10. Ward DS, Evenson KR, Vaughn A, *et al.* (2005) Accelerometer use in physical activity: best practices and research recommendations. *Med Sci Sports Exerc* **37**, S582–S588.
11. Richardson CR, Newton TL, Abraham JJ, *et al.* (2008) A meta-analysis of pedometer-based walking interventions and weight loss. *Ann Fam Med* **6**, 69–77.
12. Bravata DM, Smith-Spangler C, Sundaram V, *et al.* (2007) Using pedometers to increase physical activity and improve health: a systematic review. *JAMA* **298**, 2296–2304.
13. Bassett DR Jr, Ainsworth BE, Swartz AM, *et al.* (2000) Validity of four motion sensors in measuring moderate intensity physical activity. *Med Sci Sports Exerc* **32**, S471–S480.
14. Matthews CE (2005) Calibration of accelerometer output for adults. *Med Sci Sports Exerc* **37**, S512–S522.
15. Welk GJ, Blair SN, Wood K, *et al.* (2000) A comparative evaluation of three accelerometry-based physical activity monitors. *Med Sci Sports Exerc* **32**, S489–S497.
16. Rothney MP, Schaefer EV, Neumann MM, *et al.* (2008) Validity of physical activity intensity predictions by ActiGraph, Actical, and RT3 accelerometers. *Obesity (Silver Spring)* **16**, 1946–1952.
17. Chen KY & Sun M (1997) Improving energy expenditure estimation by using a triaxial accelerometer. *J Appl Physiol* **83**, 2112–2122.
18. Crouter SE & Bassett DR Jr (2008) A new 2-regression model for the Actical accelerometer. *Br J Sports Med* **42**, 217–224.
19. Crouter SE, Churilla JR, Bassett DR, *et al.* (2006) Estimating energy expenditure using accelerometers. *Eur J Appl Physiol* **98**, 601–612.
20. Crouter SE, Churilla JR & Bassett DR Jr (2008) Accuracy of the Actiheart for the assessment of energy expenditure in adults. *Eur J Clin Nutr* **62**, 704–711.
21. Crouter SE, Clowers KG & Bassett DR Jr (2006) A novel method for using accelerometer data to predict energy expenditure. *J Appl Physiol* **100**, 1324–1331.
22. Midorikawa T, Tanaka S, Kaneko K, *et al.* (2007) Evaluation of low-intensity physical activity by triaxial accelerometry. *Obesity (Silver Spring)* **15**, 3031–3038.
23. Pober DM, Staudenmayer J, Raphael C, *et al.* (2006) Development of novel techniques to classify physical activity mode using accelerometers. *Med Sci Sports Exerc* **38**, 1626–1634.
24. Rothney MP, Neumann M, Beziat A, *et al.* (2007) An artificial neural network model of energy expenditure using nonintegrated acceleration signals. *J Appl Physiol* **103**, 1419–1427.
25. Tanaka C, Tanaka S, Kawahara J, *et al.* (2007) Triaxial accelerometry for assessment of physical activity in young children. *Obesity (Silver Spring)* **15**, 1233–1241.
26. Zhang K, Werner P, Sun M, *et al.* (2003) Measurement of human daily physical activity. *Obes Res* **11**, 33–40.
27. Oshima Y, Kawaguchi K, Tanaka S, *et al.* (2010) Classifying household and locomotive activities using a triaxial accelerometer. *Gait Posture* **31**, 370–374.
28. Ohkawara K, Tanaka S, Ishikawa-Takata K, *et al.* (2008) Twenty-four-hour analysis of elevated energy expenditure after physical activity in a metabolic chamber: models of daily total energy expenditure. *Am J Clin Nutr* **87**, 1268–1276.
29. Weir JB (1949) New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* **109**, 1–9.
30. Bland JM & Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1**, 307–310.
31. Corder K, Brage S, Mattocks C, *et al.* (2007) Comparison of two methods to assess PAEE during six activities in children. *Med Sci Sports Exerc* **39**, 2180–2188.
32. Institute of Medicine (2005) *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty, Acids, Cholesterol, Protein and Amino Acids*. Washington, DC: The National Academies Press.
33. Westerterp KR (2001) Pattern and intensity of physical activity. *Nature* **410**, 539.
34. Crouter SE, Kuffel E, Haas JD, *et al.* (2010) Refined two-regression model for the ActiGraph accelerometer. *Med Sci Sports Exerc* **42**, 1029–1037.
35. Bonomi AG, Plasqui G, Goris AH, *et al.* (2009) Improving assessment of daily energy expenditure by identifying types of physical activity with a single accelerometer. *J Appl Physiol* **107**, 655–661.
36. Staudenmayer J, Pober D, Crouter S, *et al.* (2009) An artificial neural network to estimate physical activity energy expenditure and identify physical activity type from an accelerometer. *J Appl Physiol* **107**, 1300–1307.
37. Maiolo C, Melchiorri G, Iacopino L, *et al.* (2003) Physical activity energy expenditure measured using a portable telemetric device in comparison with a mass spectrometer. *Br J Sports Med* **37**, 445–447.
38. McLaughlin JE, King GA, Howley ET, *et al.* (2001) Validation of the COSMED K4 b2 portable metabolic system. *Int J Sports Med* **22**, 280–284.
39. Melanson EL, Freedson PS, Hendelman D, *et al.* (1996) Reliability and validity of a portable metabolic measurement system. *Can J Appl Physiol* **21**, 109–119.
40. Wideman L, Stoudemire NM, Pass KA, *et al.* (1996) Assessment of the aerosport TEEM 100 portable metabolic measurement system. *Med Sci Sports Exerc* **28**, 509–515.
41. Duffield R, Dawson B, Pinnington HC, *et al.* (2004) Accuracy and reliability of a Cosmed K4b2 portable gas analysis system. *J Sci Med Sport* **7**, 11–22.
42. Littlewood RA, White MS, Bell KL, *et al.* (2002) Comparison of the Cosmed K4 b(2) and the Deltatrac II metabolic cart in measuring resting energy expenditure in adults. *Clin Nutr* **21**, 491–497.
43. Mc Naughton LR, Sherman R, Roberts S, *et al.* (2005) Portable gas analyser Cosmed K4b2 compared to a laboratory based mass spectrometer system. *J Sports Med Phys Fitness* **45**, 315–323.
44. Byrne NM, Hills AP, Hunter GR, *et al.* (2005) Metabolic equivalent: one size does not fit all. *J Appl Physiol* **99**, 1112–1119.
45. Jette M, Sidney K & Blumchen G (1990) Metabolic equivalents (METS) in exercise testing, exercise prescription, and evaluation of functional capacity. *Clin Cardiol* **13**, 555–565.
46. Leenders NY, Sherman WM & Nagaraja HN (2006) Energy expenditure estimated by accelerometry and doubly labeled water: do they agree? *Med Sci Sports Exerc* **38**, 2165–2172.
47. Yamazaki T, Gen-No H, Kamijo Y, *et al.* (2009) A new device to estimate VO_2 during incline walking by accelerometry and barometry. *Med Sci Sports Exerc* **41**, 2213–2219.

British Journal of Nutrition

Original Article

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

Yuko Gando[1,2], Kenta Yamamoto[2], Hiroshi Kawano[3], Haruka Murakami[2], Yumi Ohmori[2], Ryoko Kawakami[2], Kiyoshi Sanada[4], Mitsuru Higuchi[3], Izumi Tabata[2,4], and Motohiko Miyachi[2]

[1]Sport Science Research Center, Waseda University, Saitama, Japan
[2]Health Promotion and Exercise Program, National Institute of Health and Nutrition, Tokyo, Japan
[3]Faculty of Sport Sciences, Waseda University, Saitama, Japan
[4]College of Sport and Health Science, Ritsumeikan University, Shiga, Japan

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.

J Atheroscler Thromb, 2011; 18:248-254.

