厚生労働科学研究費補助金（循環器疾患等生活習慣病対策総合研究事業）
分担研究報告書

筋力トレーニングは動脈スティフネスや血圧の増加と関連しているが寒冷刺激に対する頸動脈径反応で評価された内皮機能には影響しない

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筋力トレーニングは、中心動脈スティフネスの増加を引き起こす可能性がある。この硬化の一要因とされている中心動脈内皮機能に対して筋力トレーニングが及ぼす影響は検討されていない。我々は、12名の筋力トレーニング従事者と17名の対照者の血圧、ティフネスおよび局所的寒冷刺激（Cold Pressor Test, CPT）に対する中心動脈の反応性によって評価した中心動脈内皮機能を比較した。血圧および中心動脈スティフネスは、筋力トレーニング者が対照者と比較して有意に高かったが（収縮期血圧：131対116mmHg、平均血圧：95対86mmHg、中心動脈スティフネス：7.65対5.98AU、いずれもP<0.05）、CPTに対する中心動脈の反応性に有意な差は認められなかった（変化量：0.33対0.37mm、変化率：5.2対5.8%、いずれもNS）。これらの結果は、筋力トレーニングが血圧や動脈スティフネスの増大と関係するが、内皮機能の評価が可能であるCPTに対する中心動脈の反応性の異常とは関係がないことを示唆している。

A．研究目的

中心動脈スティフネスの増加を引き起こす筋力トレーニングが中心動脈内皮機能を低下させるかどうかを明らかにするために、12名の筋力トレーニング従事者と17名の対照者の血圧、中心動脈コンプライアンスおよびスティフネス、さらにCPTに対する中心動脈の反応性によって評価した中心動脈内皮機能を比較する横断研究をデザインした。

B．研究方法

被験者は、筋力トレーニングを10年以上継続して行っている男性12名（筋トレ群；平均38.7歳）と同一年齢層の非活動的な17名（対照群；平均36.8歳）とした。測定項目は、身長、体重、体脂肪率（DEXA）、血中脂質、最大酸素摂取量、握力、脚伸展パワー、上腕動脈血圧、中心動脈である頸動脈の血圧、コンプライアンス、βスティフネスおよび内皮機能であった。頸動脈コンプライアンスおよびβスティフネスは、超音波診断装置とトノメトリーセンサーを用いて測定した。頸動脈内皮機能は、片足を氷水（4℃）に90秒間浸けたときの頸動脈径の変化量および変化率によって評価した（CPT）。

結果は、平均値±標準偏差で表し、有意差検定には対応のないt検定を用い、頸動脈コンプライアンスとβスティフネスにおける筋トレ群と対照群の間に有意差があった場合、平均血圧を共変量として共分散分析を行った。有意水準は5%未満とした。

C. D. 研究結果と考察

被験者特性をTable1に示した。体脂肪率は、筋トレ群で有意に低かった。両群の血中脂質は正常範囲内であったが、HDLコレステロールのみ筋トレ群で有意に高かった。握力および脚伸展パワーは、筋トレ群で有意に高かった。その他の項目に有意な差は認められなかった。

Table 1. Subject Characteristics

	Control	Resistance-Trained
N	17	12
Age, years	36.8 ± 1.2	38.7 ± 1.7
Height, cm	171.0 ± 1.2	171.0 ± 1.8
Body weight, kg	71.9 ± 1.9	74.9 ± 2.1
%Fat, %	19.4 ± 1.2	12.3 ± 0.9*
Total cholesterol, mmol/l	5.0 ± 0.2	4.7 ± 0.2
HDL cholesterol, mmol/l	1.3 ± 0.1	1.6 ± 0.1*
Plasma Glucose, mmol/l	5.0 ± 0.1	5.1 ± 0.1
Triglycerides, mmol/l	1.5 ± 0.3	0.9 ± 0.1
Resting heart rate, bpm	58 ± 2	56 ± 2
Maximal heart rate, bpm	186 ± 3	183 ± 4
Vo_{2max}, l/min	2.7 ± 0.1	2.8 ± 0.1
Vo_{2max}/body weight, ml/kg/r	37.7 ± 1.4	36.9 ± 1.3
Leg press power, W	1719 ± 91	2293 ± 155*
Handgrip, kg	45.6 ± 1.6	51.0 ± 2.0*

Data are Means ± SEM; N, no. of subjects; VO_{2max}, maximal oxygen consumption. *Significant at $P < 0.05$ vs Control.

拡張期血圧を除いて、上腕および頸動脈における全てのパラメーターは、対照群と比較して筋トレ群において有意に高かった。頸動脈の内膜中膜複合体（IMT）や直径に有意な差はなかった（Table2）。

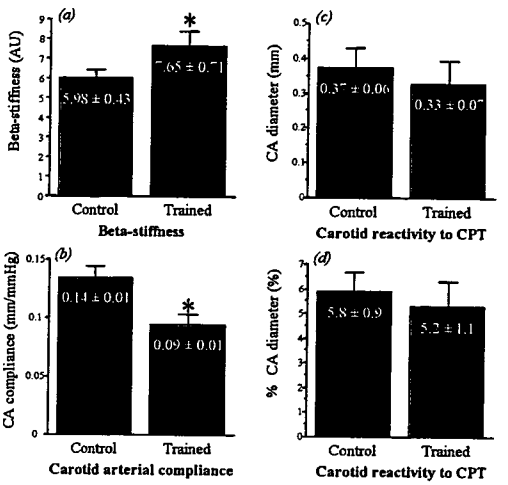
Table 2. Cardiovascular Measures

	Control	Resistance-Trained
Brachial systolic BP, mmHg	116 ± 2	131 ± 4*
Brachial mean BP, mmHg	86 ± 2	95 ± 3*
Brachial diastolic BP, mmHg	71 ± 2	74 ± 3
Brachial PP, mmHg	45 ± 1	57 ± 2*
Carotid systolic BP, mmHg	104 ± 2	123 ± 5*
Carotid PP, mmHg	33 ± 2	48 ± 4*
CA diameter, mm	6.4 ± 0.1	6.2 ± 0.1
CA IMT, mm	0.64 ± 0.02	0.65 ± 0.03

Data are Means ± SEM; BP, blood pressure; PP, pulse pressure; CA, carotid artery; IMT, intima-media thickness.
*Significant at $P < 0.05$ vs Control.

