitness based on the median value of $\dot{V}O_{2peak}$ for sex and decade.
Results Levels of body fat, triglycerides, and plasma glucose were lower and high-density lipoprotein cholesterol levels and $\dot{V}O_{2peak}$ were higher in High-Fit subjects than Low-Fit subjects. *ADRB3* Trp64Arg polymorphism did not significantly affect ccIMT or blood lipid profiles. In Low-Fit subjects, ccIMT was higher in individuals with the Arg/Arg genotype compared to the Trp/Trp and Trp/Arg genotypes (each $P < 0.0001$); however, *ADRB3* polymorphism had no effect in High-Fit subjects.
Conclusion Higher levels of cardiorespiratory fitness may attenuate the risk of atherosclerosis associated with *ADRB3* Trp64Arg polymorphism.

Keywords Peak oxygen uptake · Carotid artery · Intima-media thickness · Arterial stiffness

Abbreviations

ADRB3	β3-Adrenergic receptor
Arg	Arginine
ccIMT	Common carotid intima-media thickness
DBP	Diastolic blood pressure
eNOS	Endothelial nitric oxide synthase
HDL	High-density lipoprotein cholesterol
High-Fit	High cardiorespiratory fitness
L-NAME	*N*(G)-nitro-L-arginine methyl ester
Low-Fit	Low cardiorespiratory fitness
RPE	Rating of perceived exertion
SBP	Systolic blood pressure
SNP	Single nucleotide polymorphism
TC	Total cholesterol
TG	Triglyceride
Trp	Tryptophan
$\dot{V}O_{2peak}$	Peak oxygen uptake
β-stiffness	Carotid β-stiffness

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Introduction

Common carotid intima-media thickness (ccIMT) is an independent predictor of cardiovascular risk as well as all-cause and cardiovascular mortality. Increased arterial wall IMT is associated with higher morbidity involving coronary and peripheral atherosclerosis (Agewall et al. 1996; Burke et al. 1995; Lekakis et al. 2000; Tanaka et al. 2002) which is a risk factor of myocardial infarction and stroke (Moreau et al. 2002; O'Leary et al. 1999). In a cross-sectional study, ccIMT was found to be lower in individuals with high levels of cardiorespiratory fitness compared with individuals with low levels of cardiorespiratory fitness (Gando et al. 2011). Furthermore, regular aerobic exercise reduces femoral IMT and ccIMT in healthy subjects (Dinenno et al. 2001; Tanaka et al. 2002). Thus, regular exercise attenuates the risk of atherosclerosis.

β3-Adrenergic receptor (*ADRB3*) signaling mainly occurs in adipose tissue and plays a critical role in energy expenditure through the regulation of lipolysis and thermogenesis (Morrison et al. 1999). *ADRB3*, expressed by endothelial cells, participates in the regulation of endothelial function by activating endothelial nitric oxide synthase (eNOS) (Kou and Michel 2007; Napp et al. 2009; Trochu et al. 1999). Consequently, *ADRB3* stimulation induces relaxation of the aorta (Kou and Michel 2007; Napp et al. 2009; Trochu et al. 1999). Moreover, chronic NOS inhibition in the form of *N*(G)-nitro-L-arginine methyl ester administration for 8 weeks causes increases in ccIMT in the coronary and carotid arteries (Kristek and Gerová 1996). NOS activation through L-arginine administration prevents atherosclerosis with a contribution from eNOS (Aji et al. 1997; Li and Förstermann 2009, Radomski and Salas 1995). *ADRB3* stimulation-induced activation of NOS in endothelial cells plays a protective role in arteriosclerosis and atherosclerosis, which may lead to cardiovascular disease risk reduction.