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-

Address for correspondence: Yuko Gando, Waseda University Sport Science Research Center, 2-579-15, Mikajima, Tokorozawa, Saitama, 359-1192, Japan
E-mail: gando-y@moegi.waseda.jp
Received: August 26, 2010
Accepted for publication: October 26, 2010

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

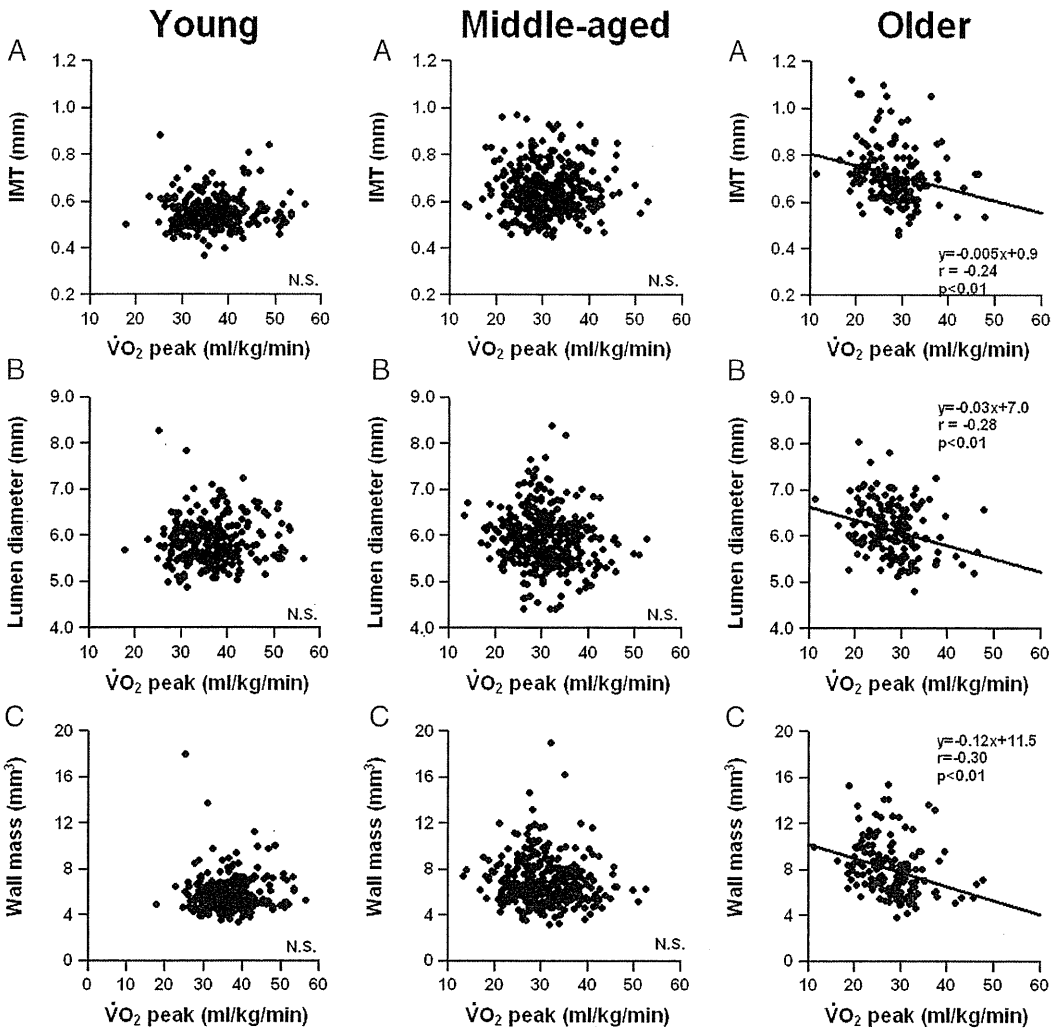

Fig. 1. Relationships between CRF and carotid IMT (A), lumen diameter (B), and wall mass (C) in each age category.

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.

Grants

This study was supported by a Grant-in-Aid for Scientific Research (#20300231, M. Miyachi, #18200041, H. Shibayama) and a Grant-in-Aid for Scientific Research from the Ministry of Health, Labor, and Welfare of Japan (M. Miyachi).

Disclosure

The authors declare no conflicts of interest.

References

1) Nagai Y, Metter EJ, Earley CJ, Kemper MK, Becker LC, Lakatta EG, Fleg JL: Increased carotid artery intimal-medial thickness in asymptomatic older subjects with exercise-induced myocardial ischemia. Circulation, 1998; 98: 1504-1509
2) Lakatta EG: Cardiovascular regulatory mechanisms in advanced age. Physiol Rev, 1993; 73: 413-467
3) Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE: Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation, 1997; 96: 1432-1437
4) O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr: Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med, 1999; 340: 14-22
5) Church TS, LaMonte MJ, Barlow CE, Blair SN: Cardiorespiratory fitness and body mass index as predictors of cardiovascular disease mortality among men with diabetes. Arch Intern Med, 2005; 165: 2114-2120
6) Lee IM, Paffenbarger RS Jr: Physical activity and stroke incidence: the Harvard Alumni Health Study. Stroke, 1998; 29: 2049-2054
7) Scuteri A, Chen CH, Yin FC, Chih-Tai T, Spurgeon HA, Lakatta EG: Functional correlates of central arterial geometric phenotypes. Hypertension, 2001; 38: 1471-1475
8) Scuteri A, Manolio TA, Marino EK, Arnold AM, Lakatta EG: Prevalence of specific variant carotid geometric patterns and incidence of cardiovascular events in older persons. The Cardiovascular Health Study (CHS E-131). J Am Coll Cardiol, 2004; 43: 187-193
9) Mourad JJ, Girerd X, Boutouyrie P, Safar M, Laurent S: Opposite effects of remodeling and hypertrophy on arterial compliance in hypertension. Hypertension, 1998; 31: 529-533
10) Bonithon-Kopp C, Touboul PJ, Berr C, Magne C, Ducimetiere P: Factors of carotid arterial enlargement in a population aged 59 to 71 years: the EVA study. Stroke, 1996; 27: 654-660
11) Reneman RS, van Merode T, Hick P, Hoeks AP: Flow velocity patterns in and distensibility of the carotid artery bulb in subjects of various ages. Circulation, 1985; 71: 500-509
12) Miyachi M, Donato AJ, Yamamoto K, Takahashi K, Gates PE, Moreau KL, Tanaka H: Greater age-related reductions in central arterial compliance in resistance-trained men. Hypertension, 2003; 41: 130-135
13) Homma S, Hirose N, Ishida H, Ishii T, Araki G: Carotid plaque and intima-media thickness assessed by b-mode ultrasonography in subjects ranging from young adults to centenarians. Stroke, 2001; 32: 830-835
14) Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R: Intimal

plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation, 1986; 74: 1399-1406
15) Miyachi M, Kawano H, Sugawara J, Takahashi K, Hayashi K, Yamazaki K, Tabata I, Tanaka H: Unfavorable effects of resistance training on central arterial compliance: a randomized intervention study. Circulation, 2004; 110: 2858-2863
16) Girerd X, Mourad JJ, Copie X, Moulin C, Acar C, Safar M, Laurent S: Noninvasive detection of an increased vascular mass in untreated hypertensive patients. Am J Hypertens, 1994; 7: 1076-1084
17) Manolio TA, Kronmal RA, Burke GL, O'Leary DH, Price TR: Short-term predictors of incident stroke in older adults. The Cardiovascular Health Study. Stroke, 1996; 27: 1479-1486
18) Armentano R, Megnien JL, Simon A, Bellenfant F, Barra J, Levenson J: Effects of hypertension on viscoelasticity of carotid and femoral arteries in humans. Hypertension, 1995; 26: 48-54
19) Perloff D, Grim C, Flack J, Frohlich ED, Hill M, McDonald M, Morgenstern BZ: Human blood pressure determination by sphygmomanometry. Circulation, 1993; 88: 2460-2470
20) Gando Y, Kawano H, Yamamoto K, Sanada K, Tanimoto M, Oh T, Ohmori Y, Miyatani M, Usui C, Takahashi E, Tabata I, Higuchi M, Miyachi M: Age and cardiorespiratory fitness are associated with arterial stiffening and left ventricular remodelling. J Hum Hypertens, 2010; 24: 197-206
21) Gando Y, Yamamoto K, Murakami H, Ohmori Y, Kawakami R, Sanada K, Higuchi M, Tabata I, Miyachi M: Longer time spent in light physical activity is associated with reduced arterial stiffness in older adults. Hypertension, 2010; 56: 540-546
22) Kozakova M, Palombo C, Morizzo C, Nolan JJ, Konrad T, Balkau B: Effect of sedentary behaviour and vigorous physical activity on segment-specific carotid wall thickness and its progression in a healthy population. Eur Heart J, 2010; 31: 1511-1519
23) Folsom AR, Eckfeldt JH, Weitzman S, Ma J, Chambless LE, Barnes RW, Cram KB, Hutchinson RG: Relation of carotid artery wall thickness to diabetes mellitus, fasting glucose and insulin, body size, and physical activity. Atherosclerosis Risk in Communities (ARIC) Study Investigators. Stroke, 1994; 25: 66-73
24) Stensland-Bugge E, Bonaa KH, Joakimsen O, Njolstad I: Sex differences in the relationship of risk factors to subclinical carotid atherosclerosis measured 15 years later: the Tromso study. Stroke, 2000; 31: 574-581
25) Rauramaa R, Vaisanen S, Mercuri M, Rankinen T, Penttila I, Bond MG: Association of risk factors and body iron status to carotid atherosclerosis in middle-aged eastern Finnish men. Eur Heart J, 1994; 15: 1020-1027
26) Schmidt-Trucksass AS, Grathwohl D, Frey I, Schmid A, Boragk R, Upmeier C, Keul J, Huonker M: Relation of leisure-time physical activity to structural and functional arterial properties of the common carotid artery in male subjects. Atherosclerosis, 1999; 145: 107-114
27) Tanaka H, Seals DR, Monahan KD, Clevenger CM, DeSouza CA, Dinenno FA: Regular aerobic exercise and the age-related increase in carotid artery intima-media thickness in healthy men. J Appl Physiol, 2002; 92: 1458-1464
28) Cuspidi C, Lonati L, Sampieri L, Leonetti G, Zanchetti A: Similarities and differences in structural and functional changes of left ventricle and carotid arteries in young borderline hypertensives and in athletes. J Hypertens, 1996; 14: 759-764
29) Galetta F, Franzoni F, Virdis A, Ghiadoni L, Taddei S, Salvetti A, Santoro G: Endothelium-dependent vasodilation and carotid artery wall remodeling in athletes and sedentary subjects. Atherosclerosis, 2006; 186: 184-192
30) Boutouyrie P, Bussy C, Lacolley P, Girerd X, Laloux B, Laurent S: Association between local pulse pressure, mean blood pressure, and large-artery remodeling. Circulation, 1999; 100: 1387-1393
31) Leung DY, Glagov S, Mathews MB: Cyclic stretching stimulates synthesis of matrix components by arterial smooth muscle cells in vitro. Science, 1976; 191: 475-477
32) Folkow B: The fourth Volhard lecture: cardiovascular structural adaptation; its role in the initiation and maintenance of primary hypertension. Clin Sci Mol Med Suppl, 1978; 4: 3s-22s
33) Dobrin PB: Mechanical factors associated with the development of intimal and medial thickening in vein grafts subjected to arterial pressure. A model of arteries exposed to hypertension. Hypertension, 1995; 26: 38-43
34) Tanaka H, Dinenno FA, Monahan KD, DeSouza CA, Seals DR: Carotid artery wall hypertrophy with age is related to local systolic blood pressure in healthy men. Arterioscler Thromb Vasc Biol, 2001; 21: 82-87
35) Park JH, Iemitsu M, Maeda S, Kitajima A, Nosaka T, Omi N: Voluntary running exercise attenuates the progression of endothelial dysfunction and arterial calcification in ovariectomized rats. Acta Physiol (Oxf), 2008; 193: 47-55
36) Matsuda M, Nosaka T, Sato M, Ohshima N: Effects of physical exercise on the elasticity and elastic components of the rat aorta. Eur J Appl Physiol Occup Physiol, 1993; 66: 122-126
37) Vaitkevicius PV, Lane M, Spurgeon H, Ingram DK, Roth GS, Egan JJ, Vasan S, Wagle DR, Ulrich P, Brines M, Wuerth JP, Cerami A, Lakatta EG: A cross-link breaker has sustained effects on arterial and ventricular properties in older rhesus monkeys. Proc Natl Acad Sci U S A, 2001; 98: 1171-1175
38) Okada S, Hiuge A, Makino H, Nagumo A, Takaki H, Konishi H, Goto Y, Yoshimasa Y, Miyamoto Y: Effect of exercise intervention on endothelial function and incidence of cardiovascular disease in patients with type 2 diabetes. J Atheroscler Thromb, 2010; 17: 828-833
39) Fukao K, Shimada K, Naito H, Sumiyoshi K, Inoue N, Iesaki T, Kume A, Kiyanagi T, Hiki M, Hirose K, Matsumori R, Ohsaka H, Takahashi Y, Toyoda S, Itoh S, Miyazaki T, Tada N, Daida H: Voluntary Exercise Ameliorates the Progression of Atherosclerotic Lesion Formation via Anti-Inflammatory Effects in Apolipoprotein E-Deficient Mice. J Atheroscler Thromb, 2010; 17: 1226-1236

Journal of
PHYSIOLOGICAL
ANTHROPOLOGY

Effects of Walking Speed and Step Frequency on Estimation of Physical Activity Using Accelerometers

Jonghoon Park[1], Kazuko Ishikawa-Takata[1], Shigeho Tanaka[1], Yuko Mekata[1] and Izumi Tabata[1),2)]

1) Health Promotion and Exercise Program, National Institute of Health and Nutrition
2) Faculty of Sport and Health Science, Ritsumeikan University

Abstract This study evaluated the accuracy of assessing step counts and energy costs under walking conditions altered by step frequency changes at given speeds using uni- (LC) and tri-axial accelerometers (AM, ASP). Healthy young men and women (n=18) volunteered as subjects. Nine tests were designed to manipulate three step frequencies, low (−15% of normal), normal, and high (+15%), at each walking speed (55, 75, and 95 m/min). A facemask connected to a Douglas bag was attached to subjects, who wore accelerometers around their waist. LC underestimated the step counts at normal or high step frequency at 55 m/min and AM also at all step frequencies at 55 m/min, whereas ASP did not in all trials. LC underestimated metabolic equivalents (METs) at low or normal step frequency at all walking speeds. AM underestimated METs at low step frequency at all walking speeds and at high step frequency of 95 m/min. ASP gave underestimates only at low step frequency of 95 m/min. The degree of the percentage error of METs for AM and ASP was affected by step frequency. Significant interaction between step frequency and speed was found that for LC. These results suggest that LC and AM can cause errors in step-count functions at a low walking speed. Furthermore, LC may show low accuracy of the METs measurement during walking altered according to step frequency and speed, whereas AM and ASP, which are tri-axial accelerometers, are more accurate but the degree of the percentage error is affected by step frequency. *J Physiol Anthropol 30(3): 119–127, 2011* http://www.jstage.jst.go.jp/browse/jpa2
[DOI: 10.2114/jpa2.30.119]

Keywords: accelerometers, step frequency, step counts, energy costs

Introduction

Walking is the most basic form of human locomotion and one of the most important components of many daily physical activities. An increased amount of energy expended in daily walking reportedly has significant effects on suppressing the progress of various diseases (Hakim et al., 1999; Lee and Buchner, 2008). Thus, walking represents a significant index of human health. Generally, human walking is complexly composed of the multidirectional characteristics of movements altered by combinations of speed and step frequency. Therefore, it would be of great value to accurately assess the number of steps or energy costs expended by various walking movements.

Pedometers are the simplest and the most inexpensive method that has been used for objective assessment of physical behaviors (Crouter et al., 2003; Schneider et al., 2004). Pedometers, especially, have the great advantage of providing immediate information on accumulated step counts as a real-time feedback tool to be more physically active (Bravata et al., 2007). Pedometers have been popularly used for normal subjects and certain epidemiological studies in Japan (Hatano 1993; Mitsui et al., 2008) and mechanisms have gradually been developed for detecting a step from using a spring-suspended lever arm (metal-on-metal contact) to using a magnetic reed proximity switch or accelerations (accelerometer). On the other hand, accelerometers are increasingly used to allow researchers to assess energy costs, and they are considered superior to other methods of measuring various physical activity categories, e.g., low or moderate-intensity activities like walking spread throughout the day (Plasqui and Westerterp, 2007; Bassett et al., 2008). Given the importance of quantifying step counts, most accelerometers made in Japan provide information not only on energy costs but also step counts. The Kenz Lifecorder EX (LC; Suzuken Co., Ltd., Nagoya, Japan), a uniaxial accelerometer using vertical acceleration, is already popular in many countries due to its reasonable cost and reliability for measuring the energy cost of walking or daily physical activity (Kumahara et al., 2004; McClain et al., 2007a, b). More recently, the Actimarker (AM; Panasonic Electronic Works Co., Ltd., Osaka, Japan) and the Active Style Pro (ASP; Omron Health Care Co., Ltd., Kyoto, Japan), newer triaxial accelerometers, have just become commercially available. Tri-axial accelerometers for measuring

daily physical activity are considered more accurate than uni-accelerometers (Plasqui and Westerterp, 2007; Yamada et al., 2009).

Many investigators have assessed the accuracy and reliability of pedometers and accelerometers, respectively, under various walking conditions (Crouter et al., 2003; Schneider et al., 2004). Pedometers or accelerometers are found to be less accurate in capturing step counts precisely at slow speeds below around 55 m·min^{-1} (Crouter et al., 2003; Le Masurier and Tudor-Locke, 2003; Le Masurier et al., 2004). Pedometers or accelerometers have their own vertical acceleration 'threshold' to capture a step. So far, low walking speed is the only factor known to lower accuracy in measuring steps; however, it remains unknown whether or not step frequency changes at a fixed walking speed (especially a slower walking speed) would affect accuracy in measuring step counts.

With respect to measuring energy costs, the uni- (vertical) or tri-axial (vertical, anteroposterior, and mediolateral) accelerometers have clearly demonstrated that output has a strong linear relationship with increasing walking speed (Bouten et al., 1994; Kumahara et al., 2004; Rowlands et al., 2007). However, in these studies, step frequency increased concurrently with walking speed. The rate of body movement changed by step length (step frequency) is likely to be proportional to the displacement in the anteroposterior direction (Dillman, 1975). In this case, a triaxial accelerometer using the integral of three accelerations (vertical, anteroposterior, and mediolateral) would be an appropriate method to quantify the energy cost from the multidirectional characteristics of human movement during walking. However, it remains unclear how much step frequency changes at a fixed walking speed would independently affect the accuracy of measuring energy costs with any accelerometer.

Accordingly, the purpose of the present study was to evaluate the accuracy of measuring step-count functions and energy costs from these three accelerometers under walking conditions altered by step frequency changes at given speeds.

Methods

Subjects

We recruited 18 healthy adult participants (9 male and 9 female, 23–41 yr) who work at the same worksites and did not have any physical impairment that affected ambulation. The descriptive characteristics of the subjects are presented in Table 1. All aspects of this study were approved by the Ethical Committee of the National Institute of Health and Nutrition. All subjects signed an informed consent document before the investigations were conducted.

Experimental Protocol

The resting metabolic rate (RMR) was measured 3 hr after each subject had lunch. Each subject became accustomed to walking on a motor-driven treadmill until it became natural. Afterward, the normal step frequency (step counts·min^{-1}) at which each subject feels comfortable walking (normal step frequency for each subject) was assessed by a researcher using a hand-tally counter at speeds of around 55, 75, and 95 m/min, which were generally used as low, normal, and high (brisk) walking speeds, respectively (Le Masurier and Tudor-Locke, 2003; Midorikawa et al., 2007). A low step frequency for each subject was calculated as −15% of normal step frequency and a high step frequency as +15%. The 15% change in step frequency during walking on a treadmill was chosen based on the range of stride length that can be easily performed and is sufficient to significantly increase metabolic costs (Holt et al., 1991; Russell et al., 2010). Before starting the measurements, each subject practiced walking while matching it to the tempo (frequency) of an electronic metronome sound. Afterward, a facemask connected to the Douglas bag was worn by subjects with three accelerometers equipped on their waist; they then took a 5-minute rest while sitting on a chair mounted on the treadmill. The experimental procedure started at a 55 m/min pace, and then was repeated at 75 and 95 m/min in order. Three trials of normal, high, and low frequency were included at each walking speed (i.e., a total of nine tests were performed). The order of the nine tests was not randomized in order to assess them from lower to higher metabolic cost. A 15% decrease in stride length, i.e., increased step frequency, requires more energy expenditure compared with normal stride length (Russell et al., 2010) and longer steps at given walking speeds increase metabolic cost even more (Cavagna and Franzetti, 1986). The subject took a 5-min rest while seated between settings of step frequency and a 30-min rest between settings of walking speed. Also, we recorded the number of steps counted by three accelerometers during each trial and recorded all trials using a digital video recorder for precise counting later.

Measurement of resting metabolic rate and metabolic equivalents

Three hr after lunch, each subject sat relaxed for 30 min to reach stable oxygen consumption. RMR was then measured using a mask and Douglas bag for 20 min (10 min×2 with a 1-minute intermission). Energy expenditure during walking was measured using a mask and Douglas bag. Each subject walked for 6 min at 55 m/min and 5 min at 75 or 95 m/min, and respiratory measurements were made during the last 3 min and 2 min at 55 m/min and 75 or 95 m/min, respectively. O_2 and CO_2 concentrations of expired gas were measured using a gas analyzer (ARCO-1000A; Arco System, Kashiwa, Japan). Before each measurement, the gas analyzer was calibrated using room air and a certified gas. Expired gas volume was measured with a certified dry gas meter (SHINAGAWA DC-5, Tokyo, Japan). Energy expenditure (kcal) was calculated from O_2 consumption and CO_2 production using Weir's equation (Weir, 1949). The metabolic equivalents (METs) were determined by energy expenditure (kcal) obtained during walking divided by the measured RMR.

Equipment

Kenz Lifecorder EX

The Kenz Lifecorder EX (LC; Suzuken Co., Ltd., Nagoya, Japan) is a uniaxial accelerometer, 70×40×25 mm in size and 30 g in mass. In this study, devices were attached on the side of the waist at the midline of the left thigh. This accelerometer samples at 32 Hz and assesses values ranging from 0.06 to 1.94 G. The LC uses only four thresholds from maximum amplitudes of vertical acceleration when determining the intensity levels. The signal is filtered by an analog bandpass filter and digitized. The maximum amplitude of the acceleration sensor and the step count generated by vertical movement determine the intensity levels. Ten intensity levels (0.5 and 1–9) are used to categorize intensities. The LC was initialized by setting the precise time and date and inputting the gender, age, height, and weight of each subject. After completing all trials, the data were downloaded using Physical Activity Analysis Software (Version 1.0, Suzuken Co., Ltd., Nagoya, Japan). The intensities obtained every 4 sec were converted into METs using Kumahara's equation obtained during progressive walking and running on a treadmill (Kumahara et al., 2004). The relationship between METs and activity level was highly significant ($r^2=0.929$):

$METs=0.043x^2+0.379x+1.361$ (Kumahara et al., 2004)

where x is the intensity level (0.5, 1–9 intensities).

Actimarker

The Actimarker (AM; Panasonic Electronic Works, Ltd., Osaka, Japan) is a triaxial accelerometer, 60×35×12 mm in size, and 30 g in mass. In the present study, devices were attached on either side of the waist at the midline of the left thigh. This device was released for sale in 2008 and has field-proven reliability for estimating various activities (Yamada et al., 2009). The AM had user-friendly software providing activity intensity categories, daily energy expenditure, steps, and METs·hr. This device obtained three-dimensional accelerations with a sensitivity of 4 mG and with a band-pass filter of 0.3 to 100 Hz. The acceleration count was calculated as the average of the absolute values from acceleration in each direction for a given interval (12 sec). The acceleration data were uploaded to a personal computer, and converted into METs by the following equation:

Physical Activity Energy Expenditure (PAEE) (kcal/min)= ax×[basal metabolic rate (BMR)]/1440+RMR

where a is a coefficient, and x is the output data from synthetic accelerations of 3 dimensions.

RMR is calculated by BMR×1.2

METs=PAEE/RMR.

The BMR was estimated according to the sixth Recommended Dietary Allowances for Japanese (Ministry of Welfare Japan, 1999). We also obtained the data of anteroposterior, mediolateral, and vertical accelerations from special software of the AM which was not available commercially.

Active Style Pro

The Active Style Pro (ASP; Omron Health Care Co., Ltd., Kyoto, Japan) is also a triaxial accelerometer, 80×20×50 mm in size, and 61 g in mass. The ASP was released for sale in Japan in 2008 and has proven reliability for estimating various activities (Oshima et al., 2010). In the present study, devices were attached on the right side of the waist. Anteroposterior, mediolateral, and vertical accelerations were obtained from the triaxial accelerometer during each activity with a sensitivity of 3 mG and at a sampling rate of 32 Hz. With a 12-bit analog to digital converter, the maximum scaling of the acceleration data was±2048 counts. Acceleration data were then uploaded to a personal computer. The signals obtained from the triaxial accelerometer were processed in the following way. Each of the 3 signals from the triaxial accelerometer was passed through a high-pass filter with a cutoff frequency at 0.7 Hz to remove the gravitational acceleration component from the signal. We calculated the integral of the absolute value of the accelerometer output of each of the 3 axes using acceleration signals over a 10-sec time interval. After the synthetic acceleration was filtered, it was categorized into either lifestyle or locomotive activity using a ratio of unfiltered to filtered synthetic acceleration. Synthetic accelerations of three dimensions were converted into METs by the follow equations:

If Counts/min: ≦A, Sedentary METs is b+ax

If Counts/min: >A and Ratio: ≦B, lifestyle activity formula METs is d+cx

If Ratio: >B, locomotive activity formula METs is f+ex

where A and B are thresholds, and a to f are coefficients.

x is output data from synthetic accelerations of 3 dimensions.

Statistics

All values are presented as means±SD. Differences were considered to be statistically significant if the p value was <0.05. Significant differences between men and women in the physical or gait characteristics were analyzed by an unpaired-sample t-test. The percentage error was calculated as [(predicted value−observed value)/observed value] ×100. Statistical comparisons of measured METs or the acceleration data obtained from the AM among step frequencies at each walking speed were performed by one-way analyses of variance (ANOVA) with repeated measures, and the Bonferroni procedure was used for *post-hoc* tests. One-way ANOVA with repeated measures was also used to compare differences between observed and predicted step counts or measured and predicted METs among accelerometers at each step frequency at a given walking speed; the Dunnett procedure was used for *post-hoc* tests. Two-way (step frequency and speed) ANOVA with repeated measures was used to determine how the factors affected the percentage error between measured and predicted METs in each accelerometer. When significant interactions were detected, simple main effect analysis was employed for each low, normal, or high step frequency to compare the effect of speed with the Bonferroni adjustment procedure. Multiple stepwise regression analysis

Table 1 Physical characteristics of subjects

	Men (N=9)	Women (N=9)	All subjects (N=18)
Age (yr)	31.0±4.9 (24–37)	28.1±5.9 (23–41)	29.6±5.5 (23–41)
Height (cm)	171.6±2.2 (167.6–173.9)	161.5±5.9 (151.4–167.9)*	166.5±6.8 (151.4–173.9)
Body mass (kg)	71.2±6.5 (62.3–78.2)	52.7±6.2 (45.3–59.2)*	71.2±6.5 (45.3–78.2)
BMI ($kg \cdot m^{-2}$)	24.2±2.1 (20.9–26.5)	20.1±1.6 (18.0–23.3)*	24.2±2.1 (18.0–26.5)
RMR ($kcal \cdot min^{-1}$)	1.1±0.3 (0.77–1.59)	0.9±0.1 (0.76–1.01)	1.1±0.3 (0.76–1.59)
Step length (cm)			
$55\ m \cdot min^{-1}$	57.2±5.2 (50.9–66.3)	58.2±5.9 (51.4–67.9)	57.2±5.2 (50.9–67.9)
$75\ m \cdot min^{-1}$	67.7±4.2 (60.0–74.3)	69.0±4.9 (59.5–74.0)	67.7±4.2 (59.5–74.3)
$95\ m \cdot min^{-1}$	79.4±4.9 (70.4 - 85.6)	78.3±6.1 (64.6 - 82.6)	79.4±4.9 (64.6–85.6)
Step frequency			
$55\ m \cdot min^{-1}$	96.8±8.5 (83–108)	95.3±9.5 (81–107)	96.8±8.5 (81–108)
$75\ m \cdot min^{-1}$	111.1±7.1 (101–125)	109.2±8.3 (101–126)	111.1±7.1 (101–126)
$95\ m \cdot min^{-1}$	120.1±7.7 (111–135)	122.1±10.6 (110–147)	120.1±7.7 (110–147)

Abbreviations: BMI, body mass index; RMR, resting metabolic rate
Values are means±s.d. (range). * indicates a significant difference from Men ($p<0.001$).

Table 2 Accuracy of step-count functions among accelerometers

walking speed	Step frequency	Visually counted steps	Step detected			p value		
			LC	AM	ASP	LC	AM	ASP
55 m/min	low	492±45	452±64	410±97	464±87	0.171	0.001	0.414
	normal	580±54	534±86	475±81	576±64	0.023	<0.001	0.984
	high	662±59	616±72	576±70	650±58	0.002	<0.001	0.695
75 m/min	low	475±31	478±47	466±48	477±32	0.968	0.693	0.997
	normal	552±40	538±49	545±43	551±40	0.069	0.590	1.000
	high	633±59	595±113	634±76	633±60	0.135	1.000	1.000
95 m/min	low	522±40	522±39	521±40	522±41	0.999	0.969	0.994
	normal	609±41	606±40	608±41	609±41	0.246	0.924	0.996
	high	705±48	692±56	702±49	701±47	0.191	0.986	0.921

Values are means±s.d. LC, Kenz Lifecorder; AM, Actimarker; ASP, Active Style Pro

was employed to determine which variables (step frequency, speed, step frequency x speed, sex, height, and body mass) contribute to the percentage error of estimating METs or step counts in each accelerometer. All statistical treatments were done using SPSS for Windows (version 16.0J; SPSS Inc., Chicago, IL, USA).

Results

The physical or gait characteristics of the subjects are shown in Table 1. Height, body mass, and BMI were significantly higher in men than in women ($p<0.001$), whereas the other factors did not differ between genders.

Accuracy of detecting step counts

The LC significantly underestimated step counts at the normal or high step frequency at 55 m/min (Table 2). The AM significantly underestimated step counts at all step frequencies at 55 m/min, whereas the ASP did not in any of the nine trials. In the percentage error between the measured and predicted step counts for each accelerometer, two-way ANOVA analysis demonstrated no significant interactions between step frequency and speed in all accelerometers (Fig. 1). Speed significantly contributed to the percentage error in all accelerometers whereas step frequency did not. A stepwise multiple regression analysis of predictors of the percentage error (including step frequency, speed, step frequency by speed, sex, height, and body mass) in the LC and AM revealed that speed was the only significant predictor (β=0.32, $p<$ 0.001 for the LC; β=0.57, $p<0.001$ for the AM), but no other factors were selected. The final models of the LC and AM accounted for 11% and 32% of the variance of the percentage error, respectively. In the case of the ASP, step frequency by speed was the only significant predictor (β=0.21, p=0.007), and the final model accounted for 5% of the variance in percentage error.

METs measured from Douglas bag method

The METs measured at low, normal, and high step frequency were shown in Table 3. There were no differences in measured METs among the three step frequencies at 55 m/min. However, at 75 and 95 m/min (p=0.003 and $p<0.001$,

respectively), the measured METs at the low step frequency were significantly higher than those of the normal step frequency.

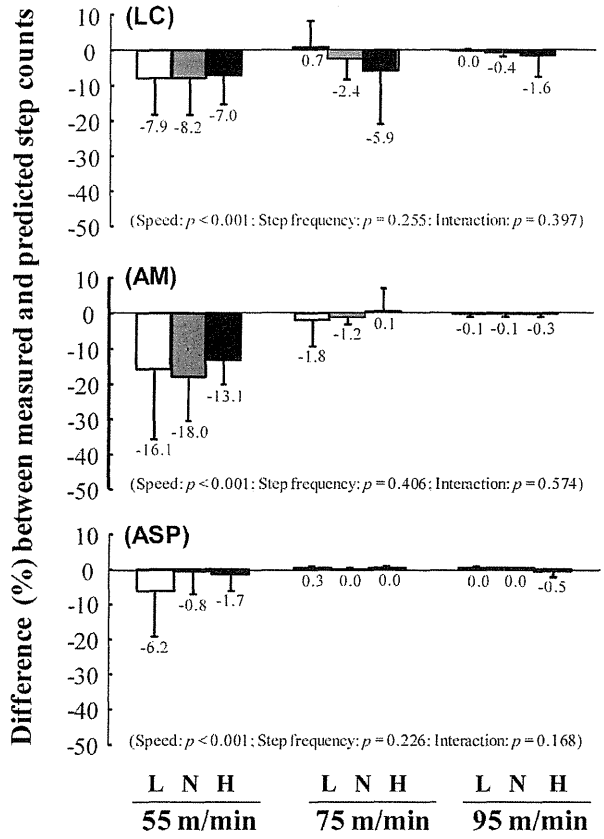

Fig. 1 Difference between measured (video records) and predicted step counts (LC, Kenz Lifecorder; AM, Actimarker; ASP, Active Style Pro) across conditions altered by three step frequencies (L, Low step frequency; N, Normal step frequency; H, High step frequency) at each walking speed (55, 75, and 95 m/min). The numbers under the bars indicate the difference (%) between measured and predicted step counts. The results of two-way ANOVA analysis were shown in parentheses for each accelerometer.

Accuracy of predicted METs in accelerometers

The LC significantly underestimated METs at the low and normal step frequencies at all walking speeds (Table 3). The AM significantly underestimated METs at the low step frequency at all walking speeds and also at the high step frequency of 95 m/min. The ASP significantly underestimated only at the low step frequency of 95 m/min. In the percentage error between the measured and predicted METs for each accelerometer, two-way ANOVA analysis demonstrated significant interaction between step frequency and speed only in the LC (Fig. 2). Simple main effect analysis in the LC showed no significant differences in the percentage errors among the three low step frequencies (55 m/min vs. 75 m/min: $p=0.788$; 75 m/min vs. 95 m/min: $p=0.647$; 55 m/min vs. 95 m/min: $p=0.060$), the three normal step frequencies (all $p=1.000$), or the three high step frequencies (55 m/min vs. 75 m/min and 75 m/min vs. 95 m/min: $p=1.000$; 55 m/min vs. 95 m/min: $p=0.612$). Step frequency significantly contributed to the percentage error in the AM and ASP, whereas speed did not. As shown in Table 4, a stepwise multiple regression analysis of predictors in the percentage error between the measured and predicted METs for each accelerometer revealed that step frequency was the strongest predictor in the LC. Speed and height were significantly associated, but step frequency by speed, sex, and body mass was not selected for the model. The final model accounted for 58% of the model variation. The percentage error in the AM showed that height and sex significantly contributed to the percentage error, but step frequency, speed, and step frequency by speed were not selected. The final model accounted for 8.8% of the model variation. With ASP, the step frequency and speed significantly contributed to the percentage error, and the final model accounted for 10% of the model variation.

Anteroposterior, mediolateral, and vertical accelerations

Figure 3 shows the absolute data of anteroposterior, mediolateral, and vertical accelerations measured by the AM. Mediolateral acceleration at 55 m/min was significantly higher at the high step frequency than at the normal step frequency. Mediolateral acceleration at 75 m/min and 95 m/min was

Table 3 Accuracy of predicted METs among accelerometers

walking speed	Step frequency	Observed METs steps	Predicted METs			*p* value		
			LC	AM	ASP	LC	AM	ASP
55 m/min	low	3.2±0.9	2.2±0.1	2.8±0.3	3.0±0.3	<0.001	0.035	0.643
	normal	2.8±0.4	2.5±0.3	2.7±0.2	3.0±0.2	0.009	0.411	0.096
	high	3.0±0.7	2.9±0.4	2.8±0.3	3.2±0.3	0.866	0.250	0.616
75 m/min	low	4.1±0.7	2.6±0.3	3.5±0.3	3.9±0.3	<0.001	0.002	0.567
	normal	3.6±0.7	3.1±0.4	3.3±0.3	3.9±0.4	0.004	0.151	0.273
	high	3.8±0.9	4.0±1.0	3.4±0.5	4.0±0.7	0.714	0.178	0.616
95 m/min	low	6.0±1.0	3.3±0.4	4.7±0.3	5.2±0.4	<0.001	<0.001	0.001
	normal	4.5±0.6	4.0±0.8	4.2±0.3	4.9±0.4	0.010	0.093	0.141
	high	4.7±0.8	5.1±0.6	4.2±0.5	5.1±0.6	0.074	0.045	0.168

Values are means±s.d. METs, Metabolic equivalents; LC, Kenz Lifecorder; AM, Actimarker; ASP, Active Style Pro

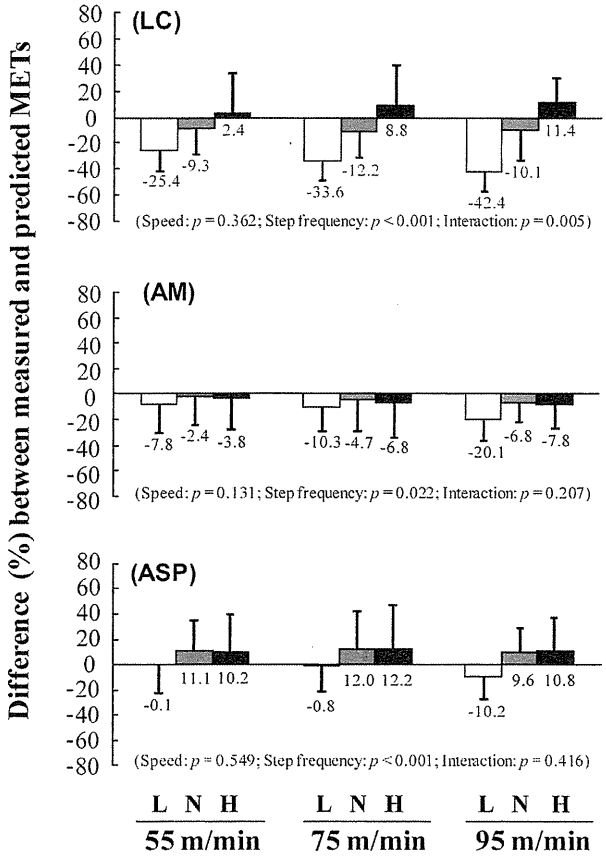

Fig. 2 Difference between measured (Douglas bag) and predicted METs (LC, Kenz Lifecorder; AM, Actimarker; ASP, Active Style Pro) across conditions altered by three step frequencies (L, Low step frequency; N, Normal step frequency; H, High step frequency) at each walking speed (55, 75, and 95 m/min). The numbers under the bars indicate the difference (%) between measured and predicted METs. The results of two-way ANOVA analysis were shown in parentheses for each accelerometer.

significantly higher at the low step frequency than at the normal frequency. Vertical acceleration was significantly higher at the high step frequency at 55 m/min or at the low step frequency at 95 m/min than at the normal frequency. Anteroposterior acceleration was significantly higher at the low step frequency at all walking speeds when compared to normal frequency. The total value of three accelerations was significantly higher at the high step frequency at 55 m/min than at the normal frequency and also higher at the low step

Table 4 Multiple regression analysis of predictors in the percentage error between the measured and predicted METs in each accelerometer

Predictors	R	R^2	Unstandardized coefficients		β	p-value
			B	Standard error		
LC						
Step frequency			1.262	0.089	0.869	<0.001
Speed			−0.857	0.102	−0.514	<0.001
Height			−0.550	0.214	−0.133	<0.001
Total	0.764	0.584				<0.001
AM						
Height			−1.409	0.370	−0.452	<0.001
Sex			−11.832	4.880	−0.288	0.016
Total	0.296	0.088				<0.001
ASP						
Step frequency			0.487	0.116	0.374	<0.001
Speed			−0.396	0.134	−0.265	0.004
Total	0.322	0.103				<0.001

Abbreviations: METs, Metabolic equivalents; R, Multiple correlation coefficient; R^2, Multiple coefficient of determination; β, Standardized partial regression coefficient; LC, Kenz Lifecorder; AM, Actimarker; ASP, Active Style Pro.

Confounding factors of step frequency, speed, step frequency x speed, sex, height, and body mass were used in the analyses of each accelerometer.

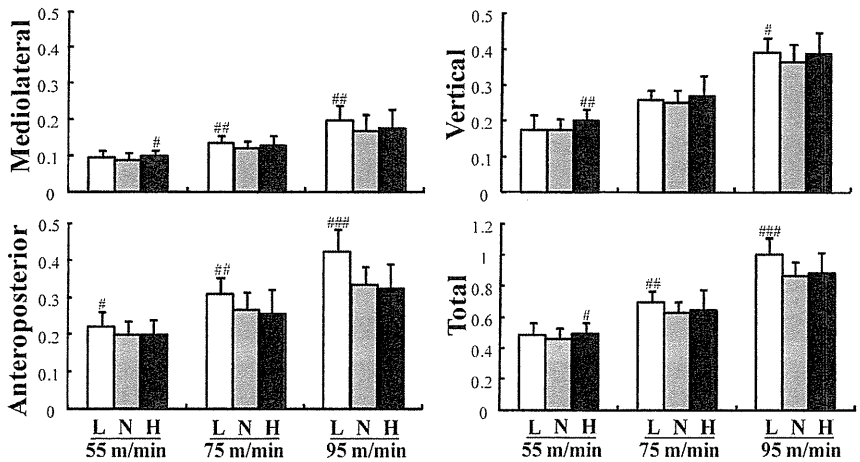

Fig. 3 Absolute accelerations in the mediolateral, vertical, anteroposterior, and total directions measured by the Actimarker across conditions altered by three step frequencies (L, Low step frequency; N, Normal step frequency; H, High step frequency) at each walking speed (55 m/min, 75 m/min, 95 m/min). Values significantly different from N (Normal step frequency) are indicated by $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$.

frequency at 75 and 95 m/min.

Discussion

This is the first study to investigate whether changes in step frequency have independent effects on the validity of step-count functions and predicted energy cost assessed by accelerometers. The LC underestimated the step counts at normal or high step frequency at low walking speed. The AM also underestimated them at all step frequencies at low walking speed, whereas the ASP did not across any of the trials. The degree of the percentage error of the step counts in all accelerometers was affected by speed, but not by step frequency. The LC underestimated METs at the low or normal step frequency at all walking speeds, whereas overall underestimation was less across trials in the AM and ASP.

The present study clearly demonstrated that LC, AM, and ASP have very accurate step-count functions at normal walking speed with normal step frequency at which each subject feels comfortable walking. Schneider et al. (2003) demonstrated that LC had more accurate step-count functions compared with 7 other pedometers during a 400-m track walk at self-selected speeds for adults, and its error in detecting actual steps taken was within±3%. Actually, Cao et al. (2010) reported no significant differences between LC and AM in daily walking step counts for adults. The error of −2.4% (LC), −1.2% (AM), and 0.0% (ASP) at normal walking speed with normal step frequency in the present study meet the Japanese Industrial Standard set by the Ministry of Industry and Trading criteria indicating that error should be within 3% (3 steps of 100) (Hatano, 1993). Therefore, it is considered that LC, AM, and ASP are among the pedometers which are very accurate and sufficiently reliable to administer to large groups.

At low walking speed, the LC underestimated the step counts at the normal and high frequency while the AM underestimated them at all step frequencies. In contrast, the ASP had accurate step-count functions across all trials. The present results correspond with those of other studies using electronic pedometers that underestimated step counts at walking speeds slower than about 55 m/min (Crouter et al., 2003; Le Masurier and Tudor-Locke, 2003; Le Masurier et al., 2004). Thus, the impact on the accelerometers (sensitivity) during slow walking for the LC and AM might be too weak to detect a "threshold" of capturing a step, whereas ASP had better reliability in detecting step counts even with slow walking. It was difficult to determine why the ASP had better accuracy than the AM in detecting step counts; however, the difference in the filtering system between the AM and the ASP might explain this. Even so, speed significantly contributed to the degree of the percentage error in all three accelerometers. Therefore, we suggest that accelerometers should be carefully used when assessing daily step counts, especially for persons who walk slowly.

The present study revealed that the LC underestimated METs at the low and normal step frequency at all walking speeds, and gross underestimation was found especially at low step frequency of high walking speeds. One possible explanation for the LC error is its own proprietary data-analyzing process, in which intensity levels are categorized using both the step counts and the maximum amplitude of vertical acceleration every four seconds. In this study, although higher energy cost was demanded at a low step frequency especially at a higher walking speed, the number of step counts by the LC would have conversely decreased due to the greater step length (lower step frequency). Therefore, a decrease in step counts at the low step frequency might cause the LC underestimation. The possibility that step frequency could strongly affect the validation of the LC was supported by the following results: Step frequency was the strongest predictor (β=0.87) and speed was the second strongest (β=−0.51) of the error between the measured and predicted METs in the multiple regression analysis. Therefore, changes in step frequency would individually and markedly affect the accuracy of the LC.

Another possible reason for the LC underestimation may be that in the data-analyzing process, it uses only four thresholds from maximum amplitudes of vertical acceleration when determining the intensity levels (i.e., noncontinuous variables). For example, if the maximum amplitudes of vertical acceleration during walking altered by both low and normal step frequency at a fixed walking speed are between 0.15–0.76 G, the difference in intensity levels between the low and normal step frequency will be determined by the difference in the step counts. However, as mentioned above, the number of step counts by the LC would have been decreased due to the low step frequency, despite the higher energy cost. This might be one explanation for the LC error.

Compared with the results of the LC, the AM and ASP showed less error in measuring METs across trials. Multiple regression analysis indicated that step frequency did not affect AM accuracy. Although ASP accuracy was affected by step frequency, it only explains 10% of the error. Better validity of the AM and ASP compared with the LC might be partly due to the higher capability of the triaxial accelerometers in assessing multiple-directional accelerations as continuous variables. In the earlier studies, anteroposterior or vertical acceleration contributed to a highly accurate estimation of physical activity under normal walking conditions in which step frequency was concurrently changed with an increment in speed (Bouten et al., 1994; Kumahara et al., 2004). However, in our experimental protocol (i.e., various step frequencies altered at a fixed walking speed), the difference in vertical acceleration between the low and normal step frequency was much less than the difference between the low and normal step frequencies in the METs measured by the Douglas bag method. Moreover, the major acceleration component at a low step frequency was in the anteroposterior direction, but in the vertical direction at a high step frequency. Based on our results, we suggest that the AM and ASP assure more accuracy than the LC for estimating intensity or energy costs under various walking conditions.

In the present study, the degree of the percentage error of METs was affected by step frequency both in the AM and ASP. Significant underestimation was found in AM at all low step frequencies of all walking speeds, but in ASP only at low step frequency of high walking speed. As shown in the raw data of the three accelerations, the total values using the output of the three accelerations at the high walking speed was around 16% higher at the low step frequency than at the normal step frequency. However, the difference in the measured METs from the Douglas bag at the high walking speed was around 25% higher at the low step frequency than at the normal step frequency. The discrepancy of 16% and 25% might therefore result in higher error at the low step frequency of high walking speed in the AM and ASP. Furthermore, the present study showed that METs estimated by the AM tended to be entirely underestimated across trials compared with METs estimated by the ASP (Fig. 2). Because the minimum amplitude of the acceleration sensor was similar between the AM (4 mG) and the ASP (3 mG), the sensitivity of the minimum amplitude of the acceleration sensor did not affect the error of the AM. Therefore, we consider that AM accuracy may be improved by using more suitable equations to precisely measure energy costs altered by the various walking patterns.

The present study has the following limitations. First, our results using young healthy subjects might not be readily generalized to children or older adults due to different characteristics such as the length and mass of legs and body movement. In addition, the elderly have been known to walk and step so slowly that their walking movements are greater in mediolateral directions (Dean et al., 2007). Therefore, further research is needed to evaluate the accuracy of an accelerometer for other aged subjects under the same conditions and to calibrate for more accurate estimations. Second, we cannot exclude differences between walking on a treadmill indoors and freely walking outdoors. However, in general, the energy costs of treadmill and over-ground walking on a firm surface are similar (Hall et al., 2004). Hence, we thought that using the treadmill in our experimental protocol was adequate to obtain more precise and reliable data as a basic study.

In conclusion, these results suggest that accelerometers can cause errors in step-count functions at a low walking speed. Furthermore, in the measurement of energy costs, LC may cause great errors especially for the group with various step frequency and speed, whereas AM and ASP, which are tri-axial accelerometers, cause fewer errors but the degree of the percentage error is affected by step frequency.

Acknowledgements The authors thank the study subjects of the National Institute of Health and Nutrition for their cooperation. We especially thank Y Hikihara, K Ohkawara, Y Azuma, L Miyake, and H Kogure for their invaluable assistance in the present experiment. This work was performed as part of the Health and Labor Sciences Research Grants (Comprehensive Research on Cardiovascular and Life-style Related Diseases) from the Ministry of Health, Labor and Welfare of Japan (to S Tanaka), and was supported, in part, by the Suzuken Memorial Foundation (to Ishikawa-Takata K).

References

Bassett DR Jr, Mahar MT, Rowe DA, Morrow JR Jr (2008) Walking and measurement. Med Sci Sports Exerc 7 (Suppl): S529–536

Bouten CV, Westerterp KR, Verduin M, Janssen JD (1994) Assessment of energy expenditure for physical activity using a triaxial accelerometer. Med Sci Sports Exerc 26: 1516–1523

Bravata DM, Smith-Spangler C, Sundaram V, Gienger AL, Lin N, Lewis R, Stave CD, Olkin I, Sirard JR (2007) Using pedometers to increase physical activity and improve health: a systematic review. JAMA 298: 2296–2304

Cao ZB, Miyatake N, Higuchi M, Miyachi M, Ishikawa-Takata K, Tabata I (2010) Predicting VO_2 max with objectively measured physical activity in Japanese women. Med Sci Sports Exerc 42: 179–186

Cavagna GA, Franzetti P (1986) The determinants of the step frequency in walking in humans. J Physiol 373: 235–242

Crouter SE, Schneider PL, Karabulut M, Bassett DR Jr (2003) Validity of 10 electronic pedometers for measuring steps, distance, and energy cost. Med Sci Sports Exerc 35: 1455–1460

Dean JC, Alexander NB, Kuo D (2007) The effect of lateral stabilization on walking in young and old adults. IEEE Trans Biomed Eng 54: 1919–1926

Dillman CJ (1975) Kinematic analyses of running. Exerc Sport Sci Rev 3: 193–218

Hakim AA, Curb JD, Petrovitch H, Rodriguez BL, Yano K, Ross GW, White LR, Abbott RD (1999) Effects of walking on coronary heart disease in elderly men: the Honolulu Heart Program. Circulation 100: 9–13

Hall C, Figueroa A, Fernhall B, Kanaley JA (2004) Energy expenditure of walking and running: comparison with prediction equations. Med Sci Sports Exerc 36: 2128–2134

Hatano Y (1993) Use of the pedometer for promoting daily walking exercise. ICHPER J 29: 4–8

Holt KG, Hamill J, Andres RO (1991) Predicting the minimal energy costs of human walking. Med Sci Sports Exerc 23: 491–498

Kumahara H, Schutz Y, Ayabe M, Yoshioka M, Yoshitake Y, Shindo M, Ishii K, Tanaka H (2004) The use of uniaxial accelerometry for the assessment of physical-activity-related energy expenditure: a validation study against whole-body indirect calorimetry. Br J Nutr 91: 235–243

Lee IM, Buchner DM (2008) The importance of walking to public health. Med Sci Sports Exerc 40(Suppl): S512–18

Le Masurier GC, Tudor-Locke C (2003) Comparison of pedometer and accelerometer accuracy under controlled conditions. Med Sci Sports Exerc 35: 867–871

Le Masurier GC, Lee SM, Tudor-Locke C (2004) Motion sensor accuracy under controlled and free-living conditions.

Med Sci Sports Exerc 36: 905–910
McClain JJ, Craig CL, Sisson SB, Tudor-Locke C (2007a) Comparison of Lifecorder EX and ActiGraph accelerometers under free-living conditions. Appl Physiol Nutr Metab 32: 753–761
McClain JJ, Sisson SB, Washington TL, Craig CL, Tudor-Locke C (2007b) Comparison of Kenz Lifecorder EX and ActiGraph accelerometers in 10-yr-old children. Med Sci Sports Exerc 39: 630–638
Midorikawa T, Tanaka S, Kaneko K, Koizumi K, Ishikawa-Takata K, Futami J, Tabata I (2007) Evaluation of low-intensity physical activity by triaxial accelerometry. Obesity 15: 3031–3038
Ministry of Welfare Japan (1999) Recommended Dietary Allowances for the Japanese 6th ed. Ministry of Welfare, Japan, 36 [*In Japanese*]
Mitsui T, Shimaoka K, Tsuzuku S, Kajioka T, Sakakibara H (2008) Pedometer-determined physical activity and indicators of health in Japanese adults. J Physiol Anthropol 27: 179–184
Oshima Y, Kawaguchi K, Tanaka S, Ohkawara K, Hikihara Y, Ishikawa-Takata K, Tabata I (2010) Classifying household and locomotive activities using a triaxial accelerometer. Gait Posture 31: 370–374
Plasqui G, Westerterp KR (2007) Physical activity assessment with accelerometers: an evaluation against doubly labeled water. Obesity 15: 2371–2379
Rowlands AV, Stone MR, Eston RG (2007) Influence of speed and step frequency during walking and running on motion sensor output. Med Sci Sports Exerc 39: 716–727
Russell EM, Braun B, Hamill J (2010) Does stride length influence metabolic cost and biomechanical risk factors for knee osteoarthritis in obese women? Clin Biomech 25: 438–443
Schneider PL, Crouter SE, Lukajic O, Bassett DR (2003) Accuracy and reliability of 10 pedometers for measuring steps over a 400-m walk. Med Sci Sports Exerc 35: 1779–1784
Schneider PL, Crouter SE, Bassett DR (2004) Pedometer measures of free-living physical activity: comparison of 13 models. Med Sci Sports Exerc 36: 331–335
Weir, JBdV (1949) New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 109: 1–9
Yamada Y, Yokoyama K, Noriyasu R, Osaki T, Adachi T, Itoi A, Naito Y, Morimoto T, Kimura M, Oda S (2009) Light-intensity activities are important for estimating physical activity energy expenditure using uniaxial and triaxial accelerometers. Eur J Appl Physiol 105: 141–152

Received: August 11, 2010
Accepted: February 15, 2011
Correspondence to: Jonghoon Park, Health Promotion and Exercise Program, National Institute of Health and Nutrition, 1–23–1 Toyama, Shinjuku-ku, Tokyo 162–8636, Japan
Phone: +81–3–3203–8061
Fax: +81–3–3203–1731
e-mail: jonghoon@nih.go.jp

J Appl Physiol 110: 1493–1496, 2011:
doi:10.1152/japplphysiol.00242.2011.

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.

REFERENCES

1. **Robergs RA.** Counterpoint: Muscle lactate and H^+ production do not have a 1:1 association in skeletal muscle. *J Appl Physiol*; doi:10.1152/japplphysiol.01506.2010a.
2. **Vinnakota KC, Kushmerick MJ.** Point: Muscle lactate and H^+ production do have a 1:1 association in skeletal muscle. *J Appl Physiol*; doi: 10.1152/japplphysiol.01506.2010..

Daniel A. Beard
Professor
Medical College of Wisconsin

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."

REFERENCES

1. **Edsall JT, Wyman J.** *Biophysical Chemistry. Thermodynamics, Electrostatics, and the Biological Significance of the Properties of Matter Volume 1.* New York: Academic, 1958.
2. **Harned HS, Owen BB.** *The Physical Chemistry of Electrolytic Solutions* (3rd ed.). New York: Reinhold Publishing Corporation, 1958.
3. **Lindinger MI, Kowalchuk JM, Heigenhauser GJ.** Applying physicochemical principles to skeletal muscle acid-base status. *Am J Physiol Regul Integr Comp Physiol* 289: R891–R894; author reply R904–R910, 2005.
4. **Robergs RA.** Counterpoint: Muscle lactate and H^+ production do not have a 1:1 association in skeletal muscle. *J Appl Physiol*; doi:10.1152/japplphysiol.01506.2010a.
5. **Vinnakota KC, Kushmerick MJ.** Point: Muscle lactate and H^+ production do have a 1:1 association in skeletal muscle. *J Appl Physiol*; doi: 10.1152/japplphysiol.01506.2010.

Michael I. Lindinger
Human Health and Nutritional Sciences
University of Guelph
George J. F. Heigenhauser
McMaster University

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

8750-7587/11 Copyright © 2011 the American Physiological Society

Downloaded from jap.physiology.org on April 10, 2012

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?

REFERENCES

1. **Böning D, Maassen N.** Point: Lactic acid is the only physicochemical contributor to the acidosis of exercise. *J Appl Physiol* 105: 358–359, 2008.
2. **Böning D, Strobel G, Beneke R, Maassen N.** Lactic acid still remains the real cause of exercise-induced metabolic acidosis. *Am J Physiol Regul Integr Comp Physiol* 289: R902–R903, 2005.
3. **Robergs RA.** Counterpoint: Muscle lactate and H^+ production do not have a 1:1 association in skeletal muscle. *J Appl Physiol*; doi:10.1152/japplphysiol.01506.2010a.
4. **Robergs RA, Ghiasvand F, Parker D.** Biochemistry of exercise-induced metabolic acidosis. *Am J Physiol Regul Integr Comp Physiol* 287: R502–R516, 2004.
5. **Sahlin K.** Intracellular pH and energy metabolism in skeletal muscle of man. With special reference to exercise. *Acta Physiol Scand Suppl* 445: 1–56, 1978.
6. **Vinnakota KC, Kushmerick MJ.** Point: Muscle lactate and H^+ production do have a 1:1 association in skeletal muscle. *J Appl Physiol*; doi: 10.1152/japplphysiol.01506.2010.

Dieter Boning
Sports Medicine
Institute of Physiology
Charite-University Medicine
Norbert Maassen
Medical School Hannover

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

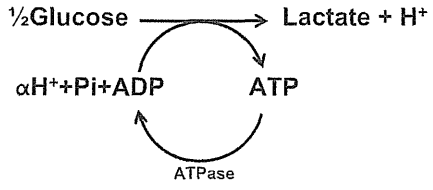

Fig. 1.

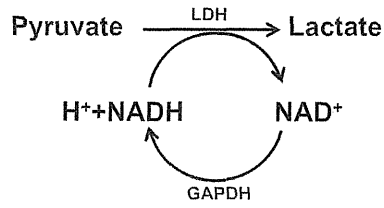

Fig. 2.

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.

REFERENCES

1. **Hochachka PW, Mommsen TP.** Protons and anaerobiosis. *Science* 219: 1391–1397, 1983.
2. **Hultman E, Sahlin K.** Acid-base balance during exercise. In: *Exercise and Sport Sciences Reviews*, edited by Hutton SR, Miller DI. The Franklin Institute, 1980, p. 41–128.
3. **Vinnakota KC, Kushmerick MJ, Robergs RA.** Point:Counterpoint: Muscle lactate and H^+ production do/do not have a 1:1 association in skeletal muscle. *J Appl Physiol*; doi:10.1152/japplphysiol.01506.2010.

Kent Sahlin
Professor
GIH, The Swedish School of Sport and Health Sciences
Astrand Laboratory

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'

Downloaded from jap.physiology.org on April 10, 2012

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.

REFERENCES

1. **Bishop D, Edge J, Mendez-Villanueva A, Thomas C, Schneiker K.** High-intensity exercise decreases muscle buffer capacity via a decrease in protein buffering in human skeletal muscle. *Pflugers Arch* 458: 929–936, 2009.
2. **Bishop D, Edge J, Thomas C, Mercier J.** Effects of high-intensity training on muscle lactate transporters and postexercise recovery of muscle lactate and hydrogen ions in women. *Am J Physiol Regul Integr Comp Physiol* 295: R1991–R1998, 2008.
3. **Hultman E, Sahlin K.** Acid-base balance during exercise. *Exerc Sport Sci Rev* 8: 41–128, 1980.
4. **Juel C.** Regulation of cellular pH in skeletal muscle fiber types, studied with sarcolemmal giant vesicles obtained from rat muscles. *Biochimica Biophysica Acta Mol Cell Res* 1265: 127–132, 1995.
5. **Vinnakota KC, Kushmerick MJ, Robergs RA.** Point:Counterpoint: Muscle lactate and H^+ production do/do not have a 1:1 association in skeletal muscle. *J Appl Physiol*; doi:10.1152/japplphysiol.01506.2010.

David J. Bishop
Professor
Institute of Sport, Exercise and Active Living (ISEAL)

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 → 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.

REFERENCES

1. **Beard DA, Lindinger MI, Heigenhauser GJF, Boning D, Maassen N, Sahlin K, Bishop DJ, Meyer RA, Wiseman RW, Crampin EJ, Tabata I.** Comments on Point:Counterpoint: Muscle lactate and H^+ production do/do not have a 1:1 association in skeletal muscle. *Appl Physiol*; doi: 10.1152/japplphysiol.00242.2011.
2. **Lindinger MI, Lowalchuk JM, Heigenhauser GJF.** Applying physicochemical principles to skeletal muscle acid-base status. *Am J Physiol Regul Integr Comp Physiol* 289: R891–R894, 2005.
3. **Sahlin K, Prakash ES, Robergs RA, Miller BF, Gladden LB, Jones N, Stringer WW, Wasserman K, Moll W, Gros G, Rowlands DS, Sahlin K, Beneke R.** Comments on Point:Counterpoint: Lactic acid is/is not the only physicochemical contributor to the acidosis of exercise. *J Appl Physiol* 105: 363–367, 2008.
4. **Vinnakota KC, Kushmerick MJ, Robergs RA.** Point:Counterpoint: Muscle lactate and H^+ production do/do not have a 1:1 association in skeletal muscle. *J Appl. Physiol*; doi:10.1152/japplphysiol.01506.2010.

Ronald A. Meyer
Professor
Robert W. Wiseman
Michigan State University

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.

REFERENCES

1. **Beard DA, Qian H.** *Chemical Biophysics*. Cambridge: Cambridge University Press, 2008.
2. **Robergs R.** Counterpoint: Muscle lactate and H^+ production do not have a 1:1 association in skeletal muscle. *J Appl Physiol*; doi:10.1152/japplphysiol.01506.2010a.
3. **Vinnakota KC, Kushmerick MJ.** Point: Muscle lactate and H^+ production do have a 1:1 association in skeletal muscle. *J Appl Physiol*; doi: 10.1152/japplphysiol.01506.2010.

Edmund J. Crampin
Associate Professor
Auckland Bioengineering Institute
The University of Auckland

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

Downloaded from jap.physiology.org on April 10, 2012