対照群と比較して筋トレ群では、頸動脈βスティフネスは有意に高く（Fig. 1a）、頸動脈コンプライアンスは有意に低かった（Fig. 1b）。CPTに対する頸動脈径の変化量（Fig. 1c）および変化率（Fig. 1d）は両群間で有意な差は認められなかった。頸動脈βスティフネスと頸動脈コンプライアンスの有意差は、平均血圧で標準化すると消失した（ANCOVA；それぞれP=0.101とP=0.081）。

Figure 1



E. 考察

本実験の結果は、血流依存性の血管拡張によって評価した末梢動脈内皮機能に筋力トレーニングが影響を及ぼさないことを報告した先行研究と一致しているが、内皮機能評価を頸動脈に代表されるような中心動脈で初めて試みたことに意義がある。

対照群と比較して筋トレ群で中心動脈スティフネスや血圧が有意に高かったが、HDLコレステロールも筋トレ群で高く、その他の血中脂質の項目や頸動脈のIMTに両群で有意な差はなかった。加齢や循環器疾患の状態悪化に伴って起こる動脈スティフネスの増加が内皮機能の低下、IMTの肥厚や血中脂質異常と関係することを考慮すると、筋力トレーニングによって引き起こされる頸動脈スティフネスの増加は加齢や循環器疾患における血管機能の変化とは異なる生理的適応かもしれない。

動脈コンプライアンスや動脈スティフネスは、交感神経による血管トーン、動脈の石灰化、エラスチン-コラーゲン比やIMTだけでなく内皮機能によっても影響を受け、有酸素性能力、年齢、血圧、体脂肪、腹部周径囲や血中脂質といった臨

床的なパラメーターと関係する。将来的に、筋力トレーニングによって内皮機能とは独立して増加する動脈スティフネスにその他の要因がどの程度影響を及ぼすかを大規模なコホート研究や介入研究等によって明らかにする必要があるだろう。

Rubenfire らは、CPT に対する頸動脈径の変化の程度によって IMT とは独立して冠状動脈疾患の状態を予測することが可能であることを報告した。CPT に対する頸動脈の血管反応は、冠状動脈疾患のリスクやその治療に対する回復状態が評価できる可能性がある。本実験では筋トレ群と対照群において、頸動脈の内皮機能および IMT に有意な差が認められない。すなわち、本実験では、筋力トレーニングが少なくとも循環器疾患の危険因子のうち 2 つの因子に影響を及ぼさないことを示唆している。加えて、HDL コレステロール、脚伸展パワーおよび握力は、対照群と比較して筋トレ群で有意に高く、好ましい状態であった。筋力トレーニングによるこのような生理的・機能的な効果を鑑みると、筋力トレーニングを避けるべきでないと主張するべきであろう。

本実験は、中心動脈スティフネスおよび血圧が対照群と比較して筋トレ群で有意に高いことを示した。しかしながら、CPT に対する頸動脈の血管反応性において筋トレ群と対照群の間で有意な差は認められなかった。これらの結果は、習慣的な筋力トレーニングが中心動脈のスティフネスおよび血圧の増大と関係するが、内皮機能の減弱を反映する交感神経刺激に対する頸動脈の血管反応における異常とは関係がないことを示唆している。

F. 健康危険情報

問題なし。

G. 研究発表

1．論文発表

Hiroshi Kawano, Michiya Tanimoto, Kenta Yamamoto, Kiyoshi Sanada, Yuko Gando, Izumi Tabata, Mitsuru Higuchi and Motohiko Miyachi: Resistance training in men is associated with increased arterial stiffness and blood pressure but does not adversely affect endothelial function as measured by arterial reactivity to the cold pressor test. Experimental Physiology 93(2): 296-302, 2008

2．学会発表

Hiroshi Kawano, Michiya Tanimoto, Kenta Yamamoto, Kiyoshi Sanada, Yuko Gando, Motoyuki Iemitsu, Mitsuru Higuchi and Motohiko Miyachi: Carotid arterial vasoreactivity to cold pressor test in resistance-trained men. The 12th Annual Congress of the European College of Sport Science, Finland, July 11-14th, 2007

河野寛、谷本道哉、山元健太、真田樹義、呉泰雄、丸藤祐子、田畑泉、樋口満、宮地元彦：筋力トレーニング者の局所的寒冷刺激に対する頸動脈径の反応性、第 62 回日本体力医学会、2007 年 9 月

H. 知的財産権の出願・登録状況

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

Eur J Appl Physiol (2007) 99:361–369
DOI 10.1007/s00421-006-0355-x

ORIGINAL ARTICLE

Central circulatory and peripheral O_2 extraction changes as interactive facilitators of pulmonary O_2 uptake during a repeated high-intensity exercise protocol in humans

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Accepted: 10 November 2006 / Published online: 13 December 2006

Abstract It has frequently been demonstrated that prior high-intensity exercise facilitates pulmonary oxygen uptake ($\dot{V}O_2$) response at the onset of subsequent identical exercise. To clarify the roles of central O_2 delivery and/or peripheral O_2 extraction in determining this phenomenon, we investigated the relative contributions of cardiac output (CO) and arteriovenous O_2 content difference (a-$\bar{v}DO_2$) to the $\dot{V}O_2$ transient during repeated bouts of high-intensity knee extension (KE) exercise. Nine healthy subjects volunteered to participate in this study. The protocol consisted of two consecutive 6-min KE exercise bouts in a supine position (work rate 70–75% of peak power) separated by 6 min of rest. Throughout the protocol, continuous-wave Doppler ultrasound was used to measure beat-by-beat CO (i.e., via simultaneous measurement of stroke volume and the diameter of the arterial aorta). The phase II $\dot{V}O_2$ response was significantly faster and the slow component (phase III) was significantly attenuated during the second KE bout compared to the first. This was a result of increased CO during the first 30 s of exercise: CO contributing to 100 and 56% of the $\dot{V}O_2$ speeding at 10 and 30 s, respectively. After this, the contribution of a-$\bar{v}DO_2$ became increasingly more predominant: being responsible to an estimated 64% of the $\dot{V}O_2$ speeding at 90 s, which rose to 100% by 180 s. This suggests that, while both CO and a-$\bar{v}DO_2$ clearly interact to determine the $\dot{V}O_2$ response, the speeding of $\dot{V}O_2$ kinetics by prior high-intensity KE exercise is predominantly attributable to increases in a-$\bar{v}DO_2$.

Keywords High-intensity exercise · Cardiac output · Arteriovenous O_2 content difference

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Introduction

It has been consistently demonstrated that the pulmonary oxygen uptake ($\dot{V}O_2$) response to a bout of high-intensity [i.e., supra-lactate threshold (LT)] exercise is faster throughout the transient when recently preceded by a similar high-intensity bout, i.e., a "double-transition" protocol (e.g., Gerbino et al. 1996; MacDonald et al. 1997; Burnley et al. 2000; Fukuba et al. 2002). The majority of these studies (using cycle ergometer exercise) point to an attenuation of the $\dot{V}O_2$ slow component as the main mediator of this phenomenon (e.g., Burnley et al. 2000; Gerbino et al. 1996; Koppo and Bouckaert 2000; MacDonald et al. 1997). However,

even for a single high-intensity transition, there is still debate regarding the relative contributions of O_2 delivery to the exercising muscles (i.e., vascular limitation) and O_2 extraction (i.e., metabolic "inertia") consequent to intramuscular enzyme-linked control mechanisms (e.g., Grassi 2001; Hughson et al. 2001; Poole et al. 1994; Whipp et al. 2002) in determining the $\dot{V}O_2$ kinetic response. The characteristics of the $\dot{V}O_2$ kinetic response to the double-transition protocol has been widely addressed (see Jones et al. 2003 for review), but the details of the potential cardiovascular determinants still remain to be elucidated. For example, to what extent does the magnitude and time course of the cardiac output (CO; reflective of the central component) and the arteriovenous O_2 content difference (a-$\bar{v}DO_2$; indicative of the peripheral O_2 extraction component) contribute to the differences in $\dot{V}O_2$ between the first and second bouts of the double-transition protocol?

