Williams *et al.* (2001) reported that whole body arterial compliance fluctuated significantly throughout the menstrual cycle. The method used to evaluate the arterial compliance throughout the whole body included both central (e.g. aorta and carotid arteries) and peripheral arteries (e.g. brachial and femoral). In this study, we separated central from peripheral measurements, demonstrating that carotid arterial compliance and stiffness fluctuated significantly, whereas leg arterial stiffness did not change significantly throughout the normal menstrual cycle. These results indicate that menstrual cycle phase only affects central arteries, whose cushioning function dampens the fluctuations in pressure and flow. This is the first study to suggest that central arterial compliance varies throughout the menstrual cycle in young women. The clinical significance of these fluctuations in central arterial elastic properties during the menstrual cycle in this population remains unclear.

In this study, the maximum difference in carotid arterial compliance was 25.1% (ovulatory *versus* late luteal phases). The fluctuations in central arterial elastic properties in healthy young women with normal menstrual cycles are probably greater than those seen in individuals following the menopause, because the menopause increases arterial stiffness by approximately 8–14% (Jonason *et al.* 1998; Staessen *et al.* 2001). In their examination of the effects of HRT on arterial elastic properties, Moreau *et al.* (2003) demonstrated that carotid arterial compliance was significantly higher, by approximately 33%, in postmenopausal women taking HRT than in age-matched women not receiving HRT. Although the changes in carotid arterial compliance throughout the menstrual cycle were smaller than those seen for HRT in the menopausal women, these differences depend on the time period of ovarian hormone encounter, with HRT administered for several months to several years and the menstrual cycle changing over several days. Regardless, our data indicate that it is necessary to control for menstrual phase when assessing central arterial elasticity in premenopausal women using these measures.

Carotid arterial compliance changed cyclically, increasing significantly from the M and F into the O phase (oestradiol high) and decreasing dramatically in the EL (oestradiol and progesterone high) and LL phases. This result is consistent with a previous report examining the variations in whole body arterial compliance throughout the menstrual cycle (Williams *et al.* 2001). Although oestrogen replacement therapy in postmenopausal women has been reported to increase arterial compliance (McGrath *et al.* 1998), the mechanisms by which hormonal fluctuations affect carotid arterial compliance are not well understood. Multiple studies, however, have documented that oestrogen can act as a both a vasodilator and as an anti-atherogenic agent. Since the changes in arterial compliance in this study occurred on a short time scale, it is likely that oestrogen rapidly modulates vascular properties by acting on either the vascular endothelium or smooth muscle cells (Orshal & Khalil, 2004). Oestrogen has been reported to enhance endothelial nitric oxide synthase (eNOS) activity, NO release (Knot *et al.* 1999; Geary *et al.* 2000), prostacyclin release (Geary *et al.* 2000) and the vasodilator activity of endothelial-dependent hyperpolarization factor (EDHF; Liu *et al.* 2001), and to decrease endothelin-1 production (Akishita *et al.* 1998). In addition, oestrogen inhibits Ca^{2+} influx into vascular smooth muscle cells (Murphy & Khalil, 2000). Sudhir *et al.* (1996) also demonstrated that endothelial function, evaluated by flow-mediated vasodilatation (FMD), improved in postmenopausal women following 8 weeks of oestradiol administration. Endothelial function in premenopausal women, evaluated by FMD, increased in the late follicular phase (O phase

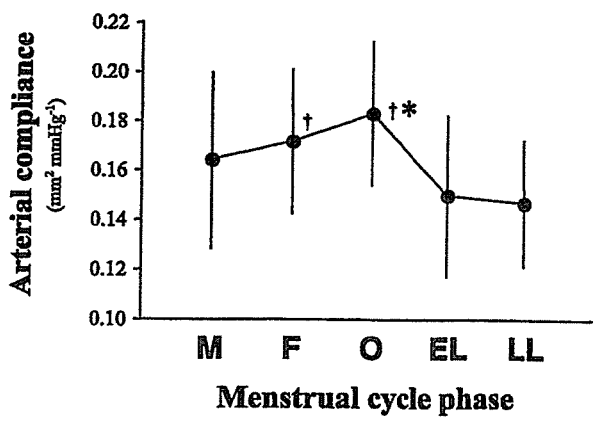

Figure 1. Changes in carotid arterial compliance during the menstrual cycle
*$P < 0.05$ *versus* M phase; †$P < 0.05$ *versus* EL and LL phases. Values are means ± S.D.

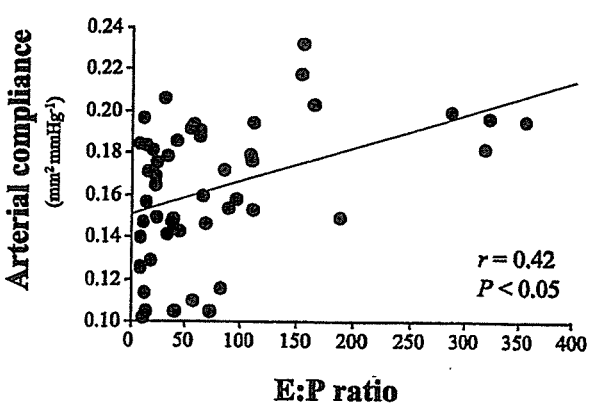

Figure 2. Relationship between carotid arterial compliance and ovarian hormone balance (E:P ratio)
r, correlation coefficient.

in this study, in which oestradiol is high) in comparison to that seen in the menstrual phase (in which oestradiol is low; Williams *et al.* 2001). Thus, the increase in carotid arterial compliance observed in the O phase probably results from the vasodilatory effects of oestrogen.

In the EL phase, carotid arterial compliance fell and stiffness rose, despite similar oestradiol levels to those seen in the O phase. The physiological mechanisms underlying these increases in arterial stiffening during the luteal phase remain poorly understood. Although progesterone levels, as well as oestrogen levels, increase during the luteal phase, the influence of progesterone on arterial functions may be complex. In animal studies, Miller & Vanhoutte (1991) reported that acetylcholine-induced relaxations in canine coronary arteries were greater in the oestrogen-treated group than in the group given both oestrogen and progesterone. Williams *et al.* (1998) reported that medroxy progesterone acetate antagonized oestrogen-mediated increases in acetylcholine-induced endothelial-dependent vasodilation in atherosclerotic monkeys. These data suggest that progesterone inhibits the endothelial-dependent vasodilatory actions of oestrogen. Recent studies, however, have indicated that progesterone also possesses vasodilatory activity, which may be mediated by modulation of Ca^{2+} channel open probabilities (Barbagallo *et al.* 2001; Minshall *et al.* 2002). Thus, increases in serum progesterone concentrations may not necessarily decrease arterial compliance. Other potential mechanisms may be related to decreases in carotid arterial compliance during the luteal phase. Minson *et al.* (2000) reported that resting muscle sympathetic nervous activity and plasma noradrenaline concentrations were higher in the midluteal phase than in the early follicular phase. Progesterone also affects fluid retention (Minson *et al.* 2000; Stachenfeld *et al.* 2003; Stachenfeld & Taylor, 2004), probably decreasing the amount of water in vascular smooth muscle cells to decrease arterial compliance (Hanke *et al.* 1996). In addition, the renin–angiotensin system and aldosterone, which are capable of altering arterial characteristics, are upregulated in the luteal phase compared with the follicular phase of the menstrual cycle (Chapman *et al.* 1997). We speculate that the additive interaction of these factors results in the decreases in carotid arterial compliance seen in the luteal phase.

We observed that the changes in carotid arterial compliance were synchronized with the balance between serum oestradiol and progesterone concentrations (E:P ratio). Although significant, these correlations were not strong. Basal individual differences in arterial compliance may influence these weak correlations, especially in the phases with a low E:P ratio. Other possible confounding factors included differences in individual efficacy of oestradiol and progesterone, and the interaction of these hormones in the regulation of arterial elasticity. Recent studies demonstrated that oestrogen receptor α polymorphisms are associated with arterial morphology (Lehtimaki *et al.* 2002*a*) and function (Lehtimaki *et al.* 2002*b*). Further study examining the interindividual differences in ovarian hormonal action on arteries will be necessary.

In our subjects, elevations of serum progesterone levels in the early luteal phase were somewhat low. The reason why progesterone levels were low might be that a substantial number of the studied women had corpus luteum deficiency but not anovular menstruation, because all the subjects had clear elevations of progesterone levels in the early luteal phase (at least > 5 nmol l^{-1}). In this regard, we should emphasize the significant change of central arterial compliance even if elevations of progesterone concentrations in the luteal phase were less than that of mature females.

In summary, this study examined the changes in young women in central and peripheral arterial elasticity at five distinct time points in the menstrual cycle. Carotid arterial compliance varied cyclically, increasing significantly from the menstrual and follicular phases into the ovulatory phase and decreasing sharply in the early and late luteal phases, but the PWV of the peripheral artery (leg) did not exhibit any significant changes throughout the menstrual cycle. Although the physiological mechanisms underlying these alterations remain unclear, these findings suggest that the menstrual cycle phase affects central, but not peripheral, arterial elasticity. Thus, it is necessity to consider the phase of the menstrual cycle when interpreting the cardiovascular disease risk of premenopausal women using carotid arterial compliance.

References

Abraham GE, Odell WD, Swerdloff RS & Hopper K (1972). Simultaneous radioimmunoassay of plasma FSH, LH, progesterone, 17-hydroxyprogesterone, and estradiol-17beta during the menstrual cycle. *J Clin Endocrinol* **34**, 312–318.

Akishita M, Kozaki K, Eto M, Yoshizumi M, Ishikawa M, Toba K, Orimo H & Ouchi Y (1998). Estrogen attenuates endothelin-1 production by bovine endothelial cells via estrogen receptor. *Biochem Biophys Res Commun* **251**, 17–21.

Armentano R, Megnien JL, Simon A, Bellenfant F, Barra J & Levenson J (1995). Effects of hypertension on viscoelasticity of carotid and femoral arteries in humans. *Hypertension* **26**, 48–54.

Barbagallo M, Dominguez LJ, Licata G, Shan J, Bing L, Karpinski E, Pang PK & Resnick LM (2001). Vascular effects of progesterone: role of cellular calcium regulation. *Hypertension* **37**, 142–147.

Blacher J, Asmar R, Djane S, London GM & Safar ME (1999). Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension* **33**, 1111–1117.

Bui MN, Arai AE, Hathaway L, Waclawiw MA, Csako G & Cannon RO III (2002). Effect of hormone replacement therapy on carotid arterial compliance in healthy postmenopausal women. *Am J Cardiol* **90**, 82–85.

Chapman AB, Zamudio S, Woodmansee W, Merouani A, Osorio F, Johnson A, Moore LG, Dahms T, Coffin C, Abraham WT & Schrier RW (1997). Systemic and renal hemodynamic changes in the luteal phase of the menstrual cycle mimic early pregnancy. *Am J Physiol* **273**, F777–F782.

Geary GG, Krause DN & Duckles SP (2000). Estrogen reduces mouse cerebral artery tone through endothelial NOS- and cyclooxygenase-dependent mechanisms. *Am J Physiol Heart Circ Physiol* **279**, H511–H519.

Giannattasio C, Failla M, Grappiolo A, Stella ML, Bo AD, Colombo M & Mancia G (1999). Fluctuations of radial artery distensibility throughout the menstrual cycle. *Arterioscler Thromb Vasc Biol* **19**, 1925–1929.

Hanke H, Hanke S, Bruck B, Brehme U, Gugel N, Finking G, Muck AO, Schmahl FW, Hombach V & Haasis R (1996). Inhibition of the protective effect of estrogen by progesterone in experimental atherosclerosis. *Atherosclerosis* **121**, 129–138.

Houtkooper LB, Going SB, Lohman TG, Roche AF & Van Loan M (1992). Bioelectrical impedance estimation of fat-free body mass in children and youth: a cross-validation study. *J Appl Physiol* **72**, 366–373.

Jonason T, Henriksen E, Kangro T, Vessby B & Ringqvist I (1998). Menopause is associated with the stiffness of the common carotid artery in 50-year-old women. *Clin Physiol* **18**, 149–155.

Knot HJ, Lounsbury KM, Brayden JE & Nelson MT (1999). Gender differences in coronary artery diameter reflect changes in both endothelial Ca^{2+} and ecNOS activity. *Am J Physiol* **276**, H961–H969.