A T-to-C transition in codon 64 of exon 1 of the gene encoding *ADRB3* on chromosome 8 resulting in a tryptophan to arginine (Trp64Arg) variation is associated with reduced lipolytic activity compared to the T genotype (Trp containing protein) (Clément et al. 1995; Umekawa et al. 1999). The Trp64Arg polymorphism in *ADRB3* is associated with hypertension in male patients with type 2 diabetes (Ringel et al. 2000) and essential hypertension (Tahara et al. 2010). Other studies, however, indicate *ADRB3* polymorphism is not associated with the risk for cardiovascular disease (Morrison et al. 1999; Rissanen et al. 1997; Stangl et al. 2001; Tamaki et al. 1999; Zafarmand et al. 2008). Therefore, there are conflicting reports on the effects of *ADRB3* Trp64Arg polymorphism on the risk of cardiovascular. Habitual exercise brings about higher cardiorespiratory fitness and results in the prevention or reduction of the risk for cardiovascular disease (Cooper et al. 1976; Dinenno et al. 2001; Ferreira et al. 2003; Iemitsu et al. 2010; Tanaka et al. 2000, 2002). However, it remains unclear whether cardiorespiratory fitness levels affect the relationship between genetic variations in *ADRB3* and risks for cardiovascular disease, such as blood pressure, ccIMT, and lipid profiles.

We hypothesize that the Trp64Arg single nucleotide polymorphism (SNP; T→C) genotype in exon 1 of *ADRB3* on chromosome 8 and cardiorespiratory fitness levels will affect the risk of cardiovascular disease in healthy Japanese subjects. The present study was a cross-sectional investigation of 877 Japanese men and women between 18 and 75 years of age to clarify the effects of cardiorespiratory fitness on the relationship between *ADRB3* Thr64Arg polymorphism and cardiovascular disease risks, including blood pressure, ccIMT, carotid β-stiffness (β-stiffness), and lipid profiles.

Methods

Subjects

A total of 877 Japanese subjects (241 men and 636 women) between 18 and 75 years of age participated in this cross-sectional study (mean 44 ± 1 years). Subjects were sedentary or moderately physically active (at least 60 min session per week), and did not participate in any other vigorous sports activity. Subjects were divided into low cardiorespiratory fitness (Low-Fit) and high cardiorespiratory fitness (High-Fit) groups based on the median value of peak oxygen uptake ($\dot{V}O_{2peak}$), an index of cardiorespiratory fitness, based on sex and decade. Subjects were recruited for the present study by advertisement. Subjects with a history of stroke, diabetes, hypertension, hyperlipidemia, cardiac disease, chronic renal failure were excluded from the study and took no medications, such as anti-hyperlipidemic, anti-hypertensive, or diabetic drugs, and did not smoke. β-Stiffness was examined as an index of arteriosclerosis and ccIMT was examined as an index of atherosclerosis. Systolic blood pressure (SBP), diastolic blood pressure (DBP), percent body fat, and genotype at *ADRB3* Trp64Arg polymorphism were also determined. Total body fat mass was determined using dual energy X-ray absorptiometry (Hologic QDR-4500A scanner; Hologic, Bedford, MA). Body composition was determined by Hologic software version 11.2:3 for Windows (Hologic). Brachial SBP and DBP were measured in the supine position at rest with a vascular testing device (Colin Medical Technology, Tokyo, Japan). Serum cholesterol and triglyceride (TG) levels and plasma glucose levels were also measured.

The study was approved by the Ethical Review Board of the National Institute of Health and Nutrition. Written informed consent was obtained from all subjects before inclusion in the study.

Measurement of $\dot{V}O_{2peak}$

$\dot{V}O_{2peak}$ was measured using an incremental cycle exercise test on a cycle ergometer (828E; Monark, Varberg, Sweden). Incremental cycle exercise testing began at a work rate of 90 W (60–120 W) for men and 60 W (30–90 W) for women, and power output was increased by 15 W min^{-1} until the subjects could no longer maintain a fixed pedaling frequency of 60 rpm. During testing, subjects were encouraged to exercise at maximum intensity. Heart rate and rating of perceived exertion (RPE) were monitored minute by minute during exercise testing. RPE was obtained using the modified Borg scale (Borg 1982). $\dot{V}O_2$ was monitored during the last 30 s of each period of increased work rate. Subjects breathed through a low-resistance 2-way valve, and the expired air was collected in Douglas bags. Expired O_2 and CO_2 concentration were measured using mass spectrometry (ARCO-1000A; Arco System, Chiba, Japan), and gas volume was determined using a dry gas meter (DC-5C; Shinagawa Seiki, Tokyo, Japan). The highest value of $\dot{V}O_2$ during the exercise test was designated as $\dot{V}O_{2peak}$ if three of the following criteria were met: (a) a plateau in $\dot{V}O_2$ with increases in external work, (b) a maximal respiratory exchange ratio $\geq$1.1, (c) a maximal heart rate $\geq$90 % of the age-predicted maximum (208 − 0.7 × age) (Tanaka et al. 2001), and (d) RPE $\geq$ 18.

Measurement of ccIMT

Common carotid intima-media thickness was measured from images obtained using a Vivid *i* ultrasound system (GE Medical Systems, Milwaukee, WI) equipped with a high-resolution broadband linear array transducer as described previously (Kawano et al. 2006; Miyachi et al. 2004). Ultrasound images were analyzed using image analysis software (Image J; National Institutes of Health, Bethesda, MD). At least ten measurements of ccIMT were obtained for each segment, and mean values were used for further analyses. This technique has excellent day-to-day reproducibility (coefficient of variation, 3 ± 1 %).

Measurement of the β-stiffness index

Pressure waveforms and amplitudes were obtained from the common carotid artery using a pencil-shaped probe with a high-fidelity strain gauge transducer (SPT-301; Millar Instruments, Houston, TX) (Tanaka et al. 2000). The β-stiffness index was calculated using the equation $[\ln(P1/P0)]/[(D1 - D0)/D0]$, where $D1$ and $D0$ are the maximum (systolic) and minimum (diastolic) diameters, and $P1$ and $P0$ are the highest (systolic) and lowest (diastolic) blood pressures, respectively. The day-to-day coefficients of variation for carotid artery diameter, pulse pressure, and β-stiffness were 2 ± 1, 7 ± 3, and 5 ± 2 %, respectively.

SNP genotyping

Genomic DNA was extracted from plasma buffy coats and buccal cells using a QIAamp DNA Blood Maxi Kit (Qiagen, Tokyo, Japan). The genotype of *ADRB3* Trp64Arg SNP was determined with real-time PCR using TaqMan probes and the Applied Biosystems 7500 Fast Real-time PCR System (Life Technologies Japan, Tokyo, Japan) as described previously with minor modifications (Iemitsu et al. 2006, 2010). Gene-specific primers and TaqMan probes for each SNP were synthesized using Primer Express v.1.5 software (Applied Biosystems, Foster City, CA) according to published DNA sequences for each SNP as follows: Trp64Arg (T → C) in exon 1 of *ADRB3* (NCBI accession rs4994). The sequences of the oligonucleotides were as follows:

ADRB3 forward	5′-GCAACCTGCTGGTCATCGT-3′
ADRB3 reverse	5′-GTTGGTCATGGTCTGGAGTCT-3′
ADRB3/T probe	5′-CATCGCCTGGACTC-3′
ADRB3/C probe	5′-ATCGCCCGGACTC-3′

PCR plates with 96 wells were examined using the Applied Biosystems 7500 Fast Real-time PCR System using the end-point analysis mode of the SDS v.1.7a software package (Life Technologies Japan). Genotypes were determined automatically using the signal processing algorithms of the software.

Measurements of serum cholesterol and TG levels and plasma glucose levels

Fasting serum concentrations of total cholesterol (TC) and high-density lipoprotein cholesterol (HDL) and TG, and plasma concentrations of glucose were determined using standard enzymatic techniques.

Statistical analysis

The frequencies of *ADRB3* alleles were calculated using a gene-counting method, and Hardy–Weinberg equilibrium was confirmed using the χ^2 test. Differences between the High-Fit and Low-Fit groups were examined using Student's *t* tests for unpaired values. One-way analysis of variance was used to evaluate differences among *ADRB3* genotypes. Differences between fitness groups stratified by genotype were

Table 1 Characteristics of subjects in the high cardiorespiratory fitness and low cardiorespiratory fitness groups

	Low-Fit ($n = 441$)	High-Fit ($n = 436$)
Age (years)	45 ± 1	44 ± 1
Body weight (kg)	60 ± 1	58 ± 1*
Height (cm)	162 ± 1	162 ± 1
Body fat (%)	26.9 ± 0.3	22.4 ± 0.3*
BMI (kg/m^2)	22.9 ± 0.2	21.9 ± 0.1*
SBP (mmHg)	116.0 ± 0.8	115.0 ± 0.6
DBP (mmHg)	69.5 ± 0.6	68.3 ± 0.5
β-Stiffness (AU)	8.3 ± 0.2	7.7 ± 0.2
ccIMT (mm)	0.63 ± 0.01	0.62 ± 0.01
Total cholesterol (mg/dl)	202 ± 2	198 ± 2
HDL cholesterol (mg/dl)	63 ± 1	69 ± 1*
Triglycerides (mg/dl)	90 ± 3	75 ± 2*
Glucose (mg/dl)	91 ± 1	89 ± 1*
$\dot{V}O_{2peak}$ (ml/kg/min)	28.2 ± 0.3	37.5 ± 0.4*

Values are means and SE

High-Fit high cardiorespiratory fitness, *Low-Fit* low cardiorespiratory fitness, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *β-stiffness* carotid β-stiffness, *AU* arbitrary units, *ccIMT* common carotid intima-media thickness, *HDL* high-density lipoprotein, $\dot{V}O_{2peak}$ peak oxygen uptake

* $P < 0.05$ vs. Low-Fit

assessed using a two-way analysis of covariance model that included age and sex as covariates, and a Fisher's post hoc test was applied when the difference was significant. Values are expressed as mean ± SE. $P < 0.05$ was defined as statistically significant. All statistical analyses were performed with StatView 5.0 (SAS Institute, Tokyo, Japan).

Results

Comparison of characteristics among subjects with low and high cardiorespiratory fitness

The High-Fit group had significantly lower body weight, percent body fat, body mass index (BMI), and blood glucose and TG levels than the Low-Fit group. HDL levels and $\dot{V}O_{2peak}$ values were significantly higher in the High-Fit group (Table 1). No significant differences between the High-Fit and Low-Fit groups were noted for age, height, SBP, DBP, β-stiffness, ccIMT, or TC levels (Table 1).

Comparison of characteristics in subjects of different genotypes

Table 2 shows the distribution of *ADRB3* genotypes among the study subjects. No significant differences were found between males and females in the frequencies of alleles at *ADRB3* Trp64Arg. In addition, allele frequencies did not deviate from the expected Hardy–Weinberg equilibrium.

Table 2 Distribution of gene polymorphisms of ADRB3 Trp64Arg and allele frequency in the study subjects

	Total	Male	Female
Genotypes (%) (*n*)			
Trp/Trp	67 (590)	71 (172)	66 (418)
Trp/Arg	29 (252)	26 (62)	30 (190)
Arg/Arg	4 (35)	3 (7)	4 (28)
Allele frequency ADRB3 64 (Arg allele)	0.18	0.16	0.19

The genotype frequencies did not deviate from Hardy–Weinberg equilibrium. No difference was found between genders

ADRB3 β3-adrenergic receptor

Table 3 Genotypes of ADRB3 Trp64Arg and subject characteristics

	ADRB3 Trp64Arg		
	Trp/Trp ($n = 590$)	Trp/Arg ($n = 252$)	Arg/Arg ($n = 35$)
Age (years)	44 ± 1	44 ± 1	43 ± 3
Body weight (kg)	59 ± 1	59 ± 1	59 ± 2
Height (cm)	162 ± 1	161 ± 1	162 ± 1
Body fat (%)	24.5 ± 0.3	25.0 ± 0.4	24.8 ± 1.2
BMI (kg/m^2)	22.3 ± 0.1	22.6 ± 0.2	22.5 ± 0.6
SBP (mmHg)	115.8 ± 0.6	114.7 ± 1.0	116.4 ± 2.5
DBP (mmHg)	68.9 ± 0.4	68.9 ± 0.7	67.9 ± 1.9
β-Stiffness (AU)	7.9 ± 0.2	8.0 ± 0.2	8.7 ± 1.3
ccIMT (mm)	0.63 ± 0.01	0.62 ± 0.01	0.64 ± 0.02
Total cholesterol (mg/dl)	200 ± 1	202 ± 2	192 ± 6
HDL cholesterol (mg/dl)	65 ± 1	67 ± 1	64 ± 2
Triglycerides (mg/dl)	82 ± 2	84 ± 3	80 ± 7
Glucose (mg/dl)	90 ± 1	90 ± 1	88 ± 1
$\dot{V}O_{2peak}$ (ml/kg/min)	33.2 ± 0.3	32.2 ± 0.5	33.1 ± 1.3

Values are means and SE

ADRB3 β3-adrenergic receptor, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *β-stiffness* carotid β-stiffness, *AU* arbitrary units, *ccIMT* common carotid intima-media thickness, *HDL* high-density lipoprotein, $\dot{V}O_{2peak}$ peak oxygen uptake

We then compared the characteristics of subjects with different *ADRB3* alleles (Table 3). No significant differences were found among subjects with different genotypes for all parameters examined.

Comparison of characteristics between subjects of various genotypes and cardiorespiratory fitness levels

We compared the characteristics of subjects between different genotypes and levels of fitness (Table 4). All High-Fit

Table 4 Characteristics of subjects in each cardiorespiratory fitness and genotypes of ADRB3 Trp64Arg group

	Low-Fit			High-Fit		
	Trp/Trp ($n = 306$)	Trp/Arg ($n = 117$)	Arg/Arg ($n = 18$)	Trp/Trp ($n = 284$)	Trp/Arg ($n = 135$)	Arg/Arg ($n = 17$)
Age (years)	44 ± 1	45 ± 1	49 ± 4	44 ± 1	43 ± 1	37 ± 3
Body weight (kg)	61 ± 1	60 ± 1	62 ± 3	58 ± 1	57 ± 1	57 ± 3
Height (cm)	163 ± 1	160 ± 1	161 ± 2	162 ± 1	161 ± 1	163 ± 2
Body fat (%)	26.8 ± 0.4	26.9 ± 0.5	29.2 ± 1.6	22.3 ± 0.3	22.8 ± 0.6	20.5 ± 1.0
BMI (kg/m^2)	22.8 ± 0.2	23.1 ± 0.3	23.6 ± 0.9	21.9 ± 0.1	22.0 ± 0.2	21.3 ± 0.8
SBP (mmHg)	115.6 ± 0.9	116.4 ± 1.5	119.9 ± 3.7	115.9 ± 0.8	112.8 ± 1.1	113.1 ± 3.3
DBP (mmHg)	69.1 ± 0.7	70.1 ± 1.1	70.1 ± 2.8	68.8 ± 0.6	67.5 ± 0.9	65.9 ± 2.5
Total cholesterol (mg/dl)	200 ± 2	205 ± 3	205 ± 10	199 ± 2	199 ± 4	180 ± 8
HDL cholesterol (mg/dl)	62 ± 1	65 ± 1	62 ± 3	69 ± 1	69 ± 2	67 ± 4
Triglycerides (mg/dl)	89 ± 4	93 ± 5	91 ± 9	76 ± 2	73 ± 3	70 ± 10
Glucose (mg/dl)	91 ± 1	90 ± 1	91 ± 2	89 ± 1	90 ± 1	86 ± 2
$\dot{V}O_{2peak}$ (ml/kg/min)	28.5 ± 0.4	27.9 ± 0.5	27.4 ± 1.3	37.5 ± 0.4	37.2 ± 0.7	38.4 ± 1.3

Values are means and SE

ADRB3 β3-adrenergic receptor, *High-Fit* high cardiorespiratory fitness, *Low-Fit* low cardiorespiratory fitness, *HDL* high-density lipoprotein, $\dot{V}O_{2peak}$ peak oxygen uptake

subjects had lower percentages of body fat and higher $\dot{V}O_{2peak}$ than Low-Fit subjects; there was a significant main effect of fitness level independent of *ADRB3* genotype ($P < 0.001$). No significant differences were noted between individuals according to cardiorespiratory fitness and genotype with respect to age, body weight, height, BMI, blood pressure, TC, HDL, TG, and glucose levels.

Comparison of ccIMT and β-stiffness between subjects of various genotypes and cardiorespiratory fitness levels

The interaction between *ADRB3* genotype and fitness level was significant for ccIMT ($P = 0.025$). The ccIMT values of Low-Fit subjects with the Arg/Arg genotype were significantly higher than those of individuals with the Trp/Trp or Trp/Arg genotype, whereas no significant differences were observed in ccIMT among the three genotypes in the High-Fit group (Fig. 1). In the Arg/Arg genotype, the ccIMT values of High-Fit subjects were significantly lower than those of Low-Fit subjects (Fig. 1).

On the other hand, the interaction between *ADRB3* genotype and fitness level was not significant for β-stiffness. β-Stiffness was not different among subjects with the three Trp64Arg genotypes in either the Low-Fit or High-Fit group (Fig. 2).

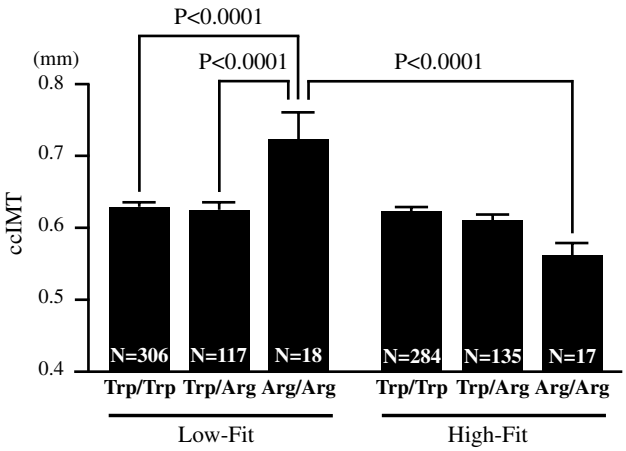

Fig. 1 Common carotid intima-media thickness (ccIMT) values are shown for each fitness group and for individuals with various genotypes in *ADRB3* (encoding Trp/Trp, Trp/Arg, or Arg/Arg at amino acid position 64). Data are expressed as mean ± SE. Low-Fit, low cardiorespiratory fitness group; High-Fit, high cardiorespiratory fitness group

Discussion

The present study investigated the relationship among a risk factor for atherosclerosis (ccIMT), cardiorespiratory fitness, and genotype at *ADRB3* Trp64Arg in healthy Japanese subjects. Interestingly, Low-Fit subjects with the Arg/Arg genotype had a higher ccIMT than subjects at a comparable level of fitness with the Trp/Trp genotype and in a higher level of fitness with the Arg/Arg genotype. However, this difference was not observed in High-Fit subjects. Conversely, the combined effects of *ADRB3* Trp64Arg genotype and cardiorespiratory fitness did not affect β-stiffness, an index of arterial stiffness, and blood pressure. Moreover, no significant differences were noted for lipid parameters such as TC, HDL, and TG levels, based on *ADRB3* Trp64Arg genotype and cardiorespiratory

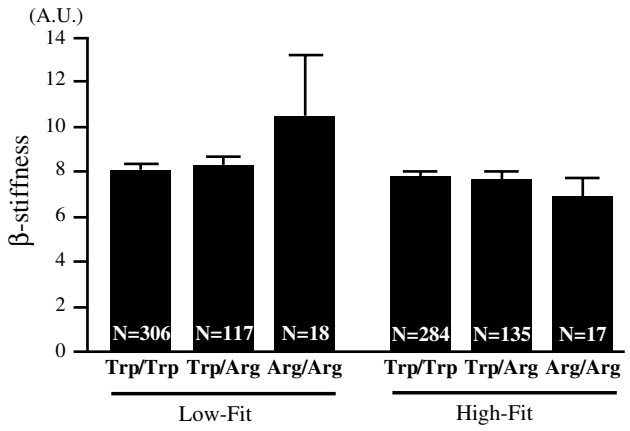

Fig. 2 Carotid β-stiffness values are shown for each fitness group and for individuals with various genotypes in *ADRB3* (encoding Trp/Trp, Trp/Arg, or Arg/Arg at amino acid position 64). Data are expressed as mean ± SE. *Low-Fit* low cardiorespiratory fitness group; *High-Fit* high cardiorespiratory fitness group

fitness. Thus, the beneficial effects of cardiorespiratory fitness on the risk of atherosclerosis, i.e., ccIMT, may be related to *ADRB3* Trp64Arg genotype.

In this study of healthy Japanese subjects, genotypes at Trp64Arg of the *ADRB3* gene were not associated with percent body fat, BMI, fasting glucose level, and blood pressure. Arg64-containing *ADRB3* has reduced lipolytic activity compared to Trp64-containing protein, which results in intra-individual differences in visceral obesity and insulin resistance (Kurokawa et al. 2001; Sheu et al. 1999; Umekawa et al. 1999). Moreover, the frequency of the Arg64 allele was higher in hypertensives than normotensives in the Sardinian population (Tonolo et al. 1999), and Arg64 allele of white male patients with type2 diabetes mellitus has higher blood pressure than Trp/Trp genotype (Ringel et al. 2000). However, in noninsulin-dependent diabetes mellitus patients, the frequency of the Arg64 allele was not greater in hypertensive patients than normotensive patients (Baba et al. 1998), and no significant differences were observed in SBP and DBP between genotypes at Trp64Arg of the *ADRB3* gene in the Finnish population with type2 diabetes mellitus (Ghosh et al. 1999) and in Caucasian patients with obese or type2 diabetes mellitus (Büettner et al. 1998). Thus, reports on the relationship between *ADRB3* Trp64Arg polymorphisms and body composition or the risk of diabetes and hypertension have not been consistent. Differences in various population and health characteristics or medication status may explain, at least in part, the conflicting results observed in prior studies.

Differences in ccIMT were not seen among individuals with different *ADRB3* Trp64Arg genotypes unless they were divided into High-Fit and Low-Fit groups. A comparison of patients with increased ccIMT (≥1.0 mm) and thin-walled controls (≤0.676 mm) by multivariate logistic regression analysis suggested that the Arg64 allele was not a significant predictor of ccIMT when ccIMT alone or ccIMT, BMI, and fasting insulin and glucose levels were evaluated in the model (Morrison et al. 1999). Furthermore, *ADRB3* Trp64Arg polymorphism did not significantly predict cardiovascular disease in Japanese subjects (Tamaki et al. 1999) and is not associated with coronary artery disease (Stangl et al. 2001). In a case-cohort study and meta-analysis, *ADRB3* polymorphism was not associated with a risk of coronary heart disease (Zafarmand et al. 2008). Consequently, differences in lifestyle associated with fitness level may be responsible for these differences in ccIMT. Thus, the genetic effect of *ADRB3* Trp64Arg polymorphism on the risk of cardiovascular disease may be detected when subjects are stratified by fitness level.

Although ccIMT were lower in Low-Fit individuals with the Arg/Arg genotype compared to those with a Trp-coding allele, these differences were not observed in High-Fit subjects. The mechanism underlying the combined effects of cardiorespiratory fitness and *ADRB3* Trp64Arg genotype on arterial stiffness is unclear. *ADRB3* is expressed in endothelial cells and regulates NO production via eNOS upregulation by Rac1-mediated activation of cAMP-dependent protein kinase (PKA) and Akt in endothelial cells (Kou and Michel 2007; Napp et al. 2009; Trochu et al. 1999). Chronic NOS inhibition induces increased ccIMT in the coronary and carotid arteries (Kristek and Gerová 1996), whereas NOS activation reduces atherosclerosis (Aji et al. 1997; Li and Förstermann 2009; Radomski and Salas 1995). Regular exercise prevents or ameliorates high ccIMT (Dinenno et al. 2001; Tanaka et al. 2002). Exercise training has also been reported to cause increases in plasma levels of nitrites and nitrates, markers of NO production (Maeda et al. 2001). In an animal study, exercise training resulted in elevated eNOS mRNA and protein expression in the aorta (Maeda et al. 2005). Therefore, regulation of eNOS via *ADRB3* in arteries may be related to the molecular mechanisms underlying the effect of exercise training on ccIMT. Based on these observations, in Low-Fit individuals with the Arg/Arg genotype, reduction of NOS activation via modulation of the *ADRB3* signal by a missense Trp64Arg variation may affect ccIMT. However, in High-Fit subjects, differences in ccIMT across individuals were not related to this polymorphism. eNOS activation is upregulated by increased laminar flow along the endothelium by acute exercise, and chronic exercise stimulation consequently elevates basal eNOS levels in the aorta (Gielen et al. 2011; Maeda et al. 2005). Furthermore, exercise training alters the expression of not only eNOS, but also other vasodilation-related molecules, including endothelin-1,

C-type natriuretic peptide, and prostacyclin (Maeda et al. 2005, 2001; Zoladz et al. 2009). Thus, in High-Fit subjects with the Arg/Arg genotype, we speculate that exercise training induces improvements in endothelial function via other mechanisms. Taken together, individuals may need to exercise regularly to improve cardiorespiratory fitness in order to counteract the negative effects of the Arg/Arg genotype in *ADRB3*. However, this study did not assess NO production in each genotype. Further study need to examine a mechanism of the combined effects of cardiorespiratory fitness and *ADRB3* Trp64Arg genotype on arterial stiffness.

In this cross-sectional study of Japanese subjects, the effect of cardiorespiratory fitness on the relationship between cardiovascular disease risk and *ADRB3* Trp64Arg gene polymorphism was assessed. Additional studies may be needed to examine the relationship between *ADRB3* Trp64Arg gene polymorphism and changes in cardiovascular disease risk due to exercise training with an interventional design. Additionally, since our study examined only Asian subjects, translating our results to other populations may not be applicable due to genotypic differences among ethnicities. Allele frequency of Arg/Arg genotype in *ADRB3* Trp64Arg was 0.18 and the number of participants in the Arg/Arg genotype was small. Therefore, it would be important to investigate various polymorphisms in *ADRB3* using a vast number of subjects in the current experimental setting. Furthermore, the combined effects of *ADRB2* Gln27Glu, *ADRB3* Trp64Arg, and *ADRA2B* 12Glu9 (3 amino acid deletion/insertion) variation are associated with intra-individual differences in body fat response to exercise training in older adults (Phares et al. 2004). Recent study reported that *ADRB3* Ser165Pro and Ser257Pro variation were associated with diabetic risks in the Chinese population (Huang et al. 2013). Consequently, further studies of the combined effects of *ADRB3* Trp64Arg and other gene polymorphisms or other *ADRB3* genotypes are warranted. Finally, although this study examined for the healthy subjects, patients with stroke, diabetes, hypertension, hyperlipidemia, or cardiac disease did not investigate about these effects. Further study need to examine for patients.

We investigated associations among cardiorespiratory fitness, cardiovascular disease risk, and *ADRB3* Trp64Arg genotype in healthy Japanese subjects. In conclusion, we identified a higher risk of atherosclerosis, i.e., ccIMT, associated with Arg homozygosity at *ADRB3* Trp64Arg in subjects with low levels of cardiorespiratory fitness, regardless of other arteriosclerosis and atherosclerosis risk factors such as β-stiffness and lipid profile parameters in Arg homozygotes. Sufficient cardiovascular fitness may influence cardiovascular adaptations to molecular variations in *ADRB3* in Japanese individuals.

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