There are, to our knowledge, no studies that have tracked central cardiovascular changes throughout the double-transition protocol to establish their potential proportional contributions. Recent advances in continuous-wave Doppler ultra-sonography provide the opportunity for CO to be assessed continuously throughout the transients from measurements at the ascending aorta. We have, therefore, determined the magnitude and time course of the CO response simultaneously with that of $\dot{V}O_2$ throughout a high-intensity knee-extension double-transition exercise protocol in humans, thereby also allowing the dynamic features of the a-$\bar{v}DO_2$ response to be calculated via the Fick equation.

Methods

Subjects

Nine healthy Japanese subjects (5 women and 4 men: age = 29.1 ± 9.1 years; height = 165.8 ± 7.6 cm; body weight = 58.9 ± 14.0 kg, mean ± SD) were selected for the study on the basis of being able to provide high-quality Doppler signals from the ascending aorta. The subjects were all volunteers and were aware of all the testing procedures, having given informed consent to participate as approved by the ethics committee of the local institution (in accordance with the Declaration of Helsinki).

Exercise protocols

During preliminary investigations using high-intensity cycle ergometry, we found that the continuous measurement of loud, high-pitched audio signals and bright visual signals (required for accurate determination of the ascending aortic flow) was technically challenging due to the cardiac movement and interference from respiratory and body movements. In this study, we therefore adopted knee extension (KE) exercise with the subject strapped to the table by belts placed across the iliac spines and shoulder that allowed stable measurement of blood flow in the ascending aorta from continuous echo-Doppler applied via the supra-sternal notch. This both minimized the effects of body (especially thoracic) movement and allowed the subject to perform bilateral KE exercise in the supine position with the hips flexed and stabilized at an angle of approximately 150°: the lower leg being free to move over the required range of motion. The bilateral KE exercise involved lifting and lowering a weight at 1-s intervals (i.e., 60 cycles per min) for each leg in an alternating pattern. The weight was connected to the ankle by a wire-and-pulley mechanism. Timed audio signals provided the subjects with a constant rhythm to cue exercise cadence. Soft rubber was used to cushion the heel during knee flexion and to minimize eccentric muscle activation and maximize concentric muscle activation. A bar (that the subjects were required to touch with their toes on each leg excursion) was used to set the range of motion during the KE exercise, which was continuously monitored (and verbal feedback provided) to ensure a consistent lifting distance. The average lifting distance for this KE exercise protocol was 16.5 cm.

Initially, the subjects each performed a stepwise incremental KE exercise test (0.5 kg each 30 s, from a baseline of 0.5 kg) to the limit of tolerance, which occurred at a peak work rate of 18.3 ± 3.3 W for each leg. The main components of the protocol (each performed on different days) consisted of an initial 3-min resting control phase immediately followed by two consecutive 6-min KE exercise bouts separated by a 6-min resting recovery phase (a double-transition protocol). This was followed by a 6-min resting recovery. The work rate selected was 70–75% of the peak power achieved on the incremental test that consistently resulted in the development of a $\dot{V}O_2$ slow component but also ensured that each subject was able to sustain the 2 × 6-min exercise durations required by the double-transition protocol. Each subject performed the protocol on two occasions at the same time on different days.

Measurements

Ventilatory and gas exchange responses were determined breath-by-breath using a computerized meta-

bolic measuring system (RM-300, Minato Medical Co., Japan). Prior to each exercise test, a hot-wire flow-sensor and gas analyzers were calibrated by inputting a known volume of air (at several mean flow rates) and gas mixtures of known concentrations, respectively.

The time-serial CO was obtained using continuous-wave Doppler, a two-dimensional and M-mode echocardiography apparatus (SSD-2000, Aloka, Japan) to measure the mean blood velocity (V_{mean}) and diameter of the ascending aorta (just above the aortic valve), i.e., similar to standard, previously described, methods (Christie et al. 1987; Miyachi et al. 1998; Rowland et al. 1998; Nottin et al. 2002; Sugawara et al. 2003). Briefly, the ascending aorta was assumed to be circular, and its cross-sectional area (CSA) was calculated using the diameter measured by two-dimensional and M-mode echocardiography during supine rest. The insertion point of the aortic valve tips at end-diastole was set using two-dimensional imaging in the parasternal long axis view. The subsequent M-mode echocardiogram was recorded at the same level. The aortic diameter was measured at mid-systole and end-diastole from the mean of 3–5 consecutive cardiac cycles by the leading edge to leading edge method. Continuous-wave Doppler echocardiographic recordings of the ascending aortic blood velocity were obtained with a small-dedicated 2.0 MHz non-imaging transducer (SSD-870, Aloka, Japan) held in the supra-sternal notch. The ascending aortic flow was identified by a loud, high-pitched audio signal and a bright well-defined video display. The Doppler and simultaneous ECG signals were stored on S-VHS video during the protocol. They were subsequently digitally converted and analyzed using image analysis software (NIH image). The V_{mean} throughout a cardiac cycle was determined between consecutive R spikes by planimetry. The velocity integral was calculated as the product of V_{mean} and ejection time during a cardiac cycle. The stroke volume (SV) was calculated as the product of the velocity integral and aortic CSA. The CO was, therefore, calculated as the product of the SV and the simultaneous heart rate (HR). The arteriovenous O_2 content difference (a-$\bar{v}DO_2$) was calculated from the Fick equation by dividing $\dot{V}O_2$ by CO. A second-by-second time course was calculated for each variable by interpolation and then stored on disk for further analysis.

Data analysis

The temporal profiles of variables at the onset of both bouts of high-intensity KE exercise were displayed by 10-s averaged data. We did not, however, perform further model-based analyses of the response transients (such as time constants, gains or amplitudes) due to the limited confidence of the estimation resulting from the small-step increment of work rate and limited number of repetitions (Lamarra et al. 1987). Instead, the summarized data from all subjects were displayed at distinct time points: 0, 10, 30, 50, 90, 180 and 360 s after the onset of each bout (where time = 0 is the baseline value averaged from the 30 s immediately preceding the exercise onset). The value at each representative time was determined as average for a 10-s bin placed equidistant around each corresponding time point. Then, the difference between the $\dot{V}O_2$ values in bout 1 and bout 2 were determined [$\dot{V}O_2$ (second)/$\dot{V}O_2$ (first)] and used to calculate the relative contributions of CO and a-$\bar{v}DO_2$ to this difference in each subject i.e., similar to the method previously used by De Cort et al. (1991) and MacDonald et al. (2001). Briefly, the relative contribution of a-$\bar{v}DO_2$ to the speeding of the $\dot{V}O_2$ kinetics observed in bout 2 can be evaluated by assuming that the CO kinetic profile is unchanged between bouts. Consequently, the relative contribution of a-$\bar{v}DO_2$ can be calculated from a-$\bar{v}DO_2$ (second)/a-$\bar{v}DO_2$ (first). The same process can be applied to a-$\bar{v}DO_2$ (i.e., assuming both bouts follow the measured bout 1 profile) in order to calculate the relative contribution of CO [CO(second)/CO(first)] to the speeding of the $\dot{V}O_2$ kinetics observed in bout 2. The difference between the $\dot{V}O_2$ kinetic profiles during the double-transition protocol (i.e., the degree of speeding of the $\dot{V}O_2$ kinetics) can thereby be attributed to the quantitative contributions of CO and/or a-$\bar{v}DO_2$.