Lehtimaki T, Kunnas TA, Mattila KM, Perola M, Penttila A, Koivula T & Karhunen PJ (2002*a*). Coronary artery wall atherosclerosis in relation to the estrogen receptor 1 gene polymorphism: an autopsy study. *J Mol Med* **80**, 176–180.

Lehtimaki T, Laaksonen R, Mattila KM, Janatuinen T, Vesalainen R, Nuutila P, Laakso J, Jaakkola O, Koivula T & Knuuti J (2002*b*). Oestrogen receptor gene variation is a determinant of coronary reactivity in healthy young men. *Eur J Clin Invest* **32**, 400–404.

Liu MY, Hattori Y, Fukao M, Sato A, Sakuma I & Kanno M (2001). Alterations in EDHF-mediated hyperpolarization and relaxation in mesenteric arteries of female rats in long-term deficiency of oestrogen and during oestrus cycle. *Br J Pharmacol* **132**, 1035–1046.

McGrath BP, Liang YL, Teede H, Shiel LM, Cameron JD & Dart A (1998). Age-related deterioration in arterial structure and function in postmenopausal women: impact of hormone replacement therapy. *Arterioscler Thromb Vasc Biol* **18**, 1149–1156.

Miller VM & Vanhoutte PM (1991). Progesterone and modulation of endothelium-dependent responses in canine coronary arteries. *Am J Physiol* **261**, R1022–R1027.

Minshall RD, Pavcnik D, Browne DL & Hermsmeyer K (2002). Nongenomic vasodilator action of progesterone on primate coronary arteries. *J Appl Physiol* **92**, 701–708.

Minson CT, Halliwill JR, Young TM & Joyner MJ (2000). Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. *Circulation* **101**, 862–868.

Miyachi M, Donato AJ, Yamamoto K, Takahashi K, Gates PE, Moreau KL & Tanaka H (2003). Greater age-related reductions in central arterial compliance in resistance-trained men. *Hypertension* **41**, 130–135.

Miyachi M, Kawano H, Sugawara J, Takahashi K, Hayashi K, Yamazaki K, Tabata I & Tanaka H (2004). Unfavorable effects of resistance training on central arterial compliance: a randomized intervention study. *Circulation* **110**, 2858–2863.

Monahan KD, Tanaka H, Dinenno FA & Seals DR (2001). Central arterial compliance is associated with age- and habitual exercise-related differences in cardiovagal baroreflex sensitivity. *Circulation* **104**, 1627–1632.

Moreau KL, Donato AJ, Seals DR, DeSouza CA & Tanaka H (2003). Regular exercise, hormone replacement therapy and the age-related decline in carotid arterial compliance in healthy women. *Cardiovasc Res* **57**, 861–868.

Murphy JG & Khalil RA (2000). Gender-specific reduction in contractility and $[Ca^{2+}]_i$ in vascular smooth muscle cells of female rat. *Am J Physiol Cell Physiol* **278**, C834–C844.

Nichols W & O'Rourke M (1998). *McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles.* Aenord, London.

O'Rourke M (1990). Arterial stiffness, systolic blood pressure, and logical treatment of arterial hypertension. *Hypertension* **15**, 339–347.

Orshal JM & Khalil RA (2004). Gender, sex hormones, and vascular tone. *Am J Physiol Regul Integr Comp Physiol* **286**, R233–R249.

Stachenfeld NS & Taylor HS (2004). Effects of estrogen and progesterone administration on extracellular fluid. *J Appl Physiol* **96**, 1011–1018.

Stachenfeld NS, Taylor HS, Leone CA & Keefe DL (2003). Oestrogen effects on urine concentrating response in young women. *J Physiol* **552**, 869–880.

Staessen JA, van der Heijden-Spek JJ, Safar ME, Den Hond E, Gasowski J, Fagard RH, Wang JG, Boudier HA & Van Bortel LM (2001). Menopause and the characteristics of the large arteries in a population study. *J Hum Hypertens* **15**, 511–518.

Sudhir K, Jennings GL, Funder JW & Komesaroff PA (1996). Estrogen enhances basal nitric oxide release in the forearm vasculature in perimenopausal women. *Hypertension* **28**, 330–334.

Sugawara J, Hayashi K, Yokoi T, Cortez-Cooper MY, DeVan AE, Anton MA & Tanaka H (2005). Brachial-ankle pulse wave velocity: an index of central arterial stiffness? *J Hum Hypertens* **19**, 401–406.

Sugawara J, Maeda S, Otsuki T, Tanabe T, Ajisaka R & Matsuda M (2004). Effects of nitric oxide synthase inhibitor on decrease in peripheral arterial stiffness with acute low-intensity aerobic exercise. *Am J Physiol Heart Circ Physiol* **287**, H2666–H2669.

Tanaka H, DeSouza CA & Seals DR (1998). Absence of age-related increase in central arterial stiffness in physically active women. *Arterioscler Thromb Vasc Biol* **18**, 127–132.

Teede HJ, Liang YL, Kotsopoulos D, Zoungas S, Cravent R & McGrath BP (2001). A placebo-controlled trial of long-term oral combined continuous hormone replacement therapy in postmenopausal women: effects on arterial compliance and endothelial function. *Clin Endocrinol (Oxf)* **55**, 673–682.

Westendorp IC, de Kleijn MJ, Bots ML, Bak AA, Planellas J, Coelingh Bennink HJ, Hofman A, Grobbee DE & Witteman JC (2000). The effect of hormone replacement therapy on arterial distensibility and compliance in perimenopausal women: a 2-year randomised trial. *Atherosclerosis* **152**, 149–157.

Willekes C, Hoogland HJ, Keizer HA, Hoeks AP & Reneman RS (1997). Female sex hormones do not influence arterial wall properties during the normal menstrual cycle. *Clin Sci (Lond)* **92**, 487–491.

Williams JK, Delansorne R & Paris J (1998). Estrogens, progestins, and coronary artery reactivity in atherosclerotic monkeys. *J Steroid Biochem Mol Biol* **65**, 219–224.

Williams MR, Westerman RA, Kingwell BA, Paige J, Blombery PA, Sudhir K & Komesaroff PA (2001). Variations in endothelial function and arterial compliance during the menstrual cycle. *J Clin Endocrinol Metab* **86**, 5389–5395.

	A P S	1 5 0 5	B	Dispatch: 31.10.05	Journal: APS	CE: Ashok
	Journal Name	Manuscript No.		Author Received:	No. of pages: 8	PE: Bhuvana

Acta Physiol Scand 2006, 186, 1–8

Fluctuations in carotid arterial distensibility during the menstrual cycle do not influence cardiovagal baroreflex sensitivity

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Received 3 June 2005,
revision requested 16 July 2005,
revision received 3 October 2005,
accepted 9 October 2005
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Abstract

Aim: Fluctuations in autonomic nervous functions throughout the menstrual cycle and the underlying mechanism concerning them are not well known. This study was designed to test the hypothesis that fluctuations in cardiovagal baroreflex sensitivity (BRS) throughout the menstrual cycles of young women are due to fluctuations in carotid arterial distensibility.

Methods: In eight eumenorrhoeic healthy young women (18–24 years), we determined the variations in the carotid arterial distensibility coefficient (DC; via simultaneous ultrasonography and applanation tonometry), cardiovagal BRS (phase IV of the Valsalva manoeuvre and the sequence method; up- or down-sequence spontaneous BRS), and serum oestradiol and progesterone concentrations at five points in the menstrual cycle (menstrual = M, follicular = F, ovulatory = O, early luteal = EL, and late luteal = LL).

Results: Serum oestradiol and progesterone levels were consistent with the predicted cycle phases. Carotid arterial DC fluctuated cyclically, increasing significantly from the M ($52.4 \pm 4.9 \times 10^{-3}$ kPa^{-1}, mean ± SE) and F (52.7 ± 4.4) phases to the O (57.6 ± 4.4) phase and declining sharply in the EL (46.0 ± 4.0) and LL (45.1 ± 3.0) phases ($F = 6.37$, $P < 0.05$). Contrary to our prediction, however, cardiovagal BRS by the Valsalva manoeuvre ($P = 0.73$) or sequence method (up-sequence spontaneous BRS; $P = 0.84$: down-sequence spontaneous BRS; $P = 0.67$) did not change significantly during the menstrual cycle.

Conclusion: The results suggest that, although carotid arterial distensibility fluctuates with the changes in ovarian hormone levels that occur during the menstrual cycle, the fluctuations in carotid arterial distensibility do not influence cardiovagal BRS.

Keywords autonomic nervous system, carotid artery, elasticity, oestrogen, progesterone.

The cardiovagal baroreflex is a short-term blood pressure buffering mechanism. This baroreflex controlling heart rate can be quantified by means of baroreflex sensitivity (BRS), representing the magnitude of changes in heart rate attributable to changes in systolic blood pressure (SBP). Lower levels of cardiovagal BRS are associated with lower orthostatic tolerance (Convertino *et al.* 1991) and an increased risk of cardiovascular disease-related mortality (La Rovere *et al.* 1998).

Previous studies have demonstrated that oestrogen increased cardiovagal BRS and heart rate variability in rats (Saleh & Connell 1999, 2000). While other studies in postmenopausal and middle-aged women demonstrated that oestrogen replacement therapy increased cardiovagal BRS (Huikuri *et al.* 1996, De Meersman *et al.* 1998), the changes in cardiovagal BRS during the menstrual cycle have not been conclusive. Minson *et al.* (2000) reported that cardiovagal BRS was unchanged during the menstrual cycle. In contrast, it was reported that cardiovagal BRS estimated by the Valsalva manoeuvre was higher in the luteal phase than in the follicular phase (Fuenmayor *et al.* 2000). Recently, Tanaka *et al.* (2003) used the phenylephrine pressor test and Valsalva manoeuvre to demonstrate that cardivagal BRS during the preovulation phase (the oestradiol level is high) was significantly higher than that measured during the early follicular (the oestradiol level is low) and mid-luteal phases (the oestradiol and progesterone levels are high), and cardiovagal BRS correlated significantly with the serum oestradiol level. However, the underlying mechanism of the effects of ovarian hormones on cardiovagal BRS is not yet well understood.

It was previously reported that the age-associated decline in cardiovagal BRS was related to changes in carotid arterial compliance (Monahan *et al.* 2001). These data suggest that the key factor determining cardiovagal BRS is the compliance of the arteries in which arterial baroreceptors are located. Baroreceptors, i.e. the carotid and aortic bodies, are stretch receptors in arterial tissue. These receptors sense the strain of the arterial wall caused by blood pressure change and send the afferent signal corresponding to the strain level to the cardiovascular center (Brown 1980, Rowe 1987). There were reports that oestrogen and progesterone have vasoactive effects, i.e. oestrogen has a vasodilation/relaxation effect (e.g. enhancement of vascular endothelial functions) (Farhat *et al.* 1996) and progesterone has an antioestrogenic action (Williams *et al.* 1998). It seems natural to consider that changes in these hormone concentrations are related to the fluctuation of the arterial distensibility or compliance. However, the effects of the menstrual cycle phase on arterial elastic properties are still unclear. Willekes *et al.* (1997) did not find significant fluctuations of distensibility and compliance in both the common carotid and the common femoral artery during the menstrual cycle. In contrast, there have been reports that the whole-body (Williams *et al.* 2001) and radial (Giannattasio *et al.* 1999) arterial compliance increased significantly in the late follicular phase or ovulatory phase and returned to baseline in the luteal phase. These phenomena led us to hypothesize that cardiovagal BRS fluctuates significantly, synchronistically with the changes in carotid arterial elastic properties throughout the menstrual cycle.

In the present study, we tested the hypothesis that fluctuations in cardiovagal BRS throughout the menstrual cycle are due to corresponding fluctuations in carotid arterial distensibility. We determined the relationship between changes in cardiovagal BRS and carotid arterial distensibility throughout the menstrual cycle in young women.

Materials and methods

Subjects

We studied eight healthy sedentary or recreationally active young women, ranging in age from 18 to 24 years (20.6 ± 0.6 years, mean ± SE). All subjects were normotensive, non-diabetic, non-smoking, and did not take any form of oral contraception. All subjects had regular menstrual cycles (25–32 days) for at least two menstrual cycles prior to this study. All subjects provided their written informed consent prior to participation. All procedures were reviewed and approved by the Ethics Committee of the University of Tsukuba.

Study protocol

We measured changes in resting cardiovagal BRS, carotid and brachial haemodynamics (carotid and brachial blood pressure, carotid arterial diameter), and serum ovarian hormone (oestradiol and progesterone) concentrations in five phases of the menstrual cycle (Saeki *et al.* 1997): (1) menstrual phase (M: the 2- to 4-day period after the beginning of menstruation), (2) follicular phase (F: the period between the menstrual and ovulatory phases), (3) ovulatory phase (O: the 4-day period beginning 3 days before ovulation), (4) early luteal phase (EL: the period between the ovulatory and late luteal phases), and (5) late luteal phase (LL: the 7-day period prior to menstruation). Menstrual phases were determined from the previous cycle length, body temperature, and with a urinary ovulation kit (Rohto Pharmaceutical Co., Ltd, Osaka, Japan). The time of entry into the study was randomized. Throughout the study period, subjects were consistently tested at the same time of day. Subjects abstained from caffeine and

fasted for at least 12 h before each test. All haemodynamic and hormonal measurements were performed as follows: (1) after measuring body composition, the subject was placed in the supine position and fitted with electrocardiographic and blood pressure devices (brachial and radial); (2) after a 20-min resting period, we measured spontaneous baroreflex sensitivity (SBRS) by the sequence method, BRS by the Valsalva manoeuvre, and carotid and brachial haemodynamics as described below; (3) after a 15-min resting period, blood was drawn for the measurement of hormone levels.

Measurements

Body composition. Body composition was determined using bioelectric impedance as described elsewhere (Houtkooper *et al.* 1992).

Heart rate and brachial arterial blood pressure. We measured heart rate and brachial blood pressure at rest non-invasively using a limb-lead electrocardiograph and a semi-automated device (Form ABI; Colin Medical) in the supine position. Brachial blood pressure values were determined in triplicate (Perloff *et al.* 1993).

Cardiovagal BRS. We used the sequence method and the Valsalva manoeuvre to estimate cardiovagal BRS. To determine SBRS, we recorded R–R intervals and SBP for 5 min with the subjects in the supine position. Respiratory rate was controlled at a frequency of 0.25 Hz during the measurements. We determined arterial blood pressure waveforms and R–R intervals using arterial tonometry (Jentow-7700; Nihon Colin, Komaki, Japan) and standard lead electrocardiography (Life Scope 11; Nihon Koden, Tokyo, Japan), respectively. Both R–R intervals and arterial blood pressure waveforms were sampled at 1000 samples per second by connecting each device to a computer using an A/D converter (Maclab/400; AD Instruments, NSW, Australia). Prior to analysis, both R–R intervals and arterial blood pressure waveforms were visually inspected for artefacts.

Spontaneous baroreflex sensitivity was assessed using a modification of a previously reported procedure (Bertinieri *et al.* 1985, 1988). Briefly, we identified baroreflex sequences (three or more beats relating to R–R intervals and progressively spontaneously changing SBP of the same detection, *lag 1*) in which SBP progressively increased followed by a lengthening of the R–R interval (up-sequence) or SBP progressively decreased with a subsequent shortening of the R–R interval (down-sequence). Next, we determined the slope of the linear relationship between the R–R intervals and SBP at these points. The minimum changes observed were 1 mmHg for SBP and 1 ms for the R–R interval. Linear regressions relating SBP to the R–R interval were plotted for each sequence; only those sequences with linear r values >0.85 were accepted. The results for a 5-min period were averaged to provide a single data set for up- or down-sequence SBRS.

To perform the Valsalva manoeuvre, subjects exhaled forcefully through a mouthpiece connected via a rubber tube to an analog manometer. Subjects were instructed to exhale until reaching a mouth pressure of 40 mmHg. Three 10-s Valsalva manoeuvres were performed, separated by 3-min recovery periods. We used the slope method to assess cardiovagal BRS during phase IV arterial pressure elevations. We used linear regression analysis to calculate the magnitude of the increases in R–R intervals as a function of the elevations in systolic pressures. We used a minimum of four consecutively increasing systolic pressure levels and the corresponding changes in R–R intervals. SBP levels regressed linearly against the corresponding (*lag 1*) R–R intervals, began to lengthen, and continued to increase to the maximal SBP elevation. Only linear r values ≥0.85 were considered a valid sequence. An average of three trials was examined for each subject.

Carotid arterial distensibility and structural indices. We used the carotid arterial distensibility coefficient (DC) to estimate carotid arterial distensibility. The combination of ultrasound imaging of the common carotid artery with simultaneous applanation tonometry yielded the arterial pressure from the contralateral carotid artery. These measurements allow the non-invasive determination of carotid arterial DC (Reneman *et al.* 1986, 2005). Carotid artery diameter and intima-media thickness (IMT) were measured from images derived from ultrasonography using a machine equipped with a high-resolution linear-array transducer. A longitudinal region of the cephalic portion of the common carotid artery was imaged 1–2 cm proximal to the carotid bulb. Computer images were digitized with a media converter and analysed using image analysis software (NIH image 1.62). Minimal and maximal lumen diameters, measured from the media-adventitia border of the near wall to the intima-lumen interface of the far wall, were identified by scrolling through images acquired at 33-ms intervals. At least 10 measurements of minimal and maximal lumen diameters and carotid artery IMT were taken at each segment, and the mean values were used for analysis. All image analyses were performed by a single investigator, who was blinded to the menstrual phase assignments. Carotid arterial pressure waveforms and amplitudes were taken from the common carotid artery using a pencil-type probe incorporating a high-fidelity strain-gauge transducer

(SPT-301; Millar Instruments) (Miyachi *et al.* 2003, 2004). As the baseline levels of carotid blood pressure were subjected to hold-down forces, we calibrated the pressure signal obtained by tonometry by equating the carotid mean arterial (MAP) and diastolic blood pressure (DBP) to the values obtained for the brachial artery as described elsewhere (Armentano *et al.* 1995). The right and left carotid arterial blood pressures were almost equal when investigated previously (unpublished data). Carotid arterial DC was calculated using the following equation (Reneman *et al.* 1986, 2005):

$$DC = [(CSAs - CSAd)/CSAd]/\Delta P$$

where ΔP is the carotid arterial pulse pressure (PP) and CSAs and CSAd are the cross-sectional areas at the maximal systolic expansion and minimal diastolic relaxation of the carotid artery, respectively.

Ovarian hormones. To measure serum oestradiol and progesterone concentrations, a 5-mL fasting blood sample was taken from the antecubital vein in each menstrual phase. Blood was centrifuged at 3000 rpm for 15 min. All serum samples were distributed into appropriate preservation tubes and stored at −80 °C until analysed. Serum oestradiol and progesterone concentrations were measured using commercially available radioimmunoassay kits (Mitsubishi BCL, Tokyo, Japan). To eliminate intra-assay variability, all samples were analysed within the same batch; intra-assay variability was <5%.

Statistical analysis

All data are presented as the mean ± SE. Differences in values measured across the menstrual cycle were assessed by one-way analysis of variance (ANOVA) with repeated measures. For significant F values in ANOVA, a *post-hoc* test using the Newman–Keuls method was used to identify significant differences between the mean values. The level of significance was set at $P < 0.05$.

Results

Ovarian hormones and body composition

All subjects were nulliparous, with a mean cycle length of 28 ± 2 days. Body weight, per cent body fat, and serum ovarian hormone measurements are summarized in Table 1. Serum oestradiol and progesterone concentrations changed significantly throughout the menstrual cycle, consistent with the predicted fluctuations for each cycle phase (oestradiol: $F = 9.3$, $P < 0.05$, progesterone: $F = 13.9$, $P < 0.05$). Serum oestradiol concentrations were higher during the O and EL phases than in other phases ($P < 0.05$). Serum progesterone concentrations were significantly higher in the EL phase in comparison with the other phases ($P < 0.05$). Body weight and per cent body fat did not change significantly during the menstrual cycle.

Heart rate and arterial blood pressure

Neither the resting heart rate, brachial arterial blood pressure (SBP, DBP, MAP and PP), nor the carotid arterial blood pressure (SBP and PP) in each menstrual phase changed significantly during the menstrual cycle (Table 2).

Carotid arterial distensibility

The diastolic lumen diameter and IMT of the carotid artery did not change significantly during the menstrual cycle (Table 2). Measurements of the changes in carotid arterial DC throughout the five phases (Fig. 1) demonstrated that the carotid arterial DC decreased after ovulation (in the EL and LL phases). The carotid arterial DC values in each phase of the menstrual cycle were 52.4 ± 4.9, 52.7 ± 4.4, 57.6 ± 4.4, 46.0 ± 4.0 and 45.1 ± 3.0 ($\times 10^{-3}$ kPa^{-1}) in the M, F, O, EL and LL phases, respectively (ANOVA; $F = 6.37$, $P < 0.05$). *Post-hoc* comparisons indicated that the O phase value was significantly higher than that of the EL and LL phases ($P < 0.05$).

Table 1 Physiological characteristics of subjects

Variables	M	F	O	EL	LL
Age (years)	21 ± 1	–	–	–	–
Height (cm)	159 ± 1	–	–	–	–
Weight (kg)	52 ± 1	52 ± 1	52 ± 1	52 ± 1	52 ± 1
% Fat	26 ± 1	27 ± 1	26 ± 1	26 ± 1	26 ± 1
Oestradiol (pg mL^{-1})	41 ± 9	50 ± 7	137 ± 27*	129 ± 19*	66 ± 12
Progesterone (ng mL^{-1})	0.8 ± 0.1	0.8 ± 0.3	0.7 ± 0.1	10.7 ± 29**	3.3 ± 0.6

Data are expressed as the mean ± SE.
M, menstrual phase; F, follicular phase; O, ovulatory phase; EL, early luteal phase; LL, late luteal phase; *$P < 0.05$ vs. M, F and LL phase; **$P < 0.05$ vs. all other phases.

Table 2 Heart rate, central and peripheral arterial blood pressure, and carotid artery structural indices

Variables	M	F	O	EL	LL
Heart rate (bpm)	53 ± 1	52 ± 2	54 ± 2	54 ± 1	55 ± 1
Brachial SBP (mmHg)	102 ± 3	100 ± 3	102 ± 3	100 ± 3	101 ± 3
Brachial DBP (mmHg)	61 ± 1	57 ± 2	59 ± 2	59 ± 2	58 ± 2
Brachial MAP (mmHg)	77 ± 2	74 ± 2	76 ± 3	73 ± 3	75 ± 2
Brachial PP (mmHg)	42 ± 3	42 ± 3	43 ± 2	41 ± 2	43 ± 3
Carotid SBP (mmHg)	95 ± 2	93 ± 2	92 ± 2	94 ± 3	95 ± 2
Carotid PP (mmHg)	37 ± 2	37 ± 2	36 ± 1	39 ± 2	37 ± 1
Carotid artery diameter (mm)	5.40 ± 0.10	5.56 ± 0.10	5.46 ± 0.08	5.53 ± 0.11	5.55 ± 0.07
Carotid artery IMT (mm)	0.47 ± 0.11	0.46 ± 0.02	0.47 ± 0.02	0.46 ± 0.01	0.46 ± 0.01

Data are expressed as the mean ± SE.
M, menstrual phase; F, follicular phase; O, ovulatory phase; EL, early luteal phase; LL, late luteal phase; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; Carotid artery diameter, carotid artery diastolic lumen diameter; IMT, intima-media thickness.

Cardiovagal BRS

Up- and down-sequence SBRS did not change significantly during the menstrual cycle (Fig. 1). Up-sequence SBRS values were 35.3 ± 3.8, 38.5 ± 3.0, 38.0 ± 3.5, 36.2 ± 3.8 and 33.6 ± 3.9 (ms $mmHg^{-1}$) in the M, F, O, EL and LL phases, respectively ($F = 0.35$, $P = 0.84$). Down-sequence SBRS values were 33.0 ± 3.1, 37.7 ± 5.5, 37.3 ± 4.4, 35.1 ± 5.1 and 34.3 ± 4.7 (ms $mmHg^{-1}$) in the M, F, O, EL and LL phases, respectively ($F = 0.59$, $P = 0.67$). BRS, estimated by the Valsalva manoeuvre, also did not change significantly during the menstrual cycle (Fig. 1), with values of 9.5 ± 1.9, 9.4 ± 1.3, 8.7 ± 0.6, 10.2 ± 1.7 and 8.7 ± 0.5 (ms $mmHg^{-1}$), respectively ($F = 0.52$, $P = 0.73$).

Discussion

The salient findings of the present study were as follows. First, carotid arterial distensibility varied significantly throughout the menstrual cycle. Second, cardiovagal BRS remained constant throughout the menstrual cycle. These findings suggest that the fluctuations in carotid arterial distensibility induced by the menstrual cycle do not influence cardiovagal BRS in young women.

Cardiovagal BRS did not change significantly during the menstrual cycle. In previous animal studies, oestrogenic intravenous injections produced significant increases in vagal BRS (Mohamed *et al.* 1999, Saleh & Connell 1999). In humans, however, it is unclear if ovarian hormone variation throughout the menstrual cycle influences cardiovagal BRS. While Minson *et al.* (2000) could not observe any differences in cardiovagal BRS (Oxford method) between the early follicular and mid-luteal phases, Fuenmayor *et al.* (2000) demonstrated that the Valsalva ratio was higher in the late luteal phase than in the early follicular phase. The results in these studies cannot, however, be correlated with either ovarian hormone levels, such as oestrogen and progesterone, or the balance between these hormones, because the menstrual cycle was divided into only two phases. Using the Oxford method, the Valsalva manoeuvre and sequence methods, Tanaka *et al.* (2003) recently examined changes in cardiovagal BRS throughout the menstrual cycle, which was divided into three phases (early follicular, when oestradiol and progesterone are low; pre-ovulation, when the oestradiol is high and progesterone is low; and mid-luteal, when both oestradiol and progesterone are high). Their results demonstrated that baroreflex sensitivities to hypertensive stimuli (phenylephrine and the Valsalva manoeuvre) were enhanced in the preovulation phase. In addition, they observed a significant correlation between plasma oestradiol concentrations and BRS to hypertensive stimuli, a result that differs significantly from our data. This discrepancy may result from differences in the magnitude of oestradiol increases. As our subjects were young women (approximately 20 years old), the serum oestradiol concentrations in the

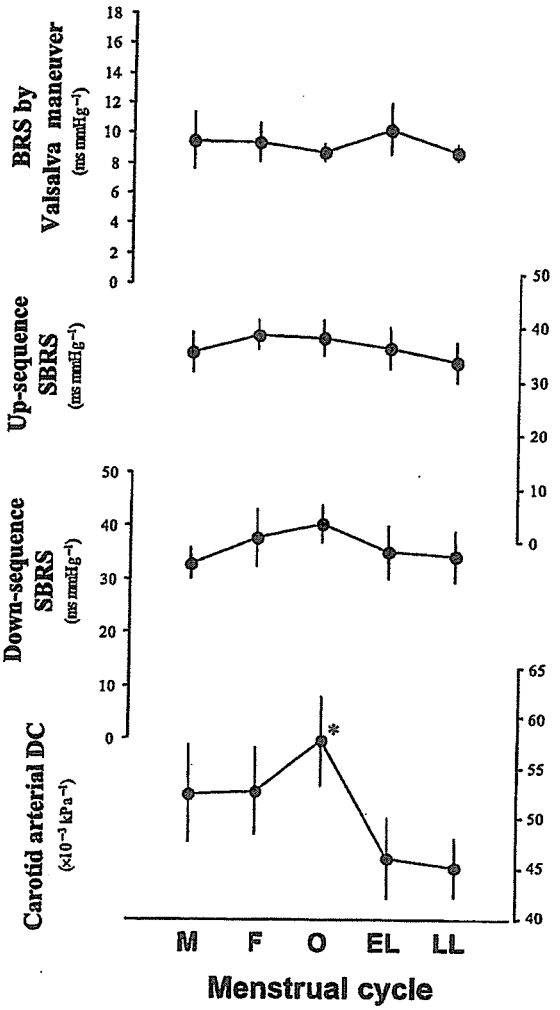

Figure 1 Changes in cardiovagal BRS and carotid arterial distensibility. $^*P < 0.05$ vs. the values measured for the EL and LL phases. M, menstrual phase; F, follicular phase; O, ovulatory phase; EL, early luteal phase; LL, late luteal phase; DC, distensibility coefficient.

ovulatory phase (O) were lower (137.2 ± 27.0 pg mL^{-1}) than those reported by Tanaka *et al.* (210.7 ± 16.5 pg mL^{-1}).

In the present study, a lack of correlation between cardiovagal BRS and carotid distensibility changes was observed. The amounts of change in carotid arterial distensibility throughout the menstrual cycle (27.7%, O phase vs. LL phase) were smaller than those of ageing (Reneman *et al.* 1986) and hormone replacement therapy (Moreau *et al.* 2003); therefore, it is likely that the amounts of change in carotid arterial distensibility during the normal menstrual cycle were not sufficient to induce significant changes in cardiovagal BRS.

As other reasons why the relationship between changes in carotid arterial distensibility and cardiovagal BRS during the menstrual cycle were lacking, the effects of ovarian hormones on neural components of the baroreflex loop (sensitivity of baroreceptor, central integration and sensitivity of effect or organ) might be related. In rats, oestrogen modulated cardiovagal BRS via oestrogen receptors in the cardiovascular centre (Saleh & Connell 2000), and the expression levels of the oestrogen receptor in the brainstem changed throughout the oestrous cycle (Haywood *et al.* 1999). A change in cardiovagal BRS may not necessarily be synchronized with the fluctuations in the blood oestrogen level if there is a temporal difference between the elevation of blood oestrogen levels and the enhancement of oestrogen receptor expression in the cardiovascular center. In this context, Kornet *et al.* (2005) provided the new elegant approach with which the component of neural control of BRS could be assessed. This was achieved by the quantification of the sensitivity of baroreflex control of the heart rate by considering the carotid arterial diameter/R–R interval relationship ('stretch-derived' BRS) through continuous monitoring of the carotid arterial diameter (by ultrasound) and the arterial waveform. Clarification of the actions of ovarian hormones on the interrelation of neural and mechanical components of the baroreflex loop will be enabled by using this method.

Carotid arterial DC changed significantly in a manner dependent on the menstrual cycle phase: increasing in the menstrual and follicular phases, peaking in the ovulatory phase, and decreasing significantly in the early and late luteal phases. These alterations in carotid arterial distensibility are consistent with changes in whole-body and radial arterial compliance varying with the menstrual cycle, as reported elsewhere (Giannattasio *et al.* 1999, Williams *et al.* 2001). However, Willekes *et al.* (1997) reported that carotid arterial distensibility did not change during the menstrual cycle. No clear explanation is at hand for this discrepancy.

Multiple studies have suggested that oestrogen improves vascular endothelial function by enhancing endothelial nitric oxide synthase activity, nitric oxide release (Knot *et al.* 1999, Geary *et al.* 2000), prostacyclin release (Orshal & Khalil 2004), and the vasodilator action of endothelial-dependent hyperpolarization factor (Liu *et al.* 2001) and also by inhibiting endothelin-1 production (Akishita *et al.* 1998). In addition, oestrogen inhibits the influx of Ca^{2+} into vascular smooth muscle cells (Murphy & Khalil 2000). All in all, these oestrogenic actions should lead to an increase of arterial distensibility. In contrast, progesterone has an inhibitory effect on oestrogen-mediated endothelial-dependent vasodilation (Williams *et al.* 1998). In addition, it was reported that, in the human female, muscle sympathetic nervous activity was significantly higher in the mid-luteal phase than in the early follicular phase

during the normal menstrual cycle (Minson *et al.* 2000). These factors might interact to change arterial distensibility.

Brachial blood pressure did not change during the menstrual cycle, although carotid arterial distensibility changed significantly. And although increased distensibility of the elastic artery should be accompanied by reduced SBP and increased DBP when the change (or difference) in arterial distensibility is great (e.g. in ageing), it is appropriate to interpret this to mean that physiological changes in arterial distensibility attributable to the menstrual cycle phase do not influence blood pressure levels. Indeed, it has been reported that whole-body arterial compliance varied significantly but the arterial blood pressure did not change during the menstrual cycle (Williams *et al.* 2001). Other factors (e.g. autonomic nervous function, vasoactive substance and blood volume) might also be associated with regulation of the blood pressure.

There are several limitations in the present study. First, only cardiovagal BRS was estimated. It will be necessary to examine the effects of ovarian hormones on sympathetic BRS. It has been reported that sympathetic BRS in the mid-luteal phase was significantly higher than that seen in the early follicular phase (Minson *et al.* 2000). Second, it is possible that the distance between the carotid bulb (where baroreceptors are located) and the common carotid artery (carotid arterial distensibility was measured) would explain the lack of correlation observed between the changes in cardiovagal BRS and carotid distensibility. However, it is difficult to evaluate the carotid arterial structures and dynamics in the bulb by means of the two-dimensional ultrasound device, and previous studies (Bonyhay *et al.* 1996, Monahan *et al.* 2001) on the association between carotid arterial distensibility (or compliance) and cardiovagal BRS have measured carotid artery elastic properties in common carotid arteries but not in the carotid bulb.

In summary, we examined during the menstrual cycles of young women, the relationship between the changes in cardiovagal BRS and carotid arterial distensibility, one of the factors controlling cardiovagal functions. Carotid arterial distensibility changed significantly in a manner dependent on ovarian hormone changes, but cardiovagal functions remained constant throughout the menstrual cycle. Therefore, these results suggest that the fluctuations in carotid arterial distensibility observed during the menstrual cycle do not influence resting cardiovagal BRS in young women.

Conflict of interest

There are no conflicts of interest for our work.

References

Akishita, M., Kozaki, K., Eto, M. *et al.* 1998. Estrogen attenuates endothelin-1 production by bovine endothelial cells via estrogen receptor. *Biochem Biophys Res Commun* 251, 17–21.

Armentano, R., Megnien, J.L., Simon, A., Bellenfant, F., Barra, J. & Levenson, J. 1995. Effects of hypertension on viscoelasticity of carotid and femoral arteries in humans. *Hypertension* 26, 48–54.

Bertinieri, G., Di Rienzo, M., Cavallazzi, A., Ferrari, A.U., Pedotti, A. & Mancia, G. 1985. A new approach to analysis of the arterial baroreflex. *J Hypertens* 3(Suppl. 3), S79–S81.

Bertinieri, G., Di Rienzo, M., Cavallazzi, A., Ferrari, A.U., Pedotti, A. & Mancia, G. 1988. Evaluation of baroreceptor reflex by blood pressure monitoring in unanesthetized cats. *Am J Physiol* 254, H377–H383.

Bonyhay, I., Jokkel, G. & Kollai, M. 1996. Relation between baroreflex sensitivity and carotid artery elasticity in healthy humans. *Am J Physiol* 271, H1139–H1144.

Brown, A.M. 1980. Receptors under pressure. An update on baroreceptors. *Circ Res* 46, 1–10.

Convertino, V.A., Adams, W.C., Shea, J.D., Thompson, C.A. & Hoffler, G.W. 1991. Impairment of carotid-cardiac vagal baroreflex in wheelchair-dependent quadriplegics. *Am J Physiol* 260, R576–R580.

De Meersman, R., Zion, A.S., Giardina, E.G., Weir, J.P., Lieberman, J.S. & Downey, J.A. 1998. Estrogen replacement, vascular distensibility, and blood pressures in postmenopausal women. *Am J Physiol* 274, H1539–H1544.

Farhat, M.Y., Lavigne, M.C. & Ramwell, P.W. 1996. The vascular protective effects of estrogen. *FASEB J* 10, 615–624.

Fuenmayor, A.J., Ramirez, L. & Fuenmayor, A.M. 2000. Left ventricular function and autonomic nervous system balance during two different stages of the menstrual cycle. *Int J Cardiol* 72, 243–246.

Geary, G.G., Krause, D.N. & Duckles, S.P. 2000. Estrogen reduces mouse cerebral artery tone through endothelial NOS- and cyclooxygenase-dependent mechanisms. *Am J Physiol Heart Circ Physiol* 279, H511–H519.

Giannattasio, C., Failla, M., Grappiolo, A. *et al.* 1999. Fluctuations of radial artery distensibility throughout the menstrual cycle. *Arterioscler Thromb Vasc Biol* 19, 1925–1929.

Haywood, S.A., Simonian, S.X., van der Beek, E.M., Bicknell, R.J. & Herbison, A.E. 1999. Fluctuating estrogen and progesterone receptor expression in brainstem norepinephrine neurons through the rat estrous cycle. *Endocrinology* 140, 3255–3263.

Houtkooper, L.B., Going, S.B., Lohman, T.G., Roche, A.F. & Van Loan, M. 1992. Bioelectrical impedance estimation of fat-free body mass in children and youth: a cross-validation study. *J Appl Physiol* 72, 366–373.

Huikuri, H.V., Pikkujamsa, S.M., Airaksinen, K.E. *et al.* 1996. Sex-related differences in autonomic modulation of heart rate in middle aged subjects. *Circulation* 94, 122–125.

Knot, H.J., Lounsbury, K.M., Brayden, J.E. & Nelson, M.T. 1999. Gender differences in coronary artery diameter reflect changes in both endothelial Ca^{2+} and ecNOS activity. *Am J Physiol* 276, H961–H969.

Kornet, L., Hoeks, A.P., Janssen, B.J., Houben, A.J., De Leeuw, P.W. & Reneman, R.S. 2005. Neural activity of the cardiac baroreflex decreases with age in normotensive and hypertensive subjects. *J Hypertens* 23, 815–823.

La Rovere, M.T., Bigger, J.T., Jr, Marcus, F.I., Mortara, A. & Schwartz, P.J. 1998. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. *Lancet* 351, 478–484.

Liu, M.Y., Hattori, Y., Fukao, M., Sato, A., Sakuma, I. & Kanno, M. 2001. Alterations in EDHF-mediated hyperpolarization and relaxation in mesenteric arteries of female rats in long-term deficiency of oestrogen and during oestrus cycle. *Br J Pharmacol* 132, 1035–1046.

Minson, C.T., Halliwill, J.R., Young, T.M. & Joyner, M.J. 2000. Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. *Circulation* 101, 862–868.

Miyachi, M., Donato, A.J., Yamamoto, K. *et al.* 2003. Greater age-related reductions in central arterial compliance in resistance-trained men. *Hypertension* 41, 130–135.

Miyachi, M., Kawano, H., Sugawara, J. *et al.* 2004. Unfavorable effects of resistance training on central arterial compliance: a randomized intervention study. *Circulation* 110, 2858–2863.

Mohamed, M.K., El-Mas, M.M. & Abdel-Rahman, A.A. 1999. Estrogen enhancement of baroreflex sensitivity is centrally mediated. *Am J Physiol* 276, R1030–R1037.

Monahan, K.D., Dinenno, F.A., Seals, D.R., Clevenger, C.M., Desouza, C.A. & Tanaka, H. 2001. Age-associated changes in cardiovagal baroreflex sensitivity are related to central arterial compliance. *Am J Physiol Heart Circ Physiol* 281, H284–H289.

Moreau, K.L., Donato, A.J., Seals, D.R., DeSouza, C.A. & Tanaka, H. 2003. Regular exercise, hormone replacement therapy and the age-related decline in carotid arterial compliance in healthy women. *Cardiovasc Res* 57, 861–868.

Murphy, J.G. & Khalil, R.A. 2000. Gender-specific reduction in contractility and [Ca(2+)](i) in vascular smooth muscle cells of female rat. *Am J Physiol Cell Physiol* 278, C834–C844.

Orshal, J.M. & Khalil, R.A. 2004. Gender, sex hormones, and vascular tone. *Am J Physiol Regul Integr Comp Physiol* 286, R233–R249.

Perloff, D., Grim, C., Flack, J. *et al.* 1993. Human blood pressure determination by sphygmomanometry. *Circulation* 88, 2460–2470.

Reneman, R.S., van Merode, T., Hick, P., Muytjens, A.M. & Hoeks, A.P. 1986. Age-related changes in carotid artery wall properties in men. *Ultrasound Med Biol* 12, 465–471.

Reneman, R.S., Meinders, J.M. & Hoeks, A.P. 2005. Noninvasive ultrasound in arterial wall dynamics in humans: –what have we learned and what remains to be solved. *Eur Heart J* 26, 960–966.

Rowe, J.W. 1987. Clinical consequences of age-related impairments in vascular compliance. *Am J Cardiol* 60, 68G–71G.

Saeki, Y., Atogami, F., Takahashi, K. & Yoshizawa, T. 1997. Reflex control of autonomic function induced by posture change during the menstrual cycle. *J Auton Nerv Syst* 66, 69–74.

Saleh, T.M. & Connell, B.J. 1999. Centrally mediated effect of 17beta-estradiol on parasympathetic tone in male rats. *Am J Physiol* 276, R474–R481.

Saleh, T.M. & Connell, B.J. 2000. 17beta-estradiol modulates baroreflex sensitivity and autonomic tone of female rats. *J Auton Nerv Syst* 80, 148–161.

Tanaka, M., Sato, M., Umehara, S. & Nishikawa, T. 2003. Influence of menstrual cycle on baroreflex control of heart rate: comparison with male volunteers. *Am J Physiol Regul Integr Comp Physiol* 285, R1091–R1097.

Willekes, C., Hoogland, H.J., Keizer, H.A., Hoeks, A.P. & Reneman, R.S. 1997. Female sex hormones do not influence arterial wall properties during the normal menstrual cycle. *Clin Sci (Lond)* 92, 487–491.

Williams, J.K., Delansorne, R. & Paris, J. 1998. Estrogens, progestins, and coronary artery reactivity in atherosclerotic monkeys. *J Steroid Biochem Mol Biol* 65, 219–224.

Williams, M.R., Westerman, R.A., Kingwell, B.A. *et al.* 2001. Variations in endothelial function and arterial compliance during the menstrual cycle. *J Clin Endocrinol Metab* 86, 5389–5395.

Eur J Appl Physiol
DOI 10.1007/s00421-006-0375-6

ORIGINAL ARTICLE

Effects of age on ventilatory threshold and peak oxygen uptake normalised for regional skeletal muscle mass in Japanese men and women aged 20–80 years

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Accepted: 30 November 2006

Abstract Ventilatory threshold (VT) is an important predictor of cardiorespiratory fitness, such as peak oxygen uptake ($\dot{V}_{O_2peak}$), and is a valuable index of aerobic exercise intensity. However, little is known about the role of skeletal muscle (SM) mass in the age-associated decline of VT. Therefore, the present study was performed to investigate the effects of age on cardiopulmonary fitness normalised for regional SM mass in 1,463 Japanese men and women, and to determine the relevance of VT normalised to SM mass based on age and gender. Total, trunk and thigh SM mass were measured using an ultrasound method, while $\dot{V}_{O_2peak}$ and VT were determined during treadmill walking. $\dot{V}_{O_2peak}$ was estimated using the predicted maximum heart rate (HR) and the HR-$\dot{V}_{O_2}$ relationship for sub-maximal treadmill walking. There were significant negative correlations between VT normalised for body mass and age in men and women ($P < 0.001$). Age-associated declines were also observed in VT normalised for body mass in both men and women; however, VT normalised for SM mass was not significantly different with age. Significant correlations were also observed between thigh SM mass and VT in both men and women. These results suggest that thigh SM mass is closely associated with $\dot{V}_{O_2peak}$ and/or VT in both men and women, and the decrease in VT with age is predominantly due to an age-related decline of SM mass. Moreover, this study provides normative cardiorespiratory fitness data regarding VT normalised SM mass in healthy men and women aged 20–80 years.

Keywords Age · Anaerobic threshold · Gender · Skeletal muscle mass · Ultrasound · $\dot{V}_{O_2peak}$

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Introduction

Low levels of cardiorespiratory fitness, such as peak oxygen uptake ($\dot{V}_{O_2peak}$), are risk factors for future cardiovascular mortality, as well as mortality of all causes in middle-aged and elderly men and women (Blair et al. 1995, 1989; Fletcher et al. 1996). Although measurement of $\dot{V}_{O_2peak}$ is important to classify an individual's health risk, the accurate determination of $\dot{V}_{O_2peak}$ requires a maximum graded exercise test (GXT) performed on a treadmill or cycle ergometer. However, GXT are accompanied by a certain degree

of risk, such as myocardial infarction, and the need to consider the subject's motivation even in healthy middle-aged and older individuals (American College of Sports Medicine 1995). Therefore, predicted maximal heart rates (HR), such as 220 minus age, are commonly used to estimate $\dot{V}_{O_2peak}$ using the HR-$\dot{V}_{O_2}$ relationship during sub-maximal exercise (McArdle 2001). The ventilatory threshold (VT) has been defined as the point when the changes in ventilation (VE) are disproportionately greater than the changes in $\dot{V}_{O_2}$ with increasing workloads which occurs at the lactate acidosis threshold (Wasserman et al. 2005). The VT can be used directly and accurately as a measure of cardiorespiratory fitness (Gaskill et al. 2001), and is also useful for evaluating the training effect in low to moderate intensity physical exercise (Zhang et al. 2003). Furthermore, it has been shown that the changes in VT in low to moderate exercise are associated with cardiac autonomic nervous function, which may be used clinically as a predictor of cardiovascular morbidity and mortality (Tuomainen et al. 2005). Thus, when studying the effects of aging on cardiorespiratory fitness, both $\dot{V}_{O_2peak}$ and VT are key factors.

The age-related decline of $\dot{V}_{O_2peak}$ has been attributed to changes in body composition, especially a loss of skeletal muscle (SM) mass, or sarcopenia (Fleg and Lakatta 1988; Frontera et al. 2000; Proctor and Joyner 1997). SM mass is important for understanding the decline in $\dot{V}_{O_2peak}$ with age, because the arterial–venous difference for oxygen in SM is one of the determinant factors of $\dot{V}_{O_2peak}$ according to the Fick principle. Previously, we reported that lower body SM mass measured by magnetic resonance imaging (MRI) was strongly correlated with $\dot{V}_{O_2peak}$ during running (Sanada et al. 2005), independent of body mass and fat-free body mass (FFM). However, to our knowledge, there is no evidence supporting the relationship between VT and total or regional SM mass as a function of age in a large population. Therefore, it is necessary to clarify what factors are important for normalisation (i.e., body mass, FFM, SM mass) in order to accurately evaluate VT.

It is difficult to accurately quantify total and regional SM mass because it requires the use of MRI or computerised tomography (the gold standard), which are costly and time-consuming for analysis. Recently, our laboratory developed several regression-based prediction equations (Sanada et al. 2006) of SM mass based on B-mode ultrasound of muscle thickness (MTH). We have further demonstrated that use of these equations are a valid method for predicting SM mass in healthy Japanese adults, and a viable alternative to costly MRI measurements. Ultrasound has been widely employed for measuring SM size in vivo (Abe et al. 1994; Kubo et al. 2003; Reimers et al. 1998). This method is practical for large-scale studies, most notably because of its portability (~10 kg) and ease of taking measurements in the field.

The purpose of the present study was twofold: (1) to investigate the effects of age on cardiorespiratory fitness normalised for regional SM mass, and (2) to determine the relevance of VT normalised to SM mass based on age and gender.

Methods

Subjects

Fourteen hundred and sixty-three healthy Japanese men and women aged 20–80 years participated in this study (807 men and 656 women, 49.3 ± 13.5 years). None of the subjects were taking any medications known to affect the study variables, such as beta-blockers or hormone replacement therapy, and all subjects were members of a fitness club. Most of the subjects routinely performed moderate aerobic and/or resistance exercises. The purpose, procedures and risks were explained to each participant, and all subjects gave their written informed consent before participating in the study approved by the Ethical Commission of Waseda University. Subjects with any of the following conditions were excluded from the study: significant cardiovascular or pulmonary disease, uncontrolled metabolic disease (diabetes, anaemia, or thyroid disease), or electrolyte abnormalities.

Measurement of $\dot{V}O_2peak$ and VT

We measured the body mass, height and waist circumference of all subjects before measurement of $\dot{V}_{O_2peak}$ and VT. $\dot{V}O_2$ during a treadmill walking test was measured using an automated breath-by-breath mass spectrometry system (Aeromonitor AE-280S; Minato Medical Science, Tokyo, Japan). Subjects warmed-up at 40 m min^{-1} on a 4% grade for 3 min. Then, the treadmill speed and grade were increased by 15 m min^{-1} or 5% alternately for each successive minute of walking until subjects reached approximately 85% of their maximum HR (220 minus age). We developed this protocol based on the metabolic equations for gross $\dot{V}O_2$ (American College of Sports Medicine 1990). Previously, we validated this protocol in 104 healthy middle-aged and older men and women (Sanada et al. 1997). Lehmann et al. (1997) confirmed that the treadmill exercise protocol designed on a

theoretical basis to span a range of 0–200 W in increments of approximately 25 W by alteration of either speed or grade from one stage to the next should correspond to a standard bicycle protocol consisting of 25-W steps. $\dot{V}O_2$ during walking was calculated every 30 s. The electrocardiograph was monitored constantly during the exercise session and was also used to measure HR at intervals of 30 s. Ratings of perceived exertion (RPE) were also recorded every minute during exercise. $\dot{V}_{O_2peak}$ was estimated from maximum HR using the HR-$\dot{V}_{O_2}$ relationship for sub-maximal exercise. VT was estimated from ventilatory equivalents for oxygen ($\dot{V}E/\dot{V}_{O_2}$) and carbon dioxide ($\dot{V}E/\dot{V}_{CO_2}$) as described previously (Caiozzo et al. 1982). VT was determined from $\dot{V}_{O_2}$ as the point of inflection where the $\dot{V}E/\dot{V}_{O_2}$ ratio was at its lowest and then increased progressively with further increments in treadmill work rate, while at the same time $\dot{V}E/\dot{V}_{O_2}$ reached a plateau or declined. The modified V-slope method where $\dot{V}_{CO_2}$ was plotted against $\dot{V}_{O_2}$ was also used to support the estimate of VT by ventilatory equivalents (Beaver et al. 1986). In this study, 1,367 (755 men and 612 women) subjects met the criteria for attainment of VT. The VT was similar with a small (< 2%) and not significant difference between the observers. The $\dot{V}_{O_2}$ should be proportional to L^2 or $M^{2/3}$, where L is length and M is body mass (Astrand and Rodahl 1977). We applied this calculation for VT and $\dot{V}_{O_2peak}$.

Ultrasound MTH and measurements

Ultrasound has been widely employed for accurate measurement of the SM size in vivo, and this method has been shown to be highly reliable and valid in previous studies involving measurement of muscle thickness—MTH (Abe et al. 1994; Fukunaga et al. 2001; Reimers et al. 1998). The MTH determined by B-mode ultrasound was assessed at six sites on the anterior and posterior surfaces of the body, as described previously (Abe et al. 1994). The sites included: the anterior and posterior upper arm, a point 60% distal between the lateral epicondyle of the humerus and the acromial process of the scapula; the abdomen, 2–3 cm to the right of the umbilicus; subscapula, 5 cm directly below the inferior angle of the scapula; anterior and posterior thigh surfaces, midway between the lateral condyle of the femur and the greater trochanter.

Ultrasonographic evaluation of MTH was performed using a real-time linear electronic scanner with a 5 MHz scanning head (SSD-500; Aloka, Tokyo, Japan). The scanning head with water-soluble transmission gel, which provided acoustic contact without depression of the skin surface, was placed perpendicular to the tissue interface at the marked sites. The MTHs were measured directly from the screen with electronic callipers, and determined as the distance from the adipose tissue-muscle interface to the muscle–bone interface. Total and regional SM mass were estimated using the equations of Sanada et al. (2005). The MTHs were converted to mass units in kilograms by ultrasound-derived prediction equations using site-matched MTH × height, which were then used to calculate arm, trunk, thigh and lower leg SM mass. Strong correlations were observed between the site-matched SM mass (total, arm, trunk body, thigh and lower leg) for the MRI measurement and MTH × height (in metres) in the model development group (r = 0.83–0.96 in men, r = 0.53–0.91 in women). In addition, the SM mass prediction equations were applied to the validation group, significant correlations were also observed between the MRI-measured and predicted SM mass in vivo(Sanada et al. 2006). Moreover, in another study the reliability of image reconstruction and distance measurements were confirmed by comparing the ultrasonic and manual measurements of tissue thickness in human cadavers, and the coefficient of variation for the MTH measurements was 1% (Kawakami et al. 1993).

Measurement of FFM

FFM was estimated from body density using the subcutaneous fat measurements from B-mode ultrasound, as described previously (Abe et al. 1994). Body density was estimated from measurements at the six subcutaneous fat layer sites, as described in the previous section. The standard error of these estimates using the ultrasound equations was ~0.006 g ml^{-1} (±2.5% body fat) for men and women. Body fat percentage was then calculated from body density using the equation described by Brozek et al. (1963) and FFM was the difference between body mass and fat mass.

Statistical analysis

All measurements and calculated values are expressed as the mean ± standard deviation. One-way ANOVA was used to compare age decade and gender differences for the following physical characteristics: total or regional SM mass and VT or $\dot{V}_{O_2peak}$, body mass, BMI, percent body fat, FFM, waist circumference, total SM mass, trunk SM mass, thigh SM mass and absolute or normalised VT and $\dot{V}_{O_2peak}$ (Tables 1, 2, 3, 4). In cases where a significant F value was obtained, Scheffe's post hoc test was performed to identify significant differences among mean values. Pearson's product correlations were calculated between SM mass and $\dot{V}_{O_2peak}$ or

Table 1 Physical characteristics of subjects

Gender and age range (years)	*n*	Body mass (kg)	Fat-free body mass (kg)	Body mass index (kg m^{-2})	Percent body fat (%)	Waist circumference (cm)
Men						
20–29	55	73.2 ± 10.7†	60.3 ± 5.9†	24.3 ± 3.3	18.2 ± 6.4	73.7 ± 6.7
30–39	110	72.0 ± 9.3†	58.2 ± 6.2†	24.3 ± 2.8	18.8 ± 5.4	75.2 ± 7.8
40–49	205	71.6 ± 9.6†	58.3 ± 6.8†	24.5 ± 3.1	18.2 ± 6.2	77.5 ± 7.2
50–59	205	70.5 ± 9.3†	57.7 ± 6.1†	24.7 ± 2.8	18.0 ± 5.3	80.1 ± 7.8
60–69	167	67.1 ± 7.3	55.3 ± 5.1	24.0 ± 2.2	17.4 ± 3.9	83.3 ± 9.3
70+	65	63.6 ± 5.8	52.9 ± 4.1	23.1 ± 1.7	16.6 ± 3.4	88.1 ± 5.6
All	807	69.9 ± 9.2	57.1 ± 6.2	24.3 ± 2.7	17.9 ± 5.3	87.4 ± 7.7
Women						
20–29	61	53.4 ± 5.8	40.6 ± 3.6	20.6 ± 2.3	23.5 ± 7.1	82.8 ± 9.8†
30–39	158	52.4 ± 7.0	40.1 ± 3.9	20.5 ± 2.5	22.9 ± 6.8	85.7 ± 7.6†
40–49	173	53.3 ± 6.6	40.0 ± 4.1	21.0 ± 2.4	24.2 ± 7.2	87.4 ± 8.3†
50–59	150	53.0 ± 6.7	40.3 ± 3.8	21.4 ± 2.4	23.3 ± 5.8	89.3 ± 7.3
60–69	101	54.0 ± 6.6	40.0 ± 4.4	22.4 ± 2.6	25.8 ± 5.0	87.6 ± 6.6
70+	13	55.4 ± 5.0	41.5 ± 3.5	22.8 ± 2.2	24.9 ± 4.8	86.9 ± 5.9
All	656	53.2 ± 6.6*	40.2 ± 4.0*	21.2 ± 2.5*	23.9 ± 6.4*	78.4 ± 8.4*

† Significant difference in the 70- to 79-year-old group ($P < 0.05$)
*Significant difference in all male subjects ($P < 0.05$)

Table 2 Total and regional SM mass in men and women

Gender and age range (years)	*n*	Total SM mass (kg)	Trunk SM mass (kg)	Thigh SM mass (kg)
Men				
20–29	55	28.1 ± 3.3†	11.6 ± 1.7†	10.5 ± 1.3†
30–39	110	26.5 ± 3.6†	10.8 ± 1.8†	9.9 ± 1.5†
40–49	205	25.7 ± 3.1†	10.4 ± 1.5†	9.6 ± 1.4†
50–59	205	24.8 ± 3.2†	9.9 ± 1.4	9.2 ± 1.4†
60–69	167	23.2 ± 2.5	9.3 ± 1.2	8.6 ± 1.1†
70+	65	21.4 ± 2.1	9.2 ± 1.3	7.8 ± 1.0
All	807	24.8 ± 3.5	10.0 ± 1.6	9.2 ± 1.5
Women				
20–29	61	15.3 ± 2.1	6.3 ± 0.8	5.8 ± 0.8
30–39	158	14.6 ± 2.0	6.0 ± 0.8	5.6 ± 0.8
40–49	173	15.0 ± 2.5	6.1 ± 0.9	5.6 ± 0.9
50–59	150	14.6 ± 2.3	5.9 ± 0.8	5.4 ± 0.8
60–69	101	14.4 ± 2.6	5.9 ± 0.9	5.2 ± 0.9
70+	13	13.9 ± 2.7	5.8 ± 0.7	4.9 ± 1.0
All	656	14.7 ± 2.3*	6.0 ± 0.8*	5.5 ± 0.9*

† Significant difference in the 70- to 79-year-old group ($P < 0.05$)
*Significant difference in all male subjects ($P < 0.05$)

VT (Table 5). Quadratic regression was performed on $\dot{V}_{O_2peak}$ normalised for body mass and linear regression was performed on VT normalised for body mass in men and women (Fig. 1). The alpha level for testing significance was set at $P < 0.05$. All statistical analyses were completed using Stat View v5.0 for windows (SAS Inc., Cary, NC, USA).

Results

The physical characteristics of the male and female subjects are listed in Table 1. Subjects varied in age from 20 to 80 years and body mass index (BMI) from 15.0 to 36.0. The waist circumference increased with age in both genders, but not the % body fat. These results suggest that the accumulation of body fat occurs in abdominal area with age. The reference values for SM mass using the ultrasound method are shown in Table 2. The men had significantly higher SM ($P < 0.001$) in comparison with the women in total, trunk and thigh. Age-associate declines were observed in total, trunk and thigh SM mass in men, but not in women. Tables 3 and 4 show the values for $\dot{V}_{O_2peak}$ and VT in each gender and age group. Age-associated declines were observed for $\dot{V}_{O_2peak}$ normalised for body mass as well as normalised for SM mass (Table 3) in both men and women. Age-associated decline of the absolute VT was observed in men, but not in women. This result is associated with gender differences in SM mass (Table 4). Despite the age-associated declines in VT normalised for body mass in both men and women, VT normalised for SM mass was not significantly different with age.

Table 5 shows simple correlation coefficients among age, and aerobic power in men and women. There were significant negative correlations between age and $\dot{V}_{O_2peak}$ normalised for body mass in men and women, and between age and VT normalised for body mass in men and women. Moreover, there were significant negative correlations between age and SM mass in both men and women.

Significant negative quadratic regression was observed between age and absolute $\dot{V}_{O_2peak}$, while there was a significant negative correlation between age and absolute VT in both men and women (Fig. 1). Signifi-

Table 3 Absolute and normalised $\dot{V}_{O_2peak}$ in various age groups

Gender and age range (years)	*n*	Absolute value (*L*)	Normalised values					
			Body mass (ml kg^{-1} min^{-1})	Body mass$^{2/3}$ (ml kg$^{-2/3}$ min^{-1})	Fat-free body mass (ml kg^{-1} min^{-1})	Total SM mass (ml kg^{-1} min^{-1})	Trunk SM mass (ml kg^{-1} min^{-1})	Thigh SM mas (ml kg^{-1} min^{-1})
Men								
20–29	55	3.44 ± 0.66†	47.2 ± 7.9†	197.1 ± 32.4†	58.7 ± 7.6†	125.9 ± 15.3†	308.5 ± 50.6†	336.4 ± 40.1†
30–39	110	3.15 ± 0.49†	44.3 ± 7.5†	183.3 ± 28.0†	54.1 ± 6.7†	119.8 ± 17.1†	296.8 ± 47.5†	322.2 ± 55.2†
40–49	205	3.04 ± 0.52†	42.6 ± 5.8†	176.3 ± 23.9†	52.3 ± 8.3†	118.7 ± 16.6†	296.7 ± 55.7†	319.9 ± 48.2†
50–59	205	2.71 ± 0.45†	38.7 ± 5.7†	159.7 ± 22.6†	47.2 ± 6.6†	110.6 ± 17.3†	279.2 ± 52.7†	298.6 ± 47.3†
60–69	167	2.39 ± 0.38†	35.7 ± 5.3†	144.8 ± 21.0†	43.2 ± 6.3†	103.7 ± 16.0	260.4 ± 48.2†	280.6 ± 48.0†
70+	65	1.94 ± 0.32	30.7 ± 4.9	122.1 ± 19.5	36.8 ± 5.8	90.9 ± 13.6	214.4 ± 42.4	251.0 ± 41.4
All	807	2.78 ± 0.61	39.8 ± 7.4	163.6 ± 30.9	48.4 ± 9.0	116.6 ± 18.8	315.7 ± 74.2	301.5 ± 53.1
Women								
20–29	61	2.15 ± 0.34†	40.5 ± 6.1†	153.2 ± 22.5†	52.4 ± 8.3†	139.6 ± 22.7†	340.1 ± 65.4†	369.6 ± 66.4†
30–39	158	2.06 ± 0.37†	39.6 ± 6.5†	147.6 ± 23.5†	51.9 ± 7.6†	144.1 ± 24.0†	354.6 ± 75.3†	376.0 ± 60.6†
40–49	173	1.90 ± 0.36	35.9 ± 6.3†	134.3 ± 23.2†	47.6 ± 8.4†	128.5 ± 25.6	317.8 ± 73.1	345.8 ± 66.5
50–59	150	1.76 ± 0.32	33.5 ± 5.7†	125.3 ± 20.8	43.7 ± 6.7†	122.1 ± 21.0	303.5 ± 60.1	332.0 ± 57.5
60–69	101	1.57 ± 0.30	29.1 ± 4.8	109.6 ± 18.0	39.3 ± 6.9	110.8 ± 21.6	270.5 ± 63.6	304.2 ± 63.1
70+	13	1.39 ± 0.26	25.3 ± 50	95.2 ± 18.7	33.6 ± 5.7	101.4 ± 28.0	242.6 ± 60.0	297.2 ± 86.4
All	656	1.87 ± 0.39*	35.4 ± 7.2*	132.5 ± 26.2*	46.5 ± 9.0*	128.2 ± 26.1*	278.2 ± 56.5*	343.8 ± 67.6*

† Significant difference in the 70- to 79-year-old group ($P < 0.05$)

*Significant difference in all male subjects ($P < 0.05$)

cant correlations were observed between the thigh SM mass and absolute $\dot{V}_{O_2peak}$ (Fig. 2) or VT (Fig. 3).

Discussion

To our knowledge, the present study is the first to normalise cardiorespiratory fitness values, including $\dot{V}_{O_2peak}$ and VT, for SM mass using a large population sample. The most notable findings of this study were that absolute $\dot{V}_{O_2peak}$ and VT were closely associated with thigh SM mass independent of age, and the study provided normative cardiorespiratory fitness data based on normalised SM mass in healthy men and women aged 20–80 years. Age-associated declines were also observed in VT normalised for body mass in both men and women; however, VT normalised for SM mass was not significantly different with age. Thus, this cross-sectional study showed that the age-associated declines in VT are markedly blunted if normalised for SM mass rather than body mass. These results suggest that SM mass is closely associated with $\dot{V}_{O_2peak}$ or VT in both men and women, and the decrease in VT with age is primarily due to an age-related decline of SM mass.

In cross-sectional studies, the rates of age-related decline in $\dot{V}_{O_2peak}$ normalised for body mass using treadmill walking or running were in the range of 0.28–0.46 ml kg^{-1} min^{-1} year^{-1} in men and 0.25–0.57 ml kg^{-1} min^{-1} year^{-1} in women (Fleg and Lakatta 1988; Jackson et al. 1995, 1996; Paterson et al. 1999; Talbot et al. 2000; Tanaka and Seals 2003; Toth et al. 1994); values for this study were 0.32 and 0.31 ml kg^{-1} min^{-1} year^{-1} in men and women, respectively (Fig. 1). In addition, previous studies have indicated that the rate of decline in VT is approximately one-third of the rate of decline in $\dot{V}_{O_2peak}$ (Babcock et al. 1992; Cunningham et al. 1985; Posner et al. 1987). Posner et al. (1987) found the rates of decline in VT were 0.08 and 0.07 ml kg^{-1} min^{-1} year^{-1} in men and women, respectively, which are similar to the values from this study (0.09 and 0.10 ml kg^{-1} min^{-1} year^{-1} Fig. 1). However, there is little scientific information about the effect of age on these cardiorespiratory fitness parameters normalised for regional SM mass. A previous study using dual energy X-ray absorptiometry (DXA) to estimate muscle mass showed some variation with a significant decrease in the $\dot{V}_{O_2peak}$ even after normalisation for appendicular muscle mass (Proctor and Joyner 1997). On the other hand, there was no evidence of a decline in VT with age, even when normalised for SM mass. However, in the present study, age-associated declines were also observed for VT normalised for body mass in both men and women. Theoretically, the $\dot{V}_{O_2}$ should be proportional to L^2 or $M^{2/3}$, where L is length and M is body mass. We applied this calculation to VT, and showed that there was an age-related decline in $\dot{V}_{O_2}$ /body mass$^{2/3}$ similarly to $\dot{V}_{O_2}$ /body mass. These results suggest that $\dot{V}_{O_2peak}$ and $\dot{V}_{O_2}$ at VT decrease with age even when taking body dimensions in consideration. This is despite this study showing VT, normalised for SM mass, did not vary

Table 4 Absolute and normalised VT in various age groups

Gender and age range (years)	*n*	Percentage of $\dot{V}_{O_2peak}$ (%)	Absolute value (*L*)	Normalised values					
				Body mass (ml kg^{-1} min^{-1})	Body mass$^{2/3}$ (ml kg$^{-2/3}$ min^{-1})	Fat-free body mass (ml kg^{-1} min^{-1})	Total SM mass (ml kg^{-1} min^{-1})	Trunk SM mass (ml kg^{-1} min^{-1})	Thigh SM mass (ml kg^{-1} min^{-1})
Men									
20–29	47	48.7 ± 7.8†	1.71 ± 0.34†	23.1 ± 4.2†	97.5 ± 16.9†	28.4 ± 4.9†	60.8 ± 9.8	150.2 ± 30.1	162.4 ± 26.7
30–39	98	47.4 ± 8.1†	1.48 ± 0.30†	20.6 ± 3.6†	85.6 ± 15.1†	25.5 ± 4.5†	56.4 ± 10.7	139.5 ± 28.1	151.7 ± 32.2
40–49	195	48.9 ± 7.4†	1.47 ± 0.28†	20.6 ± 3.3†	85.5 ± 13.5†	25.2 ± 4.2†	57.4 ± 8.8	143.8 ± 28.6	154.6 ± 24.4
50–59	185	51.7 ± 8.2†	1.40 ± 0.28†	19.8 ± 3.3	81.6 ± 14.1†	24.2 ± 4.0†	56.6 ± 10.2	142.7 ± 30.1	153.0 ± 28.5
60–69	165	53.3 ± 9 3	1.26 ± 0.22†	18.8 ± 3.2	76.1 ± 12.6	22.8 ± 3.8	54.8 ± 10.5	137.2 ± 30.3	148.2 ± 30.0
70+	65	58.0 ± 10.6	1.11 ± 0.19	17.4 ± 2.3	70.0 ± 9.7	20.9 ± 2.9	51.7 ± 7.5	122.7 ± 25.3	142.8 ± 22.9
All	755	51.1 ± 8.9	1.39 ± 0.31	19.9 ± 3.5	81.9 ± 15.0	24.3 ± 4.4	56.2 ± 9.9	140.1 ± 29.7	151.9 ± 28.1
Women									
20–29	47	51.3 ± 8.0	1.09 ± 0.20	20.5 ± 3.4†	76.9 ± 13.0†	27.2 ± 4.8†	71.6 ± 10.7	174.1 ± 34.8	190.5 ± 30.9
30–39	144	50.4 ± 7.9	1.00 ± 0.22	19.9 ± 3.3†	74.4 ± 12.8†	25.9 ± 4.6†	71.9 ± 13.1	176.9 ± 40.0	187.8 ± 35.2
40–49	161	54.5 ± 7.9	1.03 ± 0.20	19.4 ± 3.5	72.5 ± 13.2	25.7 ± 4.9†	69.4 ± 14.7	171.4 ± 41.2	186.9 ± 39.1
50–59	148	55.4 ± 8.0	0.97 ± 0.18	18.4 ± 3.2	68.5 ± 12.1	24.1 ± 4.1	67.0 ± 12.0	165.8 ± 33.2	182.3 ± 32.9
60–69	100	58.7 ± 9.0	0.90 ± 0.16	16.8 ± 2.5	63.1 ± 9.6	22.7 ± 3.7	63.9 ± 12.3	155.8 ± 34.2	175.3 ± 36.1
70+	12	60.8 ± 9.9	0.86 ± 0.23	15.4 ± 3.2	55.6 ± 10.9	20.6 ± 4.1	58.6 ± 11.2	139.6 ± 31.8	180.8 ± 68.4
All	612	54.3 ± 8.6*	1.00 ± 0.21*	18.8 ± 3.5*	70.5 ± 13.1*	24.9 ± 4.7*	68.5 ± 13.3*	168.4 ± 38.1*	183.9 ± 35.7*

† Significant difference in the 70- to 79-year-old group ($P < 0.05$)

*Significant difference in all male subjects ($P < 0.05$)

with age. These results suggest that the age-related decline of VT, defined by treadmill walking is mainly due mainly to a decline of SM mass.

This could be accounted for by the understanding that $\dot{V}_{O_2peak}$ is limited by central circulatory capacity, while changes in VT reflect peripheral/metabolic alterations with age, such as a loss of mitochondrial content for oxidative phosphorylation (Coggan et al. 1992b). It has been reported that subjects with a higher lactate threshold (LT) have a higher muscle respiratory capacity (Coggan et al. 1992a), and LT is associated with volume density of mitochondria and the surface density of mitochondrial cristae (Drexler et al. 1992) in human SM in vivo. Moreover, in rat SM, LT is determined by peripheral factors, such as mitochondrial oxidative capacity (Hepple et al. 2003). Paterson et al. (1999) suggested that the lower rate of age-associated decline in VT (compared with $\dot{V}_{O_2peak}$) may reflect preserved metabolic function of muscle oxidation and may more closely define endurance capacity, while a greater decline of $\dot{V}_{O_2peak}$ may be due to a loss of oxygen delivery capacity. Since it is well known that slow-twitch fibres have a high mitochondrial density and mitochondrial enzyme activity, these findings suggest that the age-related decline in VT defined by treadmill walking may be associated with an age-related decline of SM mass, reflecting a decrease in active tissue, especially a loss of slow-twitch fibres.

Little information is available on the age-related decline of SM mass (*i.e.*, sarcopenia) using direct measurements, such as MRI or CT, the latter of which is the gold standard. In a cross-sectional study using MRI, Janssen et al. reported an age-related decrease of total body SM mass of 0.18 kg year^{-1} in men and 0.08 kg year^{-1} in women (Janssen et al. 2000); these values were notably higher than those obtained by ultrasound in the present study (0.12 and 0.01 kg year^{-1} in men and women, respectively). Despite these variations, both studies showed the same trend with a greater decrease in total SM mass in men compared to women, and both studies had almost identical differences of ~0.1 kg year^{-1} between men and women. In contrast, a longitudinal study by Song et al. indicated that sarcopenia in total SM mass was 0.37 kg year^{-1} for African American women (Song et al. 2004). In addition to possible ethnic differences, it has been suggested that cross-sectional studies may underestimate actual rates of change in SM mass with age, because these losses may not be linear and could accelerate with age.

The observations of this study are tempered by the limitations inherent to cross-sectional studies. Sta-

Table 5 Simple correlation coefficients among age, body composition, and aerobic power in men and women

	Age (years)	Body mass (kg)	Total SM (kg)	Trunk SM (kg)	Thigh SM (kg)	$\dot{V}_{O_2peak}$ (l min^{-1})
In men						
Body mass (kg)	–0.28					
Total SM (kg)	–0.49	0.76				
Trunk SM (kg)	–0.42	0.55	0.77			
Thigh SM (kg)	–0.47	0.72	0.91	0.55		
$\dot{V}_{O_2peak}$ (l min^{-1})	–0.64	0.55	0.66	0.49	0.63	
VT (l min^{-1})	–0.45	0.57	0.59	0.43	0.58	0.68
In women						
Body mass (kg)	NS					
Total SM (kg)	–0.09	0.68				
Trunk SM (kg)	–0.11	0.42	0.69			
Thigh SM (kg)	–0.20	0.57	0.85	0.37		
$\dot{V}_{O_2peak}$ (l min^{-1})	–0.51	0.34	0.41	0.16	0.48	
VT (l min^{-1})	–0.30	0.44	0.45	0.20	0.47	0.67

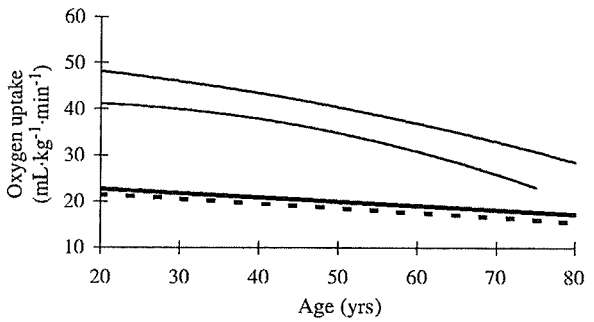

Fig. 1 Relationship between age and cardiorespiratory fitness ($\dot{V}O_2$peak and VT) are shown for men and women. The *thin line* indicates $\dot{V}_{O_2peak}$ and the *heavy line* VT. The *solid line* indicates men and the *dashed line* women. Significant quadratic age declines were observed in $\dot{V}_{O_2peak}$ in men (n = 807, R^2 = 0.34, $Y = 50.989–0.096x–0.002x^2$, $P < 0.001$) and women (n = 656, R^2 = 0.32, $Y = 40.605–0.122x–0.005x^2$, $P < 0.001$). On the other hand, VT declined linearly with age in men (n = 755, R^2 = 0.12, $Y = 24.549–0.091x$, $P < 0.001$) and women (n = 612, R^2 = 0.13, $Y = 23.623–0.102x$, $P < 0.001$)

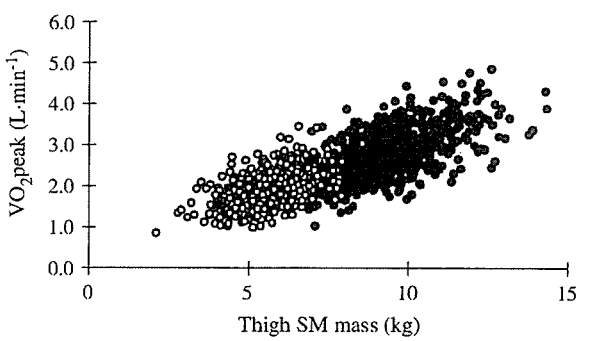

Fig. 2 Relationship between thigh SM mass and $\dot{V}_{O_2peak}$ values in men (*closed circles*) and women (*open circles*). Significant correlations were observed between the thigh SM mass and $\dot{V}_{O_2peak}$. Men; n = 755, y = 0.265x + 0.332, r = 0.63, $P < 0.001$. Women; n = 620, y = 0.215x + 0.681, r = 0.48, $P < 0.001$

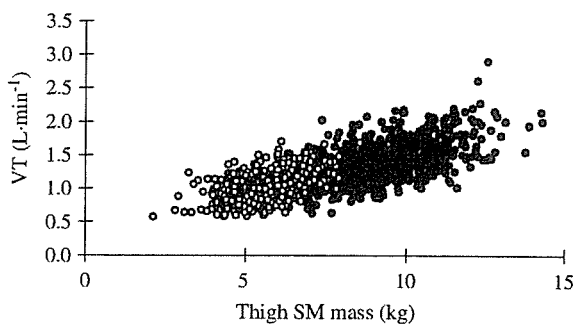

Fig. 3 Relationship between thigh SM mass and VT values in men (closed circles) and women (open circles). Significant correlations were observed between the thigh SM mass and VT. Men; n = 755, y = 0.119x + 0.297, r = 0.58, $P < 0.001$. Women; n = 612, y = 0.112x + 0.382, r = 0.47, $P < 0.001$

thokostas et al. (2004) investigated longitudinal data versus cross-sectional analysis, and showed a greater decline in VT for men (0.14 ml kg^{-1} min^{-1} year^{-1}) and women (0.11 ml kg^{-1} min^{-1} year^{-1}). Second, this study assessed the total or regional SM mass by ultrasound. MTH measurements using ultrasound may not be accurate as compared to MRI, and the measurement of SM size by B-mode ultrasound has limitations because it cannot exclude non-contractive tissue, such as the connective and intra-muscular fat tissue. Third, $\dot{V}_{O_2peak}$ was estimated at sub-maximal effort, which may introduce substantial error. However, this study had a large sample size including many middle-aged and older men and women, and there is a certain degree of risk with graded exercise tests (GXT) in subjects with low fitness levels or in the elderly (American College of Sports Medicine 1995). We configured the end point of the GXT to prevent such risks. In addition, Wasserman et al. (1995) noted that in calculating using the V-slope method, the data

above the $\dot{V}_{O_2}$ at which $VE/\dot{V}_{CO_2}$ starts to increase (respiratory compensation point) should not be included. Since we calculated the VT by this method, VT could be estimated at sub-maximal GXT. Moreover, the $\dot{V}_{O_2}$ values at VT in the present study correspond to those reported in previous studies (Posner et al. 1987; Thomas et al. 1985). Finally, the treadmill protocol in this study which alternates the speed and grade has the potential to give a non-linear increase in estimated work rate, because it uses rather large steps to increase the grade. However, we ensured a linear increase in $\dot{V}_{O_2}$ during this protocol in the majority of subjects. Therefore, we might as well to evaluate the ventilatory threshold using our protocol.

In conclusion, we have demonstrated that absolute $\dot{V}_{O_2peak}$ and VT were closely associated with thigh SM mass independent of age, body mass and FFM. Age-associated declines were observed in VT normalised for body mass in both men and women, but not VT normalised for SM mass. These results suggest that thigh SM mass was closely associated with $\dot{V}_{O_2peak}$ or VT in both men and women, and the decrease in VT with age is due, in part, to an age-related decline of SM mass. Moreover, this study provides normative cardiorespiratory fitness data regarding VT normalised SM mass in healthy men and women aged 20–80 years.

References

Abe T, Kondo M, Kawakami Y, Fukunaga T (1994) Prediction equations for body composition of Japanese adults by B-mode ultrasound. Am J Hum Biol 6:161–170

American College of Sports Medicine (1995) Guidelines for exercise testing and prescription, Lippincott Williams & Wilkins, Philadelphia

American College of Sports Medicine (1990) American College of Sports Medicine position stand. The recommended quantity and quality of exercise for developing and maintaining cardiorespiratory and muscular fitness in healthy adults. Med Sci Sports Exerc 22:265–274

Astrand PO, Rodahl K (1977) Textbook of work physiology. Physiological bases of exercise, McGraw-Hill, NY

Babcock MA, Paterson DH, Cunningham DA (1992) Influence of ageing on aerobic parameters determined from a ramp test. Eur J Appl Physiol Occup Physiol 65:138–143

Beaver WL, Wasserman K, Whipp BJ (1986) A new method for detecting anaerobic threshold by gas exchange. J Appl Physiol 60:2020–2027

Blair SN, Kohl HW III, Paffenbarger RS, Jr, Clark DG, Cooper KH, Gibbons LW (1989) Physical fitness and all-cause mortality. A prospective study of healthy men and women. JAMA 262:2395–2401

Blair SN, Kohl HW III, Barlow CE, Paffenbarger RS, Jr, Gibbons LW, Macera CA (1995) Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. JAMA 273:1093–1098

Brozek J, Grande F, Anderson JT, Keys A (1963) Densitometric analysis of body composition: revision of some quantitative assumptions. Ann NY Acad Sci 110:113–140

Caiozzo VJ, Davis JA, Ellis JF, Azus JL, Vandagriff R, Prietto CA, McMaster WC (1982) A comparison of gas exchange indices used to detect the anaerobic threshold. J Appl Physiol 53:1184–1189

Coggan AR, Kohrt WM, Spina RJ, Kirwan JP, Bier DM, Holloszy JO (1992a) Plasma glucose kinetics during exercise in subjects with high and low lactate thresholds. J Appl Physiol 73:1873–1880

Coggan AR, Spina RJ, King DS, Rogers MA, Brown M, Nemeth PM, Holloszy JO (1992b) Histochemical and enzymatic comparison of the gastrocnemius muscle of young and elderly men and women. J Gerontol 47:B71–B76

Cunningham DA, Nancekievill EA, Paterson DH, Donner AP, Rechnitzer PA (1985) Ventilation threshold and aging. J Gerontol 40:703–707

Drexler H, Riede U, Munzel T, Konig H, Funke E, Just H (1992) Alterations of skeletal muscle in chronic heart failure. Circulation 85:1751–1759

Fleg JL, Lakatta EG (1988) Role of muscle loss in the age-associated reduction in V_{O_2max}. J Appl Physiol 65:1147–1151

Fletcher GF, Balady G, Blair SN, Blumenthal J, Caspersen C, Chaitman B, Epstein S, Sivarajan Froelicher ES, Froelicher VF, Pina IL, Pollock ML (1996) Statement on exercise: benefits and recommendations for physical activity programs for all Americans. A statement for health professionals by the Committee on Exercise and Cardiac Rehabilitation of the Council on Clinical Cardiology, American Heart Association. Circulation 94:857–862

Frontera WR, Suh D, Krivickas LS, Hughes VA, Goldstein R, Roubenoff R (2000) Skeletal muscle fiber quality in older men and women. Am J Physiol Cell Physiol 279:C611–C618

Fukunaga T, Miyatani M, Tachi M, Kouzaki M, Kawakami Y, Kanehisa H (2001) Muscle volume is a major determinant of joint torque in humans. Acta Physiol Scand 172:249–255

Gaskill SE, Ruby BC, Walker AJ, Sanchez OA, Serfass RC, Leon AS (2001) Validity and reliability of combining three methods to determine ventilatory threshold. Med Sci Sports Exerc 33:1841–1848

Hepple RT, Hagen JL, Krause DJ, Jackson CC (2003) Aerobic power declines with aging in rat skeletal muscles perfused at matched convective O_2 delivery. J Appl Physiol 94:744–751

Jackson AS, Beard EF, Wier LT, Ross RM, Stuteville JE, Blair SN (1995) Changes in aerobic power of men, ages 25–70 years. Med Sci Sports Exerc 27:113–120

Jackson AS, Wier LT, Ayers GW, Beard EF, Stuteville JE, Blair SN (1996) Changes in aerobic power of women, ages 20–64 years. Med Sci Sports Exerc 28:884–891

Janssen I, Heymsfield SB, Wang ZM, Ross R (2000) Skeletal muscle mass and distribution in 468 men and women aged 18–88 years. J Appl Physiol 89:81–88

Kawakami Y, Abe T, Fukunaga T (1993) Muscle-fiber pennation angles are greater in hypertrophied than in normal muscles. J Appl Physiol 74:2740–2744

Kubo K, Kanehisa H, Azuma K, Ishizu M, Kuno SY, Okada M, Fukunaga T (2003) Muscle architectural characteristics in women aged 20–79 years. Med Sci Sports Exerc 35:39–44

Lehmann G, Schmid S, Ammer R, Schomig A, Alt E (1997) Evaluation of a new treadmill exercise protocol. Chest 112:98–106

McArdle W, Katch FI, Katch VL (2001) Exercise physiology: energy, nutrition, and human performance. Lippincott Williams & Wilkins, Philadelphia