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Original Article

Attenuated Age-Related Carotid Arterial Remodeling in Adults with a High Level of Cardiorespiratory Fitness

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Aim: Cardiorespiratory fitness (CRF) is independently associated with a reduced risk of cardiovascular disease. Carotid arterial remodeling, which is derived from the interplay between carotid luminal dilation and wall thickening, is also an independent predictor of cardiovascular events. We hypothesized that high CRF may be associated with reduced age-related carotid arterial remodeling. This cross-sectional study was performed to determine the relationships between CRF and age-related luminal dilation and wall thickening.

Methods: A total of 771 adults (180 men and 591 women), under age 40 (young), 40-59 (middle-aged), and over age 60 (older) participated in this study. Subjects in each age category were divided into either high (fit) or low (unfit) CRF groups based on $\dot{V}O_{2peak}$. Carotid artery intima-media thickness (IMT) and lumen diameter were measured on ultrasound images. Carotid wall mass was calculated as $\rho L(\pi Re^2\text{-}Ri^2)$.

Results: Two-way ANOVA indicated a significant interaction ($p<0.01$) between age and CRF in determining IMT, lumen diameter, and wall mass. In older subjects, IMT, lumen diameter, and wall mass were significantly lower ($p<0.05$) in the fit than in the unfit group (IMT, 0.69 ± 0.01 vs. 0.74 ± 0.01 mm; lumen diameter, 5.99 ± 0.06 vs. 6.28 ± 0.06 mm; wall mass, 7.41 ± 0.25 vs. 8.71 ± 0.25 mm^3). Multiple regression analysis indicated that the value of $\dot{V}O_{2peak}$ was independently correlated with carotid IMT, lumen diameter and wall mass.

Conclusion: The present study indicated that a high level of CRF is associated with reduced age-related wall thickening and luminal dilation in the carotid artery.

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Key words; Aging, Fitness, Intima-media thickness, Lumen diameter, Remodeling

Introduction

Elastic arteries undergo remodeling with advancing age (intimal and medial thickening[1]) and luminal dilation[2]). Arterial remodeling is usually an adaptive process that occurs in response to long-term changes in hemodynamic conditions, but may subsequently contribute to the pathophysiology of vascular diseases and circulatory disorders.

Carotid artery intima-media thickness (IMT) is an independent risk factor for cardiovascular disease (CVD)[3,4]. On the other hand, cardiorespiratory fitness (CRF) is independently associated with a reduced risk of CVD[5,6]. Thus, many previous studies focused mainly on the relationships between the CRF level and the age-related increase in carotid IMT. In addition to carotid IMT, carotid arterial remodeling derived from the interplay between carotid luminal dilation and wall thickening[7] is an independent predictor of cardiovascular events[8]. Previous studies sug-

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Table 1. Subject characteristics divided by age and fitness groups

	Young		Middle-aged		Older	
	Fit	Unfit	Fit	Unfit	Fit	Unfit
N	135	135	170	170	80	81
Men/Women, n	38/97	38/97	41/129	41/129	11/69	11/70
Age, years	28 ± 1	28 ± 1	50 ± 1*	51 ± 1*	63 ± 1*‡	64 ± 1*‡
Height, cm	164.2 ± 0.6	163.9 ± 0.7	160.0 ± 0.6*	160.1 ± 0.6*	156.9 ± 0.7*‡	156.9 ± 0.7*‡
Weight, kg	59.0 ± 0.9	59.3 ± 1.1	57.8 ± 0.8	61.7 ± 0.7†	54.2 ± 0.9*‡	55.9 ± 0.9‡
BMI, kg/m^2	21.6 ± 0.2	21.9 ± 0.3	22.4 ± 0.2*	24.1 ± 0.3*†	21.9 ± 0.3	22.6 ± 0.3‡
Body Fat, %	20.1 ± 0.4	24.8 ± 0.4†	23.9 ± 0.4*	30.4 ± 0.5*†	26.7 ± 0.6*	29.9 ± 0.5*†
SBP, mmHg	109 ± 1	109 ± 1	118 ± 1*	119 ± 1*	120 ± 2*	127 ± 2*‡†
DBP, mmHg	63 ± 1	64 ± 1	72 ± 1*	72 ± 1*	71 ± 1*	74 ± 1*
MAP, mmHg	81 ± 1	81 ± 1	91 ± 1*	91 ± 1*	92 ± 1*	97 ± 2*‡†
Carotid SBP, mmHg	102 ± 1	101 ± 1	117 ± 2*	118 ± 2*	121 ± 3*	131 ± 3*‡†
Plasma glucose, mmol/L	4.8 ± 0.1	4.8 ± 0.1	5.0 ± 0.1*	5.1 ± 0.1*†	5.2 ± 0.1*‡	5.3 ± 0.1*‡
Plasma insulin, μU/mL	5.1 ± 0.2	5.4 ± 0.2	4.1 ± 0.2*	5.0 ± 0.2†	4.3 ± 0.3	5.2 ± 0.5
Total cholesterol, mmol/L	4.55 ± 0.07	4.66 ± 0.06	5.39 ± 0.07*	5.39 ± 0.07*	5.78 ± 0.08*‡	5.80 ± 0.09*‡
HDL cholesterol, mmol/L	1.70 ± 0.03	1.58 ± 0.03†	1.76 ± 0.03	1.58 ± 0.03†	1.73 ± 0.04	1.64 ± 0.04
Triglycerides, mmol/L	0.72 ± 0.03	0.83 ± 0.04†	0.91 ± 0.04*	1.09 ± 0.05*†	0.95 ± 0.04*	1.04 ± 0.05*
LDL cholesterol, mmol/L	2.70 ± 0.06	2.91 ± 0.06†	3.44 ± 0.06*	3.59 ± 0.06*	3.86 ± 0.08*‡	3.95 ± 0.06*‡
$\dot{V}O_{2peak}$, mL/kg per min	41.1 ± 0.40	31.9 ± 0.3†	35.4 ± 0.4*	26.0 ± 0.3*†	32.2 ± 0.5*‡	23.7 ± 0.4*‡†

Data are the means ± SE. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; $\dot{V}O_{2peak}$, peak oxygen uptake.
*$p<0.05$ vs. young subjects within the same fitness group; ‡$p<0.05$ vs. middle-aged subjects within the same fitness group; †$p<0.05$ vs. fit subjects within the same age category.

gested that dilation of the lumen diameter is a typical vascular profile in patients with long-standing hypertension[9, 10] and may reflect the fatiguing effects of repeated intense cyclic stress[11]. Increased carotid wall mass according to luminal dilation and/or wall thickening is associated with an increased risk of cardiovascular events[8]. Thus, when considering the pathophysiological implications of vascular disease, it is also important not to overlook changes in both age-related carotid luminal dilation and wall thickening (arterial remodeling); however, the associations between the CRF level and age-related carotid arterial remodeling have attracted relatively little attention.

Accordingly, the primary aim of the present cross-sectional study was to determine the relationships between CRF and age-related carotid arterial remodeling. We hypothesized that higher CRF would be associated with reduced age-related carotid arterial remodeling.

Methods

Subjects

A total of 771 adults (180 men and 591 women), under the age of 40 (young), 40-59 years of age (middle-aged), and over the age of 60 (older) participated in this study (**Table 1**). None of the subjects smoked or were on medication for hypertension, hyperlipidemia, or diabetes. Subjects with a history of stroke, cardiac disease, chronic renal failure, or peripheral arterial disease, as well as those who regularly engaged in weight training, were excluded from the study[12]. Subjects who demonstrated significant IMT (>1.5 mm), plaque formation[13], ankle-brachial pressure index <0.90, and/or characteristics of atherosclerosis were excluded. Before testing, subjects abstained from caffeine and fasted for at least 4 hours (10-h overnight fast was used to determine metabolic risk factors and blood pressure (BP)). The purpose, procedures, and risks of the study were explained to each participant prior to inclusion, and all subjects gave their written informed consent before participating in the study, which was approved by the Human Research Committee of the National Institute of Health and Nutrition. The study was performed in accordance with the guidelines of the Declaration of Helsinki.

Carotid Artery IMT, Lumen Diameter, and Wall Mass

Carotid artery IMT and lumen diameter were

measured from ultrasound images (vivid i; GE Medical System) equipped with a high-resolution linear array transducer, as described previously[14, 15]. Longitudinal two-dimensional ultrasound images were obtained at the proximal 1- to 2-cm straight portion of the common carotid artery. These images were first recorded on an ultrasound machine for later offline analysis, and then stored on hard disk. Carotid images were obtained by two trained investigators.

Ultrasound carotid images were analyzed using Image J image analysis software (National Institutes of Health, Bethesda, MD). Carotid IMT was defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface[14]. Carotid lumen diameter was defined as the distance between the lumen and intima, and a near-wall boundary, corresponding to the interface of the adventitia and media. These measurements were made at end diastole, as described previously[14]. At least 10 measurements of IMT and lumen diameter were taken in each segment. The mean values of these 10 measurements were used for analysis. Carotid wall mass was calculated, as previously reported[16], as $\rho L(\pi Re^2\text{-}Ri^2)$, where ρ is the arterial wall density ($\rho = 1.06$)[17], L is the length of the arterial segment (L = 1 cm), and Re and Ri are the mean external and internal radii, respectively. Image analyses were performed by two investigators blinded to the group assignment of the subjects. Intraobserver and interobserver variabilities of measurements were examined in 100 subjects. Intraobserver and interobserver variabilities of measurements were 3.7% and 4.2% for carotid IMT and 2.0% and 2.2% for the lumen diameter, respectively.

Carotid Arterial Blood Pressure

The pressure waveform and amplitude were obtained from the common carotid artery with a vascular testing device (PWV/ABI; Omron Colin, Kyoto, Japan). A multielement tonometry sensor, consisting of 15 pressure-sensitive small elements aligned side by side, was coupled to the device. The carotid tonometry sensor is compact and lightweight and can be easily attached around the neck. The sensor element, located manually at the center of the carotid artery, can be identified by screening the pulse pressure (PP) levels of the 15 elements provided that the sensor element is sufficiently small compared with the vessel diameter. The quality of the carotid pulse wave and the downward force were checked visually by carotid compression tonography, and pulse waves were recorded and stored over periods of 30 s. As baseline levels of BP are subjected to hold-down force, the pressure signal obtained by tonometry was calibrated by equating the carotid mean arterial pressure (MAP) and diastolic blood pressure (DBP) to the brachial artery value[18]. Intraobserver variability of measurements was 4.0% for carotid systolic blood pressure (SBP).

Brachial Arterial Bold Pressure

Brachial BP was measured with an oscillometric device (PWV/ABI; Omron Colin) with subjects in the supine position. All measurements conformed to the American Heart Association Guidelines[19].

Cardiorespiratory Fitness

CRF, assessed from peak oxygen uptake ($\dot{V}O_{2peak}$), was measured by an incremental cycle exercise test using a cycle ergometer (Ergomedic 828E Test Cycle; Monark, Varberg, Sweden) as described previously[20, 21]. To assess the effects of CRF on carotid IMT, the subjects were categorized into high (fit) or low (unfit) CRF groups on the basis of the median value of $\dot{V}O_{2peak}$ in every decade of age in each sex.

Blood Samples

Blood samples were taken after an overnight fast of at least 10 h to determine fasting glucose and insulin levels. In the same session, serum samples were obtained to determine fasting total cholesterol, high-density lipoprotein cholesterol (HDL-cholesterol), low-density lipoprotein cholesterol (LDL-cholesterol) and triglyceride levels.

Statistical Analyses

The data were analyzed by two-way ANOVA (age × fitness level) and ANCOVA, which included sex, brachial SBP and body fat as a covariate. In cases with a significant F value, a *post hoc* test with Scheffe's method was used to identify significant differences among mean values. Univariate regression and correlation analyses were used to analyze the relationships between variables of interest. Stepwise multiple regression analysis was used to determine the independent relations of several variables to arterial remodeling values. $P < 0.05$ was considered significant. Data are presented as the mean ± SE.

Results

Table 1 shows the characteristics of the subjects. Age was associated with shorter stature, greater body fat, and higher blood pressure. The percent body fat value was lower in the fit group than in the unfit group at all ages.

Table 2 shows the effects of age and CRF on

Table 2. Arterial properties divided by age and fitness groups

	Young		Middle-aged		Older	
	Fit	Unfit	Fit	Unfit	Fit	Unfit
IMT, mm	0.56±0.01	0.55±0.01	0.66±0.01*	0.65±0.01*	0.69±0.01*‡	0.74±0.01*‡†
Lumen diameter, mm	5.88±0.04	5.85±0.04	5.85±0.05	6.03±0.05*†	5.99±0.06	6.28±0.06*‡†
Wall mass, mm^3	5.88±0.12	5.73±0.14	6.76±0.16*	7.06±0.15*	7.41±0.25*‡	8.71±0.25*‡†

Data are the means±SE. IMT, intima-media thickness; *$p<0.05$ vs. young subjects within the same fitness group; ‡$p<0.05$ vs. middle-aged subjects within the same fitness group; †$p<0.05$ vs. fit subjects within the same age category.

carotid IMT, lumen diameter, and wall mass. Two-way ANOVA indicated a significant interaction ($p<0.01$) between age and CRF in determining carotid IMT, lumen diameter, and wall mass. Carotid IMT and wall mass increased progressively with age in both fitness groups. Lumen diameter increased progressively with age in the unfit group but was not different at any age in the fit group. Carotid IMT and wall mass were lower ($p<0.05$) in fit than in unfit older subjects and lumen diameter was lower ($p<0.05$) in fit than in unfit middle-aged and older subjects. In the older group, these differences remained significant after normalizing for sex, brachial SBP and body fat as covariates; however, in the middle-aged group, the differences were abolished after normalizing for sex, brachial SBP and body fat. **Fig. 1** shows the relationships between $\dot{V}O_{2peak}$ and carotid IMT (A), lumen diameter (B), and wall mass (C) in each age category. Carotid IMT ($r=-0.24$, $p<0.05$), luminal diameter ($r=-0.28$, $p<0.01$), and wall mass ($r=-0.30$, $p<0.01$) were correlated with $\dot{V}O_{2peak}$ in older subjects. There were no significant relationships in young or middle-aged subjects.

In older subjects, the analysis also indicated that carotid IMT was correlated with brachial SBP ($r=0.29$), carotid SBP (0.28), weight (0.13), $\dot{V}O_{2peak}$ (−0.24), and HDL-cholesterol (−0.26). Stepwise multiple regression analysis revealed that brachial SBP ($\beta=0.24$), HDL-cholesterol (−0.23), and $\dot{V}O_{2peak}$ (−0.16) were independently correlated with carotid IMT.

In older subjects, the analysis also indicated that lumen diameter was correlated with brachial SBP ($r=0.43$), carotid SBP (0.39), weight (0.36), $\dot{V}O_{2peak}$ (−0.28), plasma glucose (0.24), plasma insulin (0.25), HDL-cholesterol (−0.16), and triglycerides (0.18). Stepwise multiple regression analysis revealed that brachial SBP ($\beta=0.38$), weight (0.32), and $\dot{V}O_{2peak}$ (−0.16) were independently correlated with lumen diameter.

In older subjects, the analysis also indicated that wall mass was correlated with brachial SBP ($r=0.45$), carotid SBP (0.41), weight (0.33), $\dot{V}O_{2peak}$ (−0.30), HDL-cholesterol (−0.24), plasma insulin (0.23), plasma glucose (0.19), and triglycerides (0.16). Stepwise multiple regression analysis revealed that brachial SBP ($\beta=0.42$), weight (0.28), and $\dot{V}O_{2peak}$ (−0.19) were independently correlated with wall mass.

Discussion

The key new findings of the present study were as follows. First, in the older group, carotid IMT, lumen diameter, and wall mass were significantly lower in the fit group than in the unfit group. Second, although carotid IMT and wall mass increased with age in both fitness groups, the magnitude of age-related increases was smaller in the fit group than in the unfit group. Third, carotid lumen diameter increased with advancing age in the unfit group but no differences were observed at any age in the fit group. Fourth, multiple regression analysis revealed that $\dot{V}O_{2peak}$ was independently correlated with carotid IMT, lumen diameter, or wall mass. These results suggested that higher CRF is associated with lower levels of age-related carotid arterial remodeling.

There have been many reports regarding the relationships between age-related increases in carotid IMT and CRF levels; however, these previous studies did not focus on the age-related dilation of the lumen diameter and increases in wall mass, and their findings were inconsistent. Specifically, the CRF level and habitual exercise have been reported to be associated with lower[22-24], no difference[25-27], or even greater[28] carotid IMT. Similar to previous findings by Galetta *et al.*[29], the present study also showed that a high level of CRF is related to an attenuation of age-related carotid arterial remodeling. An advantage of our study was the considerable number of subjects with a wide age range. Moreover, the strength of the present study was that CRF levels of all subjects were evaluated by maximal exercise testing. Considering the emphasis

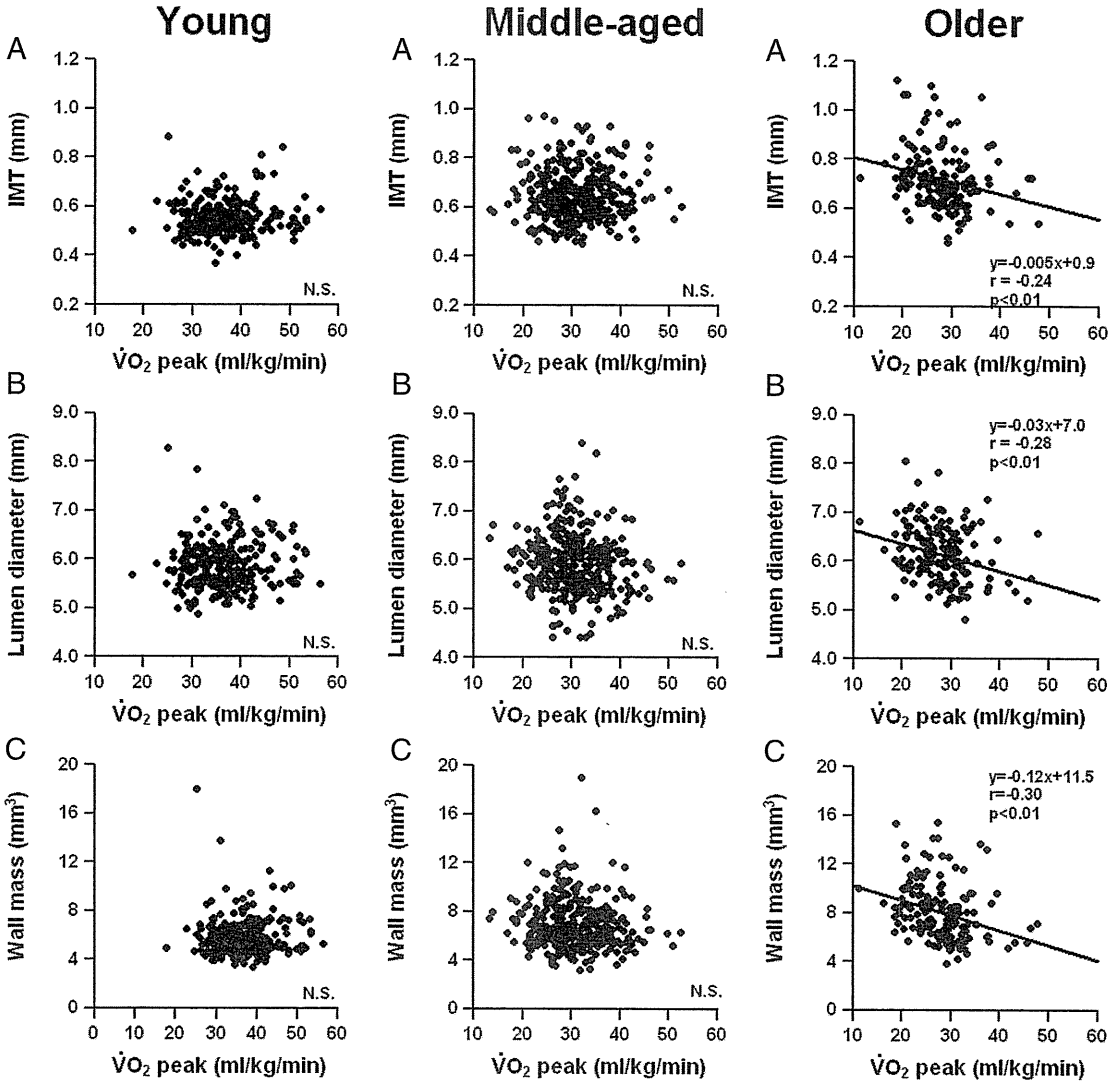

Fig. 1. Relationships between CRF and carotid IMT (A), lumen diameter (B), and wall mass (C) in each age category.

placed on dilation of the lumen diameter and increases in wall mass for prevention of CVD[8], we extended our research to age-related luminal dilation and wall thickening. Similar to some previous reports, the present study also showed that carotid IMT was lower in fit older subjects than in their unfit counterparts. More importantly, the present study demonstrated that lumen diameter and wall mass were lower in fit older subjects than in their unfit counterparts. The present findings suggested that higher CRF is associated with reduced age-related luminal dilation and wall thickening.

We can only speculate on the mechanisms responsible for the attenuation of age-related luminal dilation and wall thickening by higher CRF. Age-related arterial remodeling is primarily an adaptive response of the arterial wall to progressive elevations in chronic arterial BP[30]. The results of animal and human studies indicated that an increase in distending pressure is a major stimulus for hypertrophy of smooth muscle cells and the synthesis of extracellular matrix in the arterial wall[31-34]. Repeated intense cyclic stress may cause fracture of the load-bearing elastin fibers and thus dilation of the lumen[11]. Therefore, we propose that the smaller degree of age-related luminal dilation and increase in wall mass in fit groups may be due to a smaller age-related increase in blood pressure. Indeed, in this study, brachial SBP and carotid SBP were positively associated with carotid IMT, lumen diameter or wall mass in older subjects. However, in a

stepwise multiple regression model that included these factors, $\dot{V}O_{2peak}$ was independently related to carotid IMT, lumen diameter, or wall mass. Park *et al.*[35] reported that wall internal area and wall thickness area of the aorta were increased by menopause and improved by regular exercise in an animal study. As noted by Park *et al.*, eNOS and endothelin-1 in the aorta tissue may participate in these mechanisms. Moreover, the mechanisms by which the maintenance of higher CRF may directly influence lumen diameter and wall mass are still speculative and include the effect of an endurance-trained state on the calcium content[36] and advanced glycation end products and collagen cross-linkage in the arterial wall[37]. Exercise ameliorated the progression of endothelial dysfunction[38] and atherosclerotic lesion formation with a strong negative correlation between atherosclerotic areas and the mean running distance per day[39].

Our findings have a number of important implications. The present study showed that higher CRF was associated with smaller age-related increases in carotid IMT and wall mass and dilation of the lumen. As both luminal dilation and wall thickening are risk factors for CVD[3, 4, 8], the maintenance of higher CRF may have a protective effect against CVD in part by attenuating age-related carotid arterial remodeling; therefore, the improvement of CRF may be important for primary prevention of CVD.

A major limitation of the present study was its cross-sectional design. Due to the design of this study, we could not evaluate individual changes in age-related carotid arterial remodeling. A recent prospective study by Kozakova *et al.*[22] reported that a period of vigorous activity influenced the 3-year IMT progression in a young to middle-aged population (30-60 yr). More research will be needed to determine cause-and-effect relationships in the older population (over 60 yr).

In conclusion, the present study indicated that a high level of CRF is associated with reduced age-related wall thickening and luminal dilation in the carotid artery.

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Disclosure

The authors declare no conflicts of interest.

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Comments on Point:Counterpoint: Muscle lactate and H^+ production do/do not have a 1:1 association in skeletal muscle

Daniel A. Beard, Michael I. Lindinger, Dieter Boning, Kent Sahlin, David J. Bishop, Ronald A. Meyer, Edmund J. Crampin, Izumi Tabata, George J. F. Heigenhauser, Norbert Maassen and Robert W. Wiseman

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Comments on Point:Counterpoint: Muscle lactate and H^+ production do/do not have a 1:1 association in skeletal muscle

CALCULATIONS OF ROBERGS SUPPORT THE VIEW OF VINNAKOTA AND KUSHMERICK

TO THE EDITOR: Vinnakota and Kushmerick (2) highlight experimental evidence and computer simulations showing that net H^+ generation and lactate production occur in an ~1:1 stoichiometric ratio during anaerobic glycogenolysis coupled to ATP hydrolysis in muscle. Robergs (1) asserts that the stoichiometry is closer to 3 H^+ to 1 lactate. Frankly, while I am able to follow the simple clear logic of Vinnakota and Kushmerick, I don't fare so well with Robergs'.

Specifically, Robergs reports a value of 54 mmol of H^+/kg of muscle generated during a particular exercise protocol. He then transforms the number 54 to 100 by accounting for "the added pH-dependent H^+ metabolic buffering from LDH, CK, and PK reactions." My interpretation of this calculation is that Robergs is (loosely) estimating the proton load that would occur without those reactions present. Yet those reactions and their associated reactants do occur, both in real muscle and in the calculations of Vinnakota and Kushmerick. Admittedly, here I may be invoking a straw man; but I am at a loss to invent an alternative explanation for the mysterious calculation. In any event, if my reverse-engineered explanation of Robergs' claim is appropriate, then his estimate for what he defines as the net H^+ generated per lactate is 1.5, a value that (given the gross uncertainty in the calculation) it is perhaps not meaningfully different from 1.

Additional points made by Robergs do not speak to the debate at hand (a discussion on semantics) or are too inflammatory ("errors of science") to have a place in the scientific debate.

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TO THE EDITOR: Biochemical reactions within living muscle cells do not occur in isolation of water, nor in isolation of all other chemical and physical reactions that contribute to changes in $[H^+]$ during the progress of any one reaction or series of reactions. Importantly, biochemical reactions do not consume or produce protons—the protons are already there and what changes is the association of protons with H_20 and other proton-binding molecules (1, 2). Thus many factors simultaneously determine the $[H^+]$, or correctly $[H_3O^+]$, in physiological solutions such as sarcoplasm (3). The biochemical approaches presented in the arguments (4, 5) have measured pH and attempted to count protons "generated," "consumed," "buffered," or "released" in relation to lactate accumulation. Biochemical accounting of protons under any discrete set of conditions at select points in time may provide charge balance but only incompletely describes a more complex physicochemical series of hundreds of simultaneously occurring reactions that instantaneously affect $[H^+]$ (3). Such non-mechanistic descriptions of changes in selected variables fails to consider the importance of water in physicochemical reactions within the cells. The physical behavior of molecules in aqueous solutions depends on physicochemical interactions with water, and one must consider the associations of reaction substrates and products with water within the constraints of physical and chemical laws (maintenance of electroneutrality, conservation of mass). Therefore, when one takes a truly integrative approach to consider the physical chemistry of the intracellular environment of muscle cells, it seems moot to describe a stoichiometry between lactate and proton "production."

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NO EVIDENCE FOR THE COUNTERPOINT POSITION

TO THE EDITOR: While the contribution of Vinnakotta and Kushmerick (6) is logical and clear, Robergs (3) presents an interpretation of lactate and H^+ production by anaerobic metabolism (4) partly already criticized in a former Point:Counterpoint discussion (1).

Both Point and Counterpoint papers state that glycolysis per se finally produces no H^+, but only the lactate ion Lac^-. H^+ is however liberated by the concomitant ATP splitting. But while H^+ is again consumed during ATP resynthesis in the pyruvate kinase reaction, this does not occur during the phosphoglycerate kinase reaction: 1,3-biphosphoglycerate^{4-} + ADP^{3-} 3-phosphoglycerate^{3-} + ATP^{4-}.

The unconsumed H^+ (one per 1 Lac^-) causes acidosis. Both ions coexist and may leave the muscle fiber across monocarboxylate transporters; because of the low pK value only few combine to undissociated lactic acid.

Robergs, however, speculates about production of 3 H^+ per Lac^-. His main argument is that the physicochemical muscle buffer capacity amounts to 90 slykes according to Sahlin (5). But Sahlin has calculated only 38 slykes, applying this value

causes ~2 of the mysterious H^+ to disappear. Surprisingly Robergs continues to use a too high value as in his former review (4) despite a letter communicating this (2).

If buffering is a reversible binding of protons, Robergs' use of the term "metabolic buffering" for irreversible reactions where H^+ are transiently liberated and immediately tightly bound to other compounds is very misleading. Shall we in the future also rename oxygen as a buffer because it combines with H^+ in the mitochondria?

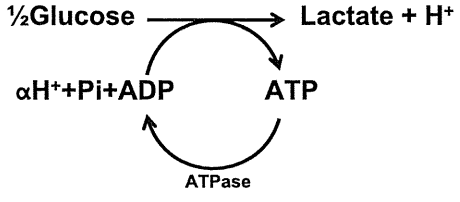

Fig. 1.

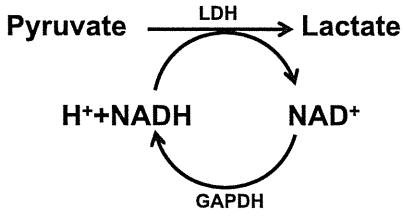

Fig. 2.

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WHY ADD COMPLEXITY/CONFUSION TO A SIMPLE ISSUE?

TO THE EDITOR: Muscle lactate and H^+ production do have a 1:1 association. From an organic chemical view, there is a simple/trivial answer: yes, there is a 1:1 association between lactate and H^+ $C_6(HOH)_6$ (glucose lactate) $\rightarrow 2CH_3CHOHCOO + 2H^+$.

Both Point:Counterpoints (3) are confusing.

Confusion-1: glycolytic ATP production has been included in the analysis (1, 3). Glycolytic ATP production cannot occur without prior ATP hydrolysis. The net change of muscle ATP content is, despite high ATP turnover, negligible and has therefore no effect on cellular acid-base balance (see Fig. 1).

Confusion-2: glycolytic reactions have been analyzed separate from each other. Although most glycolytic reactions are associated with production or consumption of H^+ they are connected in a metabolic pathway without major net changes in glycolytic intermediates (2). Robergs (3) concludes that the LDH reaction can buffer H^+. By examining the LDH reaction, isolated from the remaining glycolytic reactions, one could falsely come to this conclusion. However, oxidation of NADH in the LDH reaction equals glycolytic NADH production by glyceraldehyde-phosphate dehydrogenase (GAPDH). The conversion of pyruvate to lactate has therefore no influence on cellular acid-base balance (see Fig. 2).

Confusion-3: non-glycolytic processes/reactions have been included in the analysis (3). It is correct that the reactions catalyzed by AMP-deaminase and creatine kinase have implication for cellular acid-base balance. However, they are not linked to glycolysis and should not be included in this Point:Counterpoint discussion.

Cellular acid-base balance is complex, but I am afraid that the published Point:Counterpoints (3) have added more confusion.

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CONFUSION CONCERNING THE LACTATE PROTON RATIO: A PROBLEM OF DEFINITION?

TO THE EDITOR: In biochemistry, products can be defined as "compounds that are formed when a reaction goes to completion." But which reactions are we discussing in this Point:Counterpoint? While stating that the lactate- proton ratio from anaerobic glycolysis is close to 1:1 (5), it appears that Vinnakota and Kushmerick are actually referring to the lactate-proton ratio from ATP hydrolysis (the predominate source of H^+ in the conditions being discussed) coupled with anaerobic glycolysis. In this context, their 1:1 ratio is consistent with estimations that can be derived from skeletal muscle biopsies obtained before and after intense exercise. Using one of our studies as an example (2), lactate production (70 mmol/kg dry wt) can be shown to approximate the proton load calculated from the in vitro muscle buffer capacity [~45 mmol/kg dry wt; (1)] and PCr hydrolysis [~15 mmol/kg dry wt; (3)]; the small difference can probably be attributed to additional H^+ buffering by intracellular bicarbonate and the sodium-hydrogen exchanger (4). Robergs' opposition to this value seems to stem from his preference to calculate the lactate proton ratio as the ratio of protons released via ATP hydrolysis compared to the lactate produced via anaerobic glycolysis. While Robergs'

approach rightly emphasizes that ATP hydrolysis is the predominant source of the proton load, it is counterintuitive and distorts the total proton load when both reactions go to completion.

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LACTATE AND ACIDOSIS YET AGAIN?

TO THE EDITOR: This third debate (4) in the APS journals on the issue of lactate production and acidosis has at last narrowed the argument to a clear and manageable question: is there a 1:1 relationship between lactate and hydrogen ion production via glycolysis in muscle? The correct answer, as already noted by Dr. Sahlin both previously (see comments in Ref. 3) and today (see comments in Ref. 1), is yes, because with respect to its effect on hydrogen ion production in an aqueous solution, all that matters is the net reaction, and the only quantitatively significant net glycolytic reaction in muscle is just: glucose $\rightarrow$ 2 lactic acid. At biological pH, lactic acid is essentially all dissociated to lactate and hydrogen ion. [Or, if one prefers the alternative Stewartian way to say the same thing (2), lactate is a strong ion.] Unfortunately, both the Point and Counterpoint obscure this simple truth by their detailed analyses of the individual steps along the glycolytic pathway. Of course, if done correctly, this approach will also yield the correct answer, as shown by Drs. Vinnakota and Kushmerick. However, were a completely different set of enzymatic steps to evolve or be devised by which glucose is converted to lactic acid, the net effect would still be exactly the same. Let us not confuse our students any further. We should bring this argument to a close.

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TO THE EDITOR: Vinnakota and Kushmerick (3) report measurements and computational prediction of a near 1:1 free proton-to-lactate ratio in skeletal muscle under resting anoxic conditions. Robergs (2) disputes these results and states that the ratio is closer to 3:1. Neither protagonist gives an explanation as to why one would expect there to be a stable ratio over a range of physiological conditions.

Lactate is a product of (anaerobic) glycolysis. By contrast, protons are produced and consumed in a variety of reactions, and bind promiscuously. While there is no "structural" dependence between these reactions, they interact through common species, including protons. These interactions are described in the computational model used to infer the predominant source of protons and the 1:1 ratio.

One might then interpret this dispute as a challenge to the use of modeling for interpretation of data. The claim that their model provides "definitive answers" (3) is certainly an overstatement—the old cliche holds, that a model is only as good as the data (here the reaction species, stoichiometries, and kinetic parameters) used to make it. But the approach appears sound, including, as it should, binding constants for different phosphate moieties, cation-bound states, and so forth (1).

But why should one expect there to be a fixed relationship between free proton accumulation and lactate production? For example, protons are produced in ATP hydrolysis, which varies significantly with workload. A challenge to both authors, then, is to predict, compute, and/or measure whether and how this ratio changes with, say, exercise intensity.

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TO THE EDITOR: As Dr. Brooks described (1), a recent paper of Marcinek et al. (2) is interesting. If the 1:1 ratio of lactate and H^+ concentration change is relevant to human high-intensity exercise during which significant changes in lactate and H^+ concentration in recruited-skeletal muscle take place, discussions regarding relationship between lactate, H^+ to fatigue shall be enhanced because estimating pH

value from biopsy sampled is quite intriguing. However, several limitations should be applicable. First of all, substantial influences of circulation on concentration of metabolites in skeletal muscle should be considered. As we reported (3), difference in lactate concentration in skeletal muscle after 2–3 min of high-intensity exhaustive exercise from resting value is ~30 mmol/kg wet muscle, while muscle glycogen degradation is ~25 mmol glucosyl units/kg wet muscle (6), suggesting that two-fifths of produced lactate is removed from skeletal muscle by circulation during the exercise. Therefore, 1:1 ratio of lactate and H^+ observed in the study (2) is only relevant to anoxic condition without blood stream. Furthermore, since during such exercise, aerobic metabolism dominantly releases ATP (~60–70% of total ATP supply), an important simulation hypothesis of Marcinek et al. (2) that oxidation process does not work is not applicable to the high-intensity exhaustive exercise. Oxidation state may affect glycolysis by changing NADH and/or ATP concentrations, which may affect enzyme activity of rate limiting enzyme (presumably PFK) of glycolysis. Future research using NMR with conventional biochemical analyses should be conducted for the purpose of elucidating fatigue during high-intensity exercise.

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Association between Muscular Strength and Metabolic Risk in Japanese Women, but Not in Men

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Abstract We examined whether cardiorespiratory fitness (maximal oxygen uptake, $\dot{V}O_2max$) and muscular strength (grip strength) are associated with individual and clustered metabolic risk factors independently of abdominal adiposity in Japanese men (n=110) and women (n=110) aged 20–69 years. Blood pressure, triglycerides (TG), HDL cholesterol, and fasting plasma glucose (FPG) were assessed and metabolic risk score was calculated, which is the sum of the z scores for each individual risk factor. Waist circumference was measured and the area of visceral fat was assessed by MRI. Multiple linear regression analysis revealed that $\dot{V}O_2max$ was inversely associated with TG in men ($p<0.05$) and grip strength was negatively associated with FPG and metabolic risk score in women ($p<0.001$ and $p<0.05$, respectively), independently of waist circumference. Adjusting for visceral fat instead of waist circumference, similar results were obtained in women ($p<0.01$ and $p<0.05$, respectively), but the association between $\dot{V}O_2max$ and TG in men was attenuated to nonsignificant. This cross-sectional study demonstrates that muscular strength is inversely associated with plasma glucose levels and clustered metabolic risk factors independently of abdominal adiposity in Japanese women, but not in men. *J Physiol Anthropol 30(4): 133–139, 2011* http://www.jstage.jst.go.jp/browse/jpa2
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Keywords: metabolic syndrome, abdominal adiposity, cardiorespiratory fitness, muscular strength

Introduction

Metabolic syndrome (MeS) is a cluster of visceral obesity, insulin resistance, hypertension, glucose intolerance, and dyslipidemia that substantially increases the risk of type 2 diabetes and cardiovascular disease (Alberti et al., 2006). MeS has a rising worldwide prevalence, and is largely related to increasing obesity, especially abdominal adiposity, along with a sedentary lifestyle. A recent report from the National Health and Nutrition Examination Survey (NHANES) 2003–2006 estimated that approximately 34% of the U.S. adult population has MeS (Ervin, 2009). According to the National Health and Nutrition Survey in 2008, approximately 22% of Japanese adult men and 11% of Japanese adult women have MeS (Ministry of Health, Labour and Welfare, 2009). Given the public health significance of MeS, successful strategies are urgently needed to intervene in its development.

Both low fitness (cardiorespiratory fitness (CRF) and muscular strength) and increased adiposity have increasingly been recognized as important risk factors associated with MeS in adults (Hassinen et al., 2008; LaMonte et al., 2005; Sayer et al., 2007). However, because low fitness and excess body fat often occur in combination, it is important to distinguish the separate effects on metabolic risk factors related to MeS. A few recent studies have examined the relative contribution of abdominal adiposity and CRF to metabolic risk, demonstrating that higher CRF and lower abdominal adiposity are each independently associated with a substantial reduction in metabolic risk related to MeS (Hassinen et al., 2008; Lee et al., 2005). However, few studies have investigated the independent contribution of muscular strength and abdominal fat to individual and clustered metabolic risk factors. A recent study by Wijndaele et al. (2007) has demonstrated that muscular strength is inversely related to a metabolic risk factor in women, even after adjustment for abdominal fat and other potential confounding factors. However, their studies have characterized abdominal fat by simple anthropometric measures such as waist circumference. A more recent study has revealed that reductions in visceral and total abdominal fat may occur in the absence of changes in waist circumference (Kay and Fiatarone, 2006). Thus, the insensitivity of anthropometric indexes to reflect actual amounts of visceral fat may contribute to over/underestimating the role of muscular strength in relation to abdominal fat with regard to their effects

on metabolic risk factors.

Therefore, the purpose of the present study was to examine the relationship between fitness (CRF and muscular strength) and individual and clustered metabolic risk factors in Japanese men and women. Specifically, we investigated whether these associations are independent of abdominal adiposity quantified by direct measurement of visceral fat.

Methods

Subjects

Two hundred twenty Japanese people (110 men and 110 women) aged 20 to 69 years participated in the present study. They were recruited through advertisements from students or faculty members of Waseda University and their immediate acquaintances. None of the subjects had been diagnosed with diabetes mellitus or were taking any medications that could affect the study variables. All subjects provided written informed consent before enrollment in the study. The research project was approved by the Ethical Committee of Waseda University.

Fatness

Body weight was measured using an electronic scale (Inner Scan BC-600, Tanita Co., Tokyo, Japan) and was determined to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using a stadiometer (YL-65, YAGAMI Inc., Nagoya, Japan). Body weight and height were measured with the subjects wearing light clothing and no shoes. Waist circumference (WC) was measured at the umbilical region with an inelastic measuring tape at the end of normal expiration to the nearest 0.1 cm. Visceral fat (VF) area was measured by magnetic resonance imaging (MRI) (Signa 1.5T, General Electric Co., Milwaukee, Wisconsin, USA). The imaging conditions included a T-1 weighted spin-echo and axial-plane sequence with a slice thickness of 10 mm, a repetition time (TR) of 140 ms, and an echo time of 12.3 ms. Cross-sectional images were scanned at the umbilical region (Usui et al., 2010). During the scan, the subjects were asked to hold their breath for about 30 s after inhalation to reduce the respiratory motion artifact. The magnetic resonance images were transferred to a personal computer in the Digital Imaging and Communications in Medicine (DICOM) file format, and the cross-sectional area of the VF at the umbilical region was determined using image-analysis software (Slice-o-matic 4.3 for Windows, Tomovision, Montreal, Canada). To minimize interobserver variation, all scans and analyses were performed by the same investigator, and the coefficient of variation was 0.4% for the cross-sectional areas of the umbilical region.

Fitness

Cardiorespiratory fitness

CRF was assessed by a maximal graded exercise test on a cycle ergometer (Monark Ergomedic 828E, Varberg, Sweden) and quantified as maximal oxygen uptake ($\dot{V}O_2$max). The initial workload was adjusted to 30–60 W, and the work rate was increased thereafter by 15 W/min until the subject could not maintain the required pedaling frequency of 60 rpm (Cao et al., 2010). Heart rate (Life Scope BSM-1101, NIHON KODEN Corp., Tokyo, Japan) and a rating of perceived exertion (RPE) were monitored throughout the exercise. During the progressive exercise test, the expired gas of subjects was collected and the rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured and averaged over 30-s intervals using an automated gas analyzing system (Aeromonitor AE-280S, Minato Medical Science, Tokyo, Japan). During the latter stages of the test, each subject was verbally encouraged by the test operators to give a maximal effort. The highest observed value of $\dot{V}O_2$ during the exercise test was considered to be the maximal oxygen uptake (mL/kg/min), and achievement of $\dot{V}O_2$max was accepted if at least two of the following four criteria were achieved: the $\dot{V}O_2$ curve showed a leveling off, subject's maximal heart rate (HR) was >95% the age-predicted maximal HR (220-age), a respiratory exchange ratio in excess of 1.0, and the subject achieved an RPE of 19 or 20.

Muscular strength

Handgrip peak force was measured during maximal isometric contraction with a grip dynamometer (YX, YAGAMI Inc., Nagoya, Japan) in units of kilograms. Subjects were instructed to complete 2 handgrip contraction trials bilaterally, alternating hands between trails. Subjects held the dynamometer with the arm completely extended in standing position and were verbally encouraged by the assessor to exert the maximal force during each contraction trail. The higher grip strengths of two trials for each hand were summed to provide a single measure of grip strength. Grip strength was used as a proxy for overall strength (Rantanen et al., 1994).

Metabolic risk factors

Resting systolic and diastolic blood pressures (SBP and DBP) were measured using an automated recorder (HEM-759P, OMRON Corp., Kyoto, Japan). Blood samples were drawn between 8:00 a.m. and 11:00 a.m. after a 12-hour overnight fast for measurements of triglycerides (TG), HDL cholesterol (HDL-C), and fasting plasma glucose (FPG) levels. Serum and plasma samples were stored at −80°C until subsequent analyses. All blood parameters were analyzed by SRL, Inc. (Tokyo, Japan).

Clustered metabolic risk factors

In the present study, the gender-specific metabolic risk score (zMeS) was calculated, which is a continuous z score of 5 variables, for the risk factors of MeS (Franks et al., 2004). This variable was derived by standardizing and then summing the following values of WC, TG, inverse of HDL-C, blood pressure (SBP+DBP/2), and FPG. The standardizing of these factors was achieved by subtracting the sample mean from the individual mean and then dividing by the standard deviations

(SD). This continuously distributed metabolic risk score (zMeS) was also calculated without the adiposity component ($zMeS^{-WC}$). The calculating formula is as follows:

$$zMeS = zWC + zTG + zHDL\text{-}C + z(SBP + DBP/2) + zFPG$$

$$zMeS^{-WC} = zTG + zHDL\text{-}C + z(SBP + DBP/2) + zFPG$$

(z , standardized score)

Confounding variables

Several confounding variables were included in the analyses: age, smoking status, and daily alcohol intake. Smoking status was assessed by means of a questionnaire, and was defined as one of two categories: never or current/former smoker. Daily alcohol intake (grams per day) over the past month was assessed using a brief-type self-administered diet history questionnaire (BDHQ) (Sasaki, 2004), which asked the average frequency and the usual serving size for each type of alcoholic beverage.

Statistical analyses

Measured and calculated values are presented as means±SD. All subsequent analyses were carried out for men and women separately. The values of TG for both sexes and DBP in women were log transformed in correlation analyses due to their nonnormal distribution. The Student's *t*-test was used to determine differences between men and women. Pearson's product correlations were calculated among the outcome variables (age, BMI, WC, VF, $\dot{V}O_2$max, grip strength, TG, HDL-C, SBP, DBP, FPG, and zMeS). Multiple linear regression models were used to assess the association of fatness and fitness with metabolic risk. We first entered the fitness ($\dot{V}O_2$max or grip strength) and BMI for independent variables and zMeS for a dependent variable (model A). We then entered the fitness ($\dot{V}O_2$max or grip strength) and a parameter for abdominal fat (WC or VF) for independent variables and each metabolic risk factor or $zMeS^{-WC}$ for a dependent variable (model B and C). All models were adjusted for confounding variables: age, smoking status, and alcohol intake and are presented as standardized β coefficients. All analyses were completed using SPSS 17.0J for Windows (SPSS Japan Inc., Tokyo, Japan). The statistical significance level was set at $p<0.05$.

Results

The characteristics of men and women are shown in Table 1. Men showed higher BMI, WC, and VF in comparison with

Table 1 The characteristics of subjects

Variables	Women (n=110)	Men (n=110)	*p*
Age (yrs)	44.3±13.6	43.4±13.5	0.605
Height (cm)	158.0± 5.2	171.5± 6.4	<0.001
Body weight (kg)	53.8± 7.0	70.6±11.0	<0.001
BMI (kg/m^2)	21.6± 2.8	24.0± 3.2	<0.001
WC (cm)	76.8± 8.9	83.7± 8.8	<0.001
VF (cm^2)	53.1±31.5	93.6±47.4	<0.001
$\dot{V}O_2$max (mL/kg/min)	27.7± 4.8	35.1± 6.6	<0.001
Grip strength (kg)	54.7± 9.3	89.4±12.8	<0.001
TG (mg/dL)	72.1±38.6	108.6±67.8	<0.001
HDL-C (mg/dL)	67.8±13.6	58.6±13.1	<0.001
SBP (mmHg)	117.2±16.0	125.5±14.5	<0.001
DBP (mmHg)	74.0± 9.9	80.5±11.0	<0.001
FPG (mg/dL)	89.4± 8.3	93.0± 9.1	0.002
Current or former smokers (%)	14	41	<0.001
Alcohol intake≧20 g/day (%)	11	46	<0.001

Data are means±SD or proportions.
BMI, body mass index; WC, waist circumference; VF, visceral fat; $\dot{V}O_2$max, maximal oxygen uptake; TG, triglycerides; HDL-C, HDL cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose.

Table 2 Correlation matrix of age, fatness (BMI, WC, and VF), fitness ($\dot{V}O_2$max and grip strength), and individual and clustered metabolic risk factors

Variables	Women (n=110)						Men (n=110)					
	Age (yrs)	BMI (kg/m^2)	WC (cm)	VF (cm^2)	$\dot{V}O_2$max (mL/kg/min)	Grip strength (kg)	Age (yrs)	BMI (kg/m^2)	WC (cm)	VF (cm^2)	$\dot{V}O_2$max (mL/kg/min)	Grip strength (kg)
BMI (kg/m^2)	0.17	—					0.00	—				
WC (cm)	0.31**	0.78***	—				0.20*	0.90***	—			
VF (cm^2)	0.43***	0.74***	0.72***	—			0.26**	0.71***	0.80***	—		
$\dot{V}O_2$max (mL/kg/min)	−0.38***	−0.17	−0.32**	−0.38***	—		−0.44***	−0.47***	−0.55***	−0.59***	—	
Grip strength (kg)	−0.34***	−0.02	−0.10	−0.19*	0.35***	—	−0.20*	0.12	0.09	0.01	0.19	—
TG (mg/dL)	0.37***	0.32**	0.42***	0.43***	−0.26**	−0.15	0.15	0.49***	0.53***	0.60***	−0.44***	−0.01
HDL-C (mg/dL)	0.13	−0.22*	−0.14	−0.16	0.11	0.05	0.08	−0.36***	−0.32**	−0.26**	0.19*	0.03
SBP (mmHg)	0.53***	0.38***	0.47***	0.52***	−0.33***	−0.18	0.36***	0.24*	0.28**	0.24*	−0.18	0.04
DBP (mmHg)	0.37***	0.32**	0.44***	0.37***	−0.27**	−0.11	0.31**	0.30**	0.36***	0.40***	−0.27**	0.01
FPG (mg/dL)	0.52***	0.29**	0.37***	0.41***	−0.29**	−0.43***	0.33***	0.13	0.20*	0.18	−0.12	0.05
zMeS	0.46***	0.62***	—	—	−0.39***	−0.28*	0.29*	0.71***	—	—	−0.46***	0.08

TG and DBP were log transformed for analyses.
BMI, body mass index; WC, waist circumference; VF, visceral fat; $\dot{V}O_2$max, maximal oxygen uptake; TG, triglycerides; HDL-C, HDL cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; zMeS, metabolic risk score.
*$p<0.05$;**$p<0.01$;***$p<0.001$.

women ($p<0.001$). Both $\dot{V}O_2$max and grip strength were significantly higher in men than in women ($p<0.001$). TG, SBP, DBP, and FPG were higher in men (FPG: $p<0.01$, expect for FPG: $p<0.001$), whereas HDL-C was higher in women ($p<0.001$).

Table 2 shows the Pearson correlations matrix of individual and clustered metabolic risk factors and fatness (BMI, WC, and VF) and fitness ($\dot{V}O_2$max and grip strength) in men and women. Age correlated positively with SBP, DBP, FPG, and zMeS in men and women ($p<0.05$) and additionally with TG in women ($p<0.001$). Significant correlations were obtained between individual metabolic risk factors and all three measures of fatness except for FPG in men and HDL-C in women ($p<0.05$). Positive correlations were obtained between zMeS and BMI in men and women. $\dot{V}O_2$max was significantly correlated with TG, HDL-C, DBP, and zMeS in men ($p<0.05$) and with TG, SBP, DBP, FPG, and zMeS in women ($p<0.01$). Grip strength was negatively correlated with FPG and zMeS in women ($p<0.05$), but no significant correlations were obtained between grip strength and any of the metabolic risk factors or zMeS in men.

Table 3 presents the results of multiple linear regression analyses of clustered metabolic risk factors (zMeS) controlled for age, smoking status, and alcohol intake, including the contribution of BMI (model A). Model A revealed that $\dot{V}O_2$max and grip strength were inversely associated with zMeS independently of BMI in women ($p<0.05$), but not in men.

Table 4 presents the results of multiple linear regression analyses of individual and clustered metabolic risk factors ($zMeS^{-WC}$) controlled for age, smoking status, and alcohol intake, including the contribution of WC (model B) and VF (model C). Model B shows that $\dot{V}O_2$max was inversely associated with TG in men ($p<0.05$) and that grip strength was negatively associated with FPG ($p<0.001$) and $zMeS^{-WC}$ ($p<0.05$) in women independently of WC. Controlling for VF instead of WC (model C) revealed similar results for grip strength in women (FPG: $p<0.01$, $zMeS^{-WC}$: $p<0.05$); however, the association between $\dot{V}O_2$max and TG in men became nonsignificant ($p=0.14$).

Table 3 Association of BMI and the fitness ($\dot{V}O_2$max or grip strength) with the clustered metabolic risk factors (zMeS)

Model A	BMI (kg/m^2)	$\dot{V}O_2$max (mL/kg/min)	BMI (kg/m^2)	Grip strength (kg)
Women	0.55***	−0.19*	0.58***	−0.18*
Men	0.71***	0.00	0.70***	0.05

Date are standardized β coefficients.
All models were adjusted for age, smoking habit, and alcohol intake.
$\dot{V}O_2$max, maximal oxygen uptake; zMeS, metabolic risk score.
*$p<0.05$; ***$p<0.001$.

Table 4 Association of a measure of abdominal adiposity (WC or VF) and the fitness ($\dot{V}O_2$max or grip strength) with the individual and clustered metabolic risk factors ($zMeS^{-WC}$)

Model B	Women				Men			
	WC (cm)	$\dot{V}O_2$max (mL/kg/min)	WC (cm)	Grip strength (kg)	WC (cm)	$\dot{V}O_2$max (mL/kg/min)	WC (cm)	Grip strength (kg)
TG (mg/dL)	0.33***	−0.05	0.34***	−0.02	0.42***	−0.23*	0.54***	−0.07
HDL-C (mg/dL)	−0.19	0.15	−0.23*	0.15	−0.28**	0.12	−0.35***	0.06
SBP (mmHg)	0.31***	−0.08	0.33***	0.01	0.29**	0.13	0.22*	0.07
DBP (mmHg)	0.34***	−0.06	0.35***	0.04	0.32**	0.00	0.32***	0.01
FPG (mg/dL)	0.21*	−0.06	0.24**	−0.30***	0.21	0.16	0.12	0.11
$zMeS^{-WC}$	0.43***	−0.12	0.46***	−0.19*	0.54***	0.00	0.53***	0.05

Model C	Women				Men			
	VF (cm^2)	$\dot{V}O_2$max (mL/kg/min)	VF (cm^2)	Grip strength (kg)	VF (cm^2)	$\dot{V}O_2$max (mL/kg/min)	VF (cm^2)	Grip strength (kg)
TG (mg/dL)	0.31**	−0.05	0.33**	0.00	0.53***	−0.16	0.61***	−0.03
HDL-C (mg/dL)	−0.26*	0.13	−0.29**	0.14	−0.31**	0.09	−0.36***	0.04
SBP (mmHg)	0.34***	−0.07	0.35***	0.03	0.17	0.07	0.13	0.10
DBP (mmHg)	0.22*	−0.09	0.24*	0.05	0.33**	0.01	0.32***	0.04
FPG (mg/dL)	0.20*	−0.06	0.21*	−0.28**	0.17	0.14	0.09	0.12
$zMeS^{-WC}$	0.44***	−0.12	0.47***	−0.16*	0.55***	0.02	0.53***	0.09

Date are standardized β coefficients.
All models were adjusted for age, smoking habit, and alcohol intake.
TG and DBP were log transformed for analyses.
WC, waist circumference; VF, visceral fat; $\dot{V}O_2$max, maximal oxygen uptake; TG, triglycerides; HDL-C, HDL cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; $zMeS^{-WC}$, metabolic risk score without WC factor.
*$p<0.05$; **$p<0.01$; ***$p<0.001$.

Discussion

This cross-sectional study was performed to examine whether fitness is associated with individual and clustered metabolic risk factors independently of abdominal adiposity in Japanese men and women aged 20–69. The principal finding of this study was that both grip strength and abdominal adiposity are each independently associated with individual and clustered metabolic risk factors in women, but not in men (Table 3). Previous studies have indicated that muscular strength is a significant predictor of metabolic risk in adults (Sayer et al., 2007; Wijndaele et al., 2007). However, few studies have evaluated the independent importance of abdominal adiposity and fitness (Sayer et al., 2007). An important issue to consider is that low fitness and excess body fat often occur in combination. In fact, grip strength was negatively correlated with VF in women ($r=-0.19$, $p<0.05$) in the present study (Table 2). To elucidate the role of muscular strength in metabolic risk, it is important to examine these relationships, including the contribution of abdominal adiposity. Wijndaele et al. (2007) have reported that muscular strength assessed by measuring isometric knee extension and flexion peak torque is associated with a metabolic risk factor even after controlling for abdominal adiposity in women. This finding is roughly in accordance with our results, although in their study the abdominal fat indicator depended on simple anthropometric measures such as WC, which does not always reflect the accumulation of VF (Kay and Fiatarone, 2006). We found that muscular strength is associated with metabolic risk independent of WC and additionally with abdominal adiposity quantified by MRI, which is a direct measurement of VF. Therefore, this result supports the position that increasing muscular strength levels may be an effective strategy for preventing MeS in women.

As seen in Table 3, physical fitness ($\dot{V}O_2$max and grip strength) was found to be significantly and inversely associated with zMeS after controlling for BMI only in women (model A). In men, low levels of fitness did not appear to be a direct and primary cause of clustered metabolic risk factors. These results indicate that the effects of physical fitness, as measured by CRF and muscular strength, on clustered metabolic risk factors differ between men and women, and that the role of fitness may be more important in women than in men in the prevention of MeS. However, BMI is an indicator of general obesity and does not reflect abdominal adiposity, although these variables are highly correlated (Table 2). We therefore examined these associations using abdominal fat indicators.

In the present study, abdominal adiposity was found to be a stronger predictor of all metabolic risk factors than $\dot{V}O_2$max in men and women (Table 4). These data support a study by Christou et al. (2005), where it was reported that body fatness is a better predictor of the cardiovascular disease risk factor profile than CRF. In the present study, however, $\dot{V}O_2$max was associated with TG independently of WC in men (model B). The reason why CRF is predictive of TG only in men remains to be elucidated, but the effect of CRF on metabolic risk may be overestimated in men in the model B. Men generally have more VF than women (Boule et al., 2005) due to the lack of a possible protective effect of estrogen in men compared with women (Geer and Shen, 2009). However, WC can not distinguish VF from subcutaneous fat. Thus, the insensitivity of the anthropometric index in reflecting actual amounts of VF may contribute to overestimating the role of CRF in relation to abdominal fat with regard to metabolic risk. As such, we investigated the relative contribution of VF, as assessed by MRI, to metabolic risk (model C).

In model C, the association of $\dot{V}O_2$max with TG was attenuated to nonsignificant in men. Previous studies have indicated that abdominal fat, as measured by computed tomography, and CRF are significant predictors of several metabolic risk factors related to dyslipidemia in adults (Boule et al., 2005; Nagano et al., 2004). However, these associations were not found in the present study. The methodological difference in evaluating CRF may lead to different results. In previous studies (Boule et al., 2005; Nagano et al., 2004) CRF was not measured directly, whereas in our study $\dot{V}O_2$max was assessed directly using a respiratory gas exchange analysis during a maximal cycle ergometer test, which is an accurate and highly reproducible measure of CRF. The results of the present study suggest that preventing abdominal fat accumulation rather than increasing CRF levels appears to be a more effective and direct strategy for the primary prevention of MeS in both men and women.

The main finding of this study was that grip strength is associated with FPG independently of WC or VF in women. Grip strength is a simple and direct isometric method for the assessment of hand and forearm skeletal muscular strength, which may be representative of overall muscular strength because it is highly correlated with other muscular strength measures, including elbow flexion, knee extension, trunk flexion, and trunk extension (Rantanen et al., 1994). Sayer et al. (2005) have indicated that there is a graded association between increased glucose levels and weaker muscular strength in those with impaired glucose tolerance and normal blood glucose levels. As such, there appears to be a link between muscular strength and glucose metabolism. Because muscular strength is related to skeletal muscle mass, which is a significant site of glucose disposal (Lazarus et al., 1997), muscular strength may be important for glucose metabolism and could be a good target for the treatment of metabolic risk leading to conditions such hyperglycemia and type 2 diabetes mellitus. The amount of physical activity is also found to be related to muscular fitness (Paalanne et al., 2009). Actually, grip strength in subjects with exercise habits is known to be higher than those without exercise habits (Miyatake et al., 2009). Previous studies have shown that increase in moderate and vigorous activity results in a decrease in fasting insulin level, a marker of insulin resistance (Assah et al., 2008), indicating physical activity exerts its action on glucose metabolism in the long term.

Taken together, these results suggest that the association of muscular strength with FPG may bepartially explained by physical activity. It should be noted that in the present study, grip strength ($\beta=-0.28$, $p<0.01$) was a strong predictor of FPG as much as VF ($\beta=0.21$, $p<0.05$) in women (model C). It is therefore plausible that increasing the levels of muscular strength as well as preventing VF accumulation may be equally effective in preventing hyperglycemia and type 2 diabetes mellitus.

It is interesting that grip strength was not found to be predictive of FPG in men (Table 3). Differences in muscular strength are known to exist between men and women, and from early adulthood, women have on average 35% to 45% less muscular strength than men (Katzmarzyk and Craig, 2002; Ministry of Education, Culture, Sports, Science and Technology, 2010). Thus absolutely lower muscular strength for women compared with men might explain the sex difference in the association between grip strength and FPG. Because fasting glucose is known to be a predictor of new onset diabetes mellitus (Chen et al., 2009), poor muscular strength in women can be considered a marker for risk of type 2 diabetes.

The present study has several limitations. First, as it was a cross-sectional study; larger sample sizes and prospective and interventional studies are needed to confirm the effects of muscular strength on metabolic risk independent of abdominal fat accumulation. Second, the results may have limited generalizability due to ethnic differences in muscular strength. Japanese generally have lower muscular strength than Westerners, so the relationship between muscular strength and metabolic risk in other racial groups may differ from that found in our study. While this remains to be investigated, this study has contributed valuable information for Japanese. Third, because the prevalence of type 2 diabetes is much lower in Japanese women than in Japanese men, the findings in the present study must be adopted carefully for women. However, type 2 diabetes occurs in Asians who are less obese than those in Western countries (Chan et al., 2009) and the incidence is increasing continuously in both Japanese men and women. As such, maintenance of good muscular strength by regular daily exercise (Miyatake et al., 2009) appears to be important in the prevention of diabetes in Japanese women.

In summary, the present study suggests that there are sex differences in associations of physical fitness and fatness with metabolic risk in Japanese adults. The role of physical fitness (CRF and muscular strength) appears to be more important in women than in men in the prevention of MeS. In men, abdominal adiposity is a stronger predictor of individual and clustered metabolic risk factors than fitness. In women, muscular strength and abdominal adiposity are each independently associated with FPG and clustered metabolic risk factors. Muscular strength in women is a strong predictor of FPG as much as VF accumulation. The present study implies that effective approaches to preventing MeS may differ between men and women, and that exercise to increase muscular strength should be incorporated into individual lifestyles to reduce the risk of MeS and diabetes mellitus in women.

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