The increment in $\dot{V}O_2$ between 180 and 360 s of each exercise bout ($\Delta\dot{V}O_{2(6-3)}$) was used to estimate the magnitude of the $\dot{V}O_2$ slow component. In addition, the increment in $\dot{V}O_2$ between 120 and 360 s of each exercise bout ($\Delta\dot{V}O_{2(6-2)}$) was also calculated to aid direct comparison with other recently published data (e.g., Koppo et al. 2002).

The values are expressed as mean ± SD. The time-serial changes in the variables were tested with respect to the differences between first and second bouts by repeated-measures ANOVA with time. When a significant difference was detected, this was further examined by Tukey's post-hoc test. All statistical analyses were performed with SPSS for Windows (SPSS Inc.). The statistical significance was accepted at $P < 0.05$.

Results

Examples of the 10-s averaged $\dot{V}O_2$ and cardiac responses (i.e., HR, SV and CO) for a representative

subject during the double-transition KE protocol are shown in Fig. 1. The group mean on-transient responses are shown in Fig. 2 at 0, 10, 30, 50, 90, 180 and 360 s of each bout (where t = 0 represents the baseline preceding exercise onset). The temporal profile of $\dot{V}O_2$ after the onset of bout 2 was consistently and significantly higher than that in bout 1 for all values from 10 to 180 s, consistent with speeded $\dot{V}O_2$ kinetics. Note also that $\dot{V}O_2$ had recovered to its prior control value by the start of the second exercise bout ($\dot{V}O_2$ values at t = 0 were the same for both bouts). The $\Delta\dot{V}O_{2(6-3)}$ was significantly higher in bout 1 (78 ± 44 ml min^{-1}) compared to bout 2 (57 ± 36 ml min^{-1}). This was also the case for the $\Delta\dot{V}O_{2(6-2)}$ index (bout 1, 132 ± 51 ml min^{-1} vs. bout 2, 94 ± 42 ml min^{-1}).

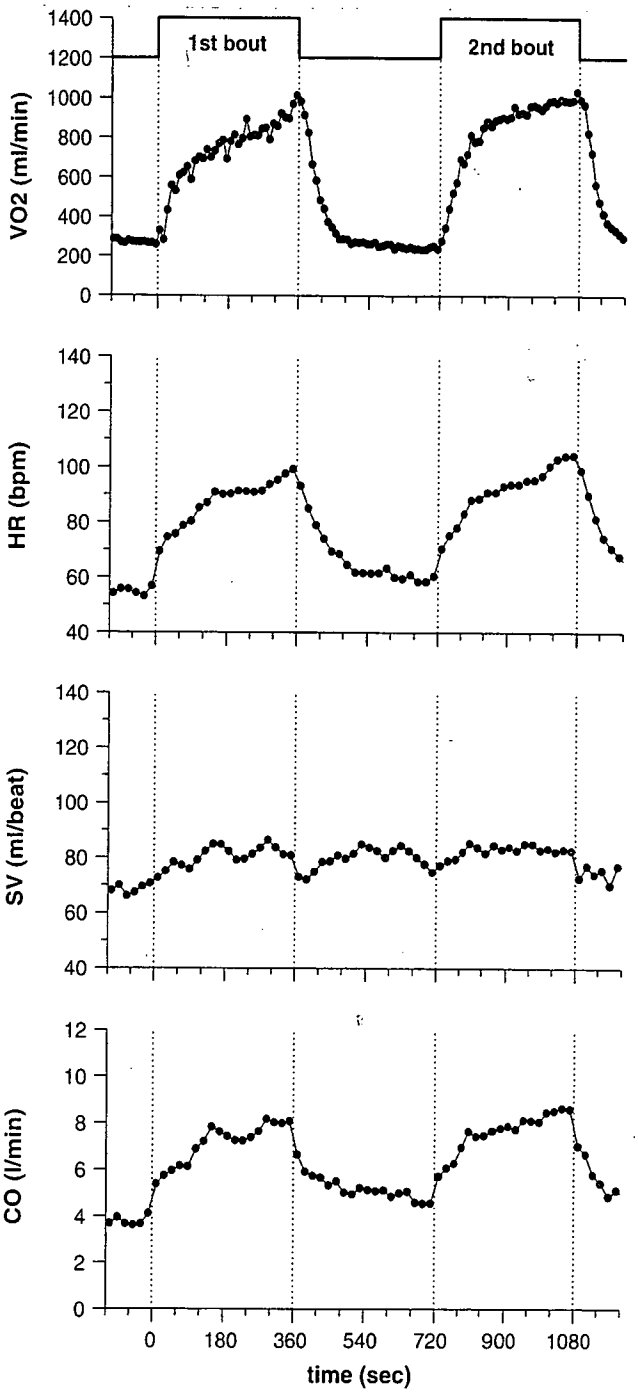

Fig. 1 The pulmonary $\dot{V}O_2$, heart rate (*HR*), stroke volume (*SV*) and cardiac output (*CO*) responses (averaged every 20 s) during repeated bouts of high-intensity knee extension exercise in a representative subject

Following the initial 20 s, CO showed a very similar temporal response during both exercise bouts, with the CO only being greater (P < 0.05) at 0 and 10 s of bout 2. In contrast, the HR remained significantly elevated throughout the bout 2, including the baseline prior to the exercise onset (Fig. 2). The a-$\bar{v}DO_2$ was significantly lower at the onset of the bout 2 as a consequence of the high CO residual from the prior exercise (Fig. 2). The difference of a-$\bar{v}DO_2$ between the bouts gradually widened after phase I and was significantly higher in bout 2 (compared to bout 1) by 180 s (Fig. 2). It is salient to note that the qualitative difference between the bouts in the $\dot{V}O_2$ profiles was closer to that of a-$\bar{v}DO_2$ than CO. We could not discern a slow component-like phase in the CO response in either bout: $\Delta CO_{(6-3)}$ bout 1, 0.21 ± 0.46 vs. bout 2, 0.40 ± 0.31 l min^{-1}. However, Δa-$\bar{v}DO_{2(6-3)}$ was significantly lower in bout 2 (–0.071 ± 0.401 ml 100 ml) compared with bout 1 (0.635 ± 0.388 ml 100 ml).

The relative contributions (expressed in %) of CO and a-$\bar{v}DO_2$ to the speeding of $\dot{V}O_2$ observed in bout 2 between 0 and 180 s (i.e., the region encompassing phase II) are displayed in Fig. 3. Using the 90 s time point as an example, the absolute mean values for $\dot{V}O_2$ increased from 617 ml min^{-1} in bout 1 to 656 ml min^{-1} in bout 2: an increase of 6.3%. The corresponding values for CO and a-$\bar{v}DO_2$ were 8.41 l min^{-1} and 73.34 ml l^{-1}, respectively, during bout 1, and 8.60 l min^{-1} and 76.31 ml l^{-1}, respectively, during bout 2. If it is assumed that CO did not change between bouts (i.e., was 8.41 l min^{-1} at 90 s in both bouts), then the expected increase in $\dot{V}O_2$ would be 8.41 l min^{-1} multiplied by 76.31 ml l^{-1}, or 642 ml min^{-1}. This can then be used to estimate the relative contribution of a-$\bar{v}DO_2$ to the speeded $\dot{V}O_2$ kinetics observed in bout 2, i.e., 642/617 ml min^{-1}, or 4%. A similar calculation for CO (assuming unchanged a-$\bar{v}DO_2$) yields an expected $\dot{V}O_2$ of 631 ml min^{-1} (i.e., 8.60 l min^{-1} multiplied by 73.34 ml l^{-1}). The relative contribution of CO was therefore estimated to be 631/617, or 2.3%.

These calculations were repeated for each individual at each time point. The increase in the $\dot{V}O_2$ of bout 2 was approximately 6–7% throughout the transient phase (i.e., until 180 s; Fig. 3). Using the approach described above, approximately 2–3% was attributable to a-$\bar{v}DO_2$ during the first 30 s, after which this con-

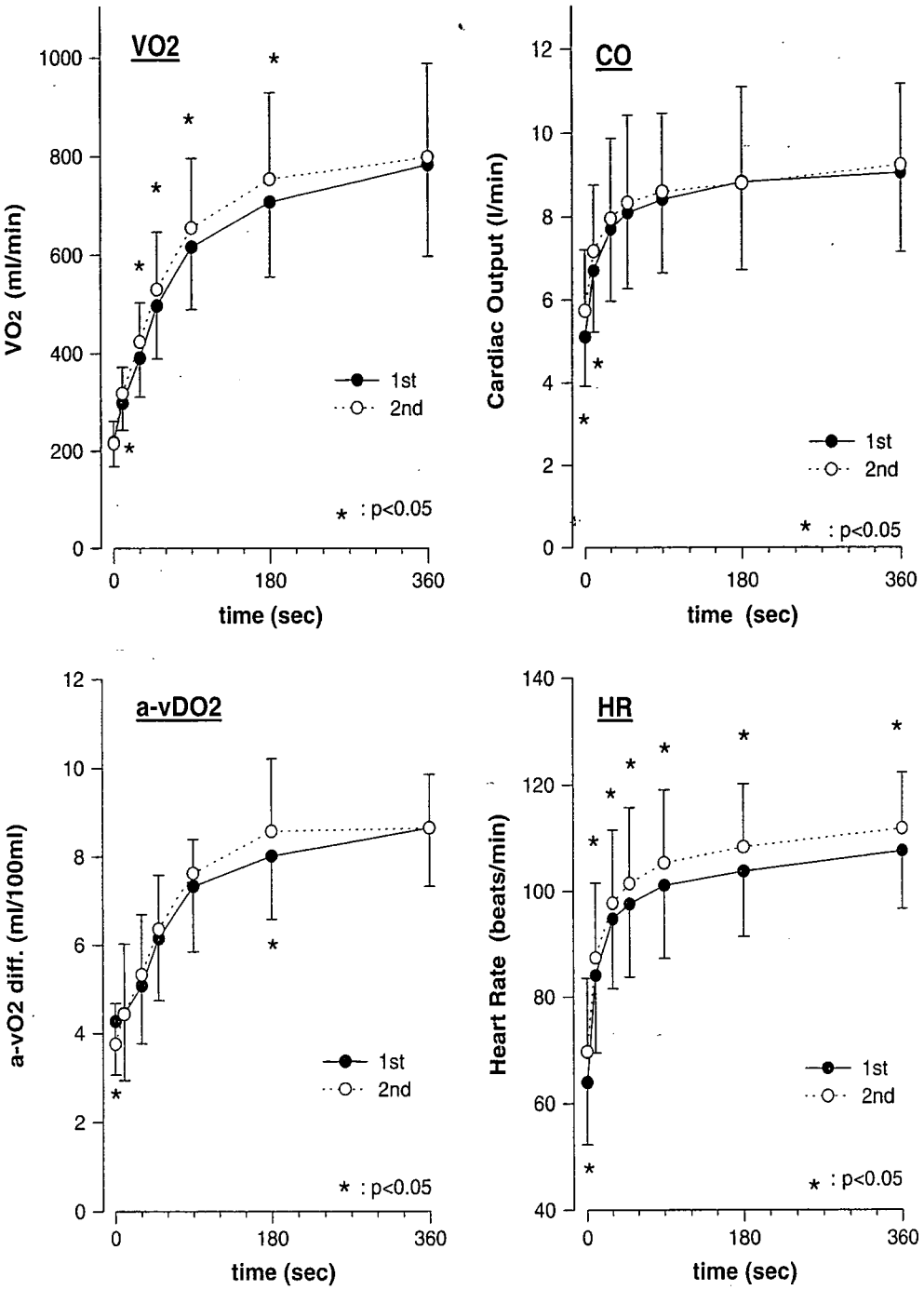

Fig. 2 The superimposition of pulmonary $\dot{V}O_2$, cardiac output (*CO*), arteriovenous O_2 content difference (a-$\bar{v}DO_2$) and hear rate (*HR*) responses determined at district time points during bout 1 (*filled circle*) and bout 2 (*open circle*) of repeated high-intensity KE exercise (group mean and SD). The values at time 0 represent the average of the 30 s baseline just prior to the exercise onset. *Asterisks* represent significant difference between first and second bouts ($P < 0.05$)

tribution continuously increased reaching 7% by 180 s. The higher $\dot{V}O_2$ measured at 180 s in bout 2 (compared to bout 1) could, therefore, be entirely attributed to a proportional increase in a-$\bar{v}DO_2$ by the end of phase II. The proportional contribution of CO showed the opposite trend. At 10 s, the higher $\dot{V}O_2$ in bout 2 could be entirely attributed to a proportionally higher CO. However, this proportional contribution fell rapidly over the initial 30 s and subsequently continued to decrease, such that by 180 s, none of the $\dot{V}O_2$ speeding could be attributed to CO (Fig. 3). There were statistical differences between contributions of both CO and a-$\bar{v}DO_2$ at 10 and 180 s (Fig. 3). Overall, following high-intensity KE exercise, the $\dot{V}O_2$ response to a second identical exercise bout 2 was ~7% greater at each time point considered from 0 to 180 s. This was consequent to interactive contributions by both O_2 delivery and extraction, with the predominant proportion attributable to a progressively increasing extraction (greater a-$\bar{v}DO_2$) following the initial 30 s of exercise (i.e., rising from 43 to 107% between 30 and 180 s of exercise).

Discussion

This investigation provides, we believe, the first description of the relative contributions of CO and

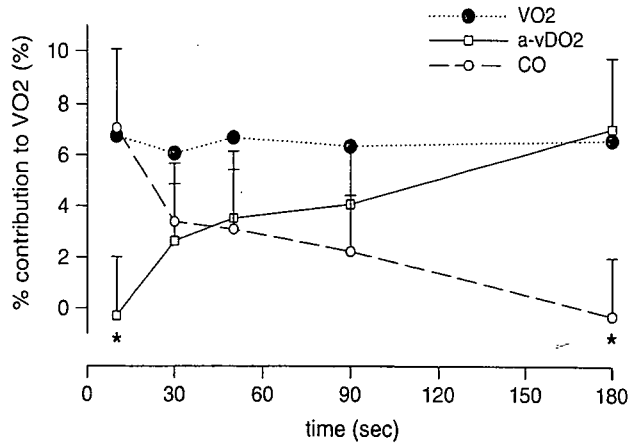

Fig. 3 The percentage contribution of arteriovenous O_2 content difference (a-$\bar{v}DO_2$) (*square*) and cardiac output (*CO*) (*circle*) throughout the transient, to the difference in $\dot{V}O_2$ (*closed circle*) between the first and second exercise bouts. See the detailed explanations in the text. *Asterisks* represent significant difference between CO and a-$\bar{v}DO_2$ contributions ($P < 0.05$)

a-$\bar{v}DO_2$ to the faster $\dot{V}O_2$ response manifest during the transient phase of the second bout of a repeated high-intensity exercise protocol. In order to determine both $\dot{V}O_2$ and CO in concert (and calculate a-$\bar{v}DO_2$) we used KE exercise, not cycle ergometry as is more typical. However, through solving the Fick equation using these measurements during KE exercise, we have elucidated the relative and interactive contributions to $\dot{V}O_2$ from central circulatory and peripheral O_2 extractive components. The findings suggest that the ~7% speeding of the $\dot{V}O_2$ kinetics in the second bout of the double-transition protocol are mainly derived from a gradually widening a-$\bar{v}DO_2$ (relative to that of the first bout). However, during a very early stage of the transition (the first 50 s after onset) the CO contributes to a greater degree to the speeding of $\dot{V}O_2$ in repeated bouts.

In the present study, we chose a conservative strategy to analyze the time-serial data without identifying kinetic parameters (see section Limitations). However, it should be noted that the relationships among CO, $\dot{V}O_2$ and a-$\bar{v}DO_2$ in the present study are compatible to those previously reported for a single transition of cycling exercise (e.g., Cummin et al. 1986; De Cort et al. 1991). Even without estimation of the kinetic parameters, it is clear from the profiles shown in Fig. 2 that the time course of CO is relatively faster than that of $\dot{V}O_2$ in both exercise transitions. Despite these faster kinetics, the magnitude of the CO increase (relative to that of $\dot{V}O_2$) is not adequate to prevent slow increases in a-$\bar{v}DO_2$ throughout the transient. By comparing the $\dot{V}O_2$ kinetics of two bouts of repeated high-intensity exercise, we were able to calculate the relative contributions of CO and a-$\bar{v}DO_2$ to the $\dot{V}O_2$ speeding in the second bout. To do this, we assumed that either of the two variables contributing to $\dot{V}O_2$ (i.e., CO or a-$\bar{v}DO_2$) would have the same absolute value throughout the bout 2 transition as that measured during bout 1. That is, with a-$\bar{v}DO_2$ assumed to respond in bout 2 precisely as it did during bout 1, we can calculate the degree to which an augmented CO may have contributed to the greater $\dot{V}O_2$ measured. Comparison of these calculated and measured $\dot{V}O_2$ values allows a relative weighting to be placed on either of the two components, CO or a-$\bar{v}DO_2$. These data suggest that CO had a relatively important contribution to the increment in $\dot{V}O_2$ during phase I and the following few seconds of bout 2 (the first ~50 s; Fig. 3). Consequently, mechanism(s) related to the peripheral extraction and utilization of oxygen seem to be relatively more important than central circulatory factors in determining the speeding of the phase II and III (i.e., 50–180 s after the exercise onset) pulmonary $\dot{V}O_2$ kinetics during the high-intensity double-transition protocol; although, of course, the interaction of both factors ultimately determine the $\dot{V}O_2$ kinetics.

We are aware of only one study with respect to "double-transition" protocol that has experimentally addressed the potential contribution of circulatory factors in determining the faster $\dot{V}O_2$ kinetics during bout 2. MacDonald et al. (2001) used a similar strategy to the present study to explore the relative contributions of peripheral "blood flow" (BF) and a-vDO_2 (where v means antecubital venous blood) to exercising limb $\dot{V}O_2$ (not pulmonary $\dot{V}O_2$) during repeated bouts of forearm exercise (lateral handgrip). In that study, the forearm $\dot{V}O_2$ was raised by ~30% at 30 s after exercise onset in bout 2 compared to bout 1. They calculated (using the same methods as those used here) that the increase in $\dot{V}O_2$ was consequent to a 25.1% increase in forearm BF and 3.7% increase in a-vDO_2. MacDonald et al. (2001) concluded that this relative contribution indicated that the major factor influencing exercising-limb $\dot{V}O_2$ was the increase in BF.

The main discrepancy between the study of MacDonald et al. (2001) and the present findings is the exercising muscle mass and the intensity of the exercise bouts. The limb $\dot{V}O_2$ in the study of MacDanolad et al. (2001) reached a plateau within 2 min and there was no evidence of a slow component, suggesting a light or moderate intensity (see Fig. 2 in MacDonald et al. 2001). During larger muscle mass exercise (such as cycling or KE), a speeded $\dot{V}O_2$ response is typically only manifest if the preceding exercise is above LT and $\dot{V}O_2$ consequently manifests a slow component. The discrepancy between the studies may, therefore, be derived from the difference in the relative intensity of

the exercise for the involved muscle mass adopted (e.g., Shephard et al. 1988). We suggest that when a larger, locomotory muscle mass is engaged in exercise, the predominant cause of speeded $\dot{V}O_2$ kinetics is an increase in a-vDO_2. In support of this notion, Endo et al. (2003) recently demonstrated that attenuation of central circulatory dynamics (reducing HR by cold face stimulation; CFS) applied at the onset of bout 2 during high-intensity cycling had no effect on the pulmonary $\dot{V}O_2$ response. This supports the notion that the speeded $\dot{V}O_2$ response during the double-transition protocol is not dominated by central factors.

Attenuation of the magnitude of the $\dot{V}O_2$ slow component by prior exercise was a characteristic of the present study. This is similar to previous observations during a double-transition protocol using cycle ergometry (e.g., Burnley et al. 2000; Gerbino et al. 1996; Koppo and Bouckaert 2000; MacDonald et al. 1997). However, in the present study the reduced $\dot{V}O_2$ slow component was not associated with an alteration of CO during KE exercise. Here, CO did not show evidence of a slow component-like phase, with $\Delta CO_{(6\text{-}3)}$ being essentially zero (bout 1: 0.21 ± 0.46 vs. bout 2: 0.40 ± 0.31 l min^{-1}). Rather the attenuation of the $\dot{V}O_2$ slow component was more closely associated with the profile of a-$\bar{v}DO_2$. This also indicates that O_2-extractive factor(s) appear to be more important in speeding the overall $\dot{V}O_2$ response dynamic using a double-transition protocol.

The present result revealed a relatively dominant contribution of a-$\bar{v}DO_2$ to the augmentation of the $\dot{V}O_2$ response during the high-intensity double-transition protocol. While it was, of course, determined by the effective interaction of both central circulatory and peripheral extractive factors, we calculated that a-$\bar{v}DO_2$ contributed to over 50% of the $\dot{V}O_2$ speeding (i.e., from 50 s onwards, or the majority of phase II). In line with previous suggestions, these findings indicate that the predominant determinants for the speeding of $\dot{V}O_2$ kinetics by prior exercise (and the consequent reduction of the $\dot{V}O_2$ slow component) is likely to be attributable to factors more proximal to the exercising limb (the lower limb in this case). One candidate was within the peripheral bulk circulation; that is a more efficient distribution of CO to the exercising limb. However, a recent study from our laboratory utilizing kinetic analyses (Endo et al. 2005) indicated that the optimization of femoral artery blood flow could not explain the faster pulmonary $\dot{V}O_2$ kinetics during the second bout of repeated high-intensity KE exercise. For blood flow to be responsible for a greater proportion of the speeded $\dot{V}O_2$ response, a flow optimization that is more peripheral than the femoral artery would be required. Such a mechanism is yet to be elucidated.

Intramuscular mechanisms, that are thought to determine $\dot{V}O_2$ kinetics, have been investigated during the double-transition protocol by Rossiter et al. (2001). These authors suggested that attenuation of the $\dot{V}O_2$ slow component following prior exercise in humans was consequent to an intramuscular "sparing" of [PCr] degradation. This suggestion that the $\dot{V}O_2$ slow component is determined by intramuscular mechanisms was in accordance with the findings of Poole et al. (1991), who showed that ~80–90% of the pulmonary $\dot{V}O_2$ slow component could be accounted for by an increase in the leg $\dot{V}O_2$. Consequently, the control of the $\dot{V}O_2$ slow component is typically ascribed to intramuscular factors in the exercising limb, rather than to the rest of the body. Therefore, the attenuation of the slow component by prior exercise (and perhaps the speeding of phase II kinetics) ought also to be determined by intramuscular events, such as the pattern of motor unit recruitment and/or fatigue (Barstow et al. 1996; Rossiter et al. 2002), intracellular factors other than O_2 availability (Hogan 2001; Behnke et al. 2002), which may arise from either activation of the pyruvate dehydrogenase complex (Timmons et al. 1998; Howlett and Hogan 2003; Rossiter et al. 2003), and/or be related to the attenuation of the blood lactate increase (Gerbino et al. 1996), or altered phosphate-mediated feedback control (Rossiter et al. 2001). Identifying the intramuscular source of the faster $\dot{V}O_2$ kinetics is, however, beyond the scope of this study.

Limitations

The development of the Doppler ultrasound technique for the measurement of time-resolved CO has made it possible to observe the kinetics CO throughout an exercise transition in greater detail than has previously been possible. Traditional non-invasive methods, such as CO_2 or acetylene rebreathing or prolonged exhalations (e.g., Sackner 1987), have poor time resolution and are therefore not ideal for kinetic observations. However, the continuous Doppler wave method is itself sensitive to signal "noise" derived from several technical and spontaneous sources. We attempted to minimize these by averaging two identical repetitions for each subject with simultaneous $\dot{V}O_2$ measurement. The $\dot{V}O_2$ kinetic responses were further enhanced by additional repetitions, with particular care not to induce significant training effects. Despite this, the CO responses were not sufficiently noise-free to confidently estimate kinetic parameters. We, therefore, chose a conservative strategy to analyze the time-serial

data without estimation of kinetic parameters in any of the measured or calculated variables. However, it should be noted that the relationships among CO, $\dot{V}O_2$ and a-$\bar{v}DO_2$ in the present study were very compatible to those previously reported for a single transition of leg dynamic cycling exercise (e.g., Cummin et al. 1986; De Cort et al. 1991).

In general, the KE exercise modality has frequently led to a reduction in the time constant of $\dot{V}O_2$ primary component (τ_p) as well as the magnitude of the $\dot{V}O_2$ slow component (e.g., Hughson et al. 2003; Fukuba et al. 2004; Rossiter et al. 2001). However, investigations using cycle ergometry typically find that prior exercise-induced changes are limited to the slow component region; in other words, the phase II $\dot{V}O_2$ τ_p is unaltered (e.g., Burnley et al. 2000; Endo et al. 2003; Gerbino et al. 1996; Koppo and Bouckaert 2000; Wilkerson et al. 2004). In addition, KE exercise can result in longer $\dot{V}O_2$ τ_p (~50 s) and higher fundamental gain ($\Delta\dot{V}O_2/\Delta W$; ~20 ml min^{-1} W^{-1}), compared to those seen during cycle erogmetry in healthy subjects (e.g., ~25–35 s; ~10 ± 1 ml min^{-1} W^{-1}) (e.g., Endo et al. 2005). The distinction between KE and cycle erogometry, therefore, may be of importance in this regard. Furthermore, because the KE exercise requires substantially high intramuscular force, there are presumably differences in circulatory adjustments (including CO) at the onset of KE exercise compared to cycle ergometry. Steady-state relationships among CO, $\dot{V}O_2$ and a-$\bar{v}DO_2$ in the present study (i.e., KE exercise) were, however, very compatible to those previously reported for a single transition of leg cycling ergometer exercise (e.g., Cummin et al. 1986; De Cort et al. 1991). Therefore, caution should be used in extrapolating the results of the present study to other modes of exercise, such as conventional cycling exercise.

In summary, this study demonstrated that: (1) the pulmonary $\dot{V}O_2$ was significantly higher between 10 and 180 s (and slow component reduced) after the onset of high-intensity KE exercise when preceded by an identical first bout of KE; (2) cardiac output manifests a faster on-transient time course than pulmonary $\dot{V}O_2$ throughout, but was unchanged between the phase II transient of the first and second exercise bouts; and (3) the apparent speeding of the $\dot{V}O_2$ response during the phase II region of bout 2 was initially greatly determined by a large contribution of CO, but later (and more predominantly), as a result of increased O_2 extraction. This suggests that the mechanism(s) modulating the speeding of the $\dot{V}O_2$ response during bout 2 of the double-transition protocol should be sought for in event(s) within the exercising muscles themselves.

Acknowledgments The authors are grateful to Professor Brian J. Whipp for the constructive criticism of the manuscript and Dr. Harry B. Rossiter for his literary contributions. This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (#16500383 to YF) and Uehara Memorial Life Science Foundation to YF.

References

Barstow TJ, Jones AM, Nguyen PH, Casaburi R (1996) Influence of muscle fiber type and pedal frequency on oxygen uptake kinetics of heavy exercise. J Appl Physiol 81:1642–1650

Behnke BJ, Kindig CA, Musch TI, Sexton WL, Poole DC (2002) Effects of prior contractions on muscle microvascular oxygen pressure at onset of subsequent contractions. J Physiol 539:927–934

Burnley M, Jones AM, Carter H, Doust JH (2000) Effects of prior heavy exercise on phase II pulmonary oxygen uptake kinetics during heavy exercise. J Appl Physiol 89:1387–1396

Christie J, Sheldahl LM, Tristani FE, Sagar KB, Ptacin MJ, Wann S (1987) Determination of stroke volume and cardiac output during exercise: comparison of two-dimensional and Doppler echocardiography, Fick oximetry, and thermodilution. Circulation 76:539–547

Cummin AR, Iyawe VI, Mehta N, Saunders KB (1986) Ventilation and cardiac output during the onset of exercise, and during voluntary hyperventilation, in humans. J Physiol 370:567–583

De Cort SC, Innes JA, Barstow TJ, Guz A (1991) Cardiac output, oxygen consumption and arteriovenous oxygen difference following a sudden rise in exercise level in humans. J Physiol 441:501–512

Endo M, Tauchi S, Hayashi N, Koga S, Rossiter HB, Fukuba Y (2003) Facial cooling-induced bradycardia does not slow pulmonary $\dot{V}O_2$ kinetics at the onset of high-intensity exercise. J Appl Physiol 95:1623–1631

Endo M, Okada Y, Rossiter HB, Ooue A, Miura A, Koga S, Fukuba Y (2005) Kinetics of pulmonary $\dot{V}O_2$ and femoral artery blood flow and their relationship during repeated bouts of heavy exercise. Eur J Appl Physiol 95:418–430

Fukuba Y, Hayashi N, Koga S, Yoshida T (2002) $\dot{V}O_2$ kinetics in heavy exercise is not altered by prior exercise with a different muscle group. J Appl Physiol 92:2467–2474

Fukuba Y, Ohe Y, Miura A, Kitano A, Endo M, Sato H, Miyachi M, Koga S, Fukuda O (2004) Dissociation between the time courses of femoral artery blood flow and pulmonary VO2 during repeated bouts of heavy knee extension exercise in humans. Exp Physiol 89:243–253

Gerbino A, Ward SA, Whipp BJ (1996) Effects of prior exercise on pulmonary gas-exchange kinetics during high-intensity exercise in humans. J Appl Physiol 80:99–107

Grassi B (2001) Regulation of oxygen consumption at exercise onset: is it really controversial? Exerc Sport Sci Rev 29:134–138

Hogan MC (2001) Fall in intracellular PO_2 at the onset of contractions in Xenopus single skeletal muscle fibers. J Appl Physiol 90:1871–1876

Howlett RA, Hogan MC (2003) Dichloroacetate accelerates the fall in intracellular PO_2 at onset of contractions in Xenopus single muscle fibers. Am J Physiol 284: R481–R485

Hughson RL, Tschakovsky ME, Houston ME (2001) Regulation of oxygen consumption at the onset of exercise. Exerc Sport Sci Rev 29:129–133

Hughson RL, Schijvens H, Burrows S, Devitt D, Betik AC, Hopman MTE (2003) Blood flow and metabolic control at the onset of heavy exercise. Int J Sport Health Sci 1:1–10

Jones AM, Koppo K, Burnley M (2003) Effects of prior exercise on metabolic and gas exchange responses to exercise. Sports Med 33:949–971

Koppo K, Bouckaert J (2000) In human the oxygen uptake slow component is reduced by prior exercise of high as well as low intensity. Eur J Appl Physiol 83:559–565

Koppo K, Jones AM, Vanden Bossche L, Bouckaert J (2002) Effect of prior exercise on VO2 slow component is not related to muscle temperature. Med Sci Sports Exerc 34:1600–1604

Lamarra N, Whipp BJ, Ward SA, Wasserman K (1987) Effect of interbreath fluctuations on characterizing exercise gas exchange kinetics. J Appl Physiol 62:2003–2012

MacDonald M, Pedersen PK, Hughson RL (1997) Acceleration of $\dot{V}O_2$ kinetics in heavy submaximal exercise by hyperoxia and prior high-intensity exercise. J Appl Physiol 83:1318–1325

MacDonald MJ, Naylor HL, Tschakovsky ME, Hughson RL (2001) Peripheral circulatory factors limit rate of increase in muscle O_2 uptake at onset of heavy exercise. J Appl Physiol 90:83–89

Miyachi M, Iemitsu M, Okutsu M, Onodera S (1998) Effects of endurance training on the size and blood flow of the arterial conductance vessels in humans. Acta Physiol Scand 163:13–16

Nottin S, Vinet A, Stecken F, N'Guyen LD, Ounissi F, Lecoq AM, Obert P (2002) Central and peripheral cardiovascular adaptations to exercise in endurance-trained children. Acta Physiol Scand 175:85–92

Poole DC, Schaffartzik W, Knight DR, Derion T, Kennedy B, Guy HJ, Prediletto R and Wagner PD (1991) Contribution of excising legs to the slow component of oxygen uptake kinetics in humans. J Appl Physiol 71:1245–1260

Poole DC, Barstow TJ, Gaesser GA, Willis WT, Whipp BJ (1994) $\dot{V}O_2$ slow component: physiological and functional significance. Med Sci Sports Exerc 26:1354–1358

Rossiter HB, Ward SA, Kowalchuk JM, Howe FA, Griffiths JR, Whipp BJ (2001) Effects of prior exercise on oxygen uptake and phosphocreatine kinetics during high-intensity knee-extension exercise in humans. J Physiol 537:291–303

Rossiter HB, Ward SA, Howe FA, Kowalchuk JM, Griffiths JR, Whipp BJ (2002) Dynamics of the intramuscular ^{31}P MRS Pi peak-splitting and the slow component of PCr and O_2 uptake during exercise. J Appl Physiol 93:2059–2069

Rossiter HB, Ward SA, Howe FA, Wood DM, Kowalchuk JM, Griffiths JR, Whipp BJ (2003) Effects of dichloroacetate on $\dot{V}O_2$ and intramuscular ^{31}P metabolite kinetics during high-intensity exercise in humans. J Appl Physiol 95:1105–1115

Rowland TW, Melanson EL, Popowski BE, Ferrone LC (1998) Test–retest reproducibility of maximum cardiac output by Doppler echocardiography. Am J Cardiol 81:1228–1230

Sackner MA (1987) Measurement of cardiac output by alveolar gas exchange. In: Fishman AP (ed) Handbook of physiology. Section 3: the respiratory System, vol IV. Gas exchange. Oxford University Press, New York pp 233–255

Shephard RJ, Bouhlel E, Vandewalle H, Monod H (1988) Muscle mass as a factor limiting physical work. J Appl Physiol 64:1472–1479

Sugawara J, Tanabe T, Miyachi M, Yamamoto K, Takahashi K, Iemitsu M, Otsuki T, Homma S, Maeda S, Ajisaka R, Matsuda M (2003) Non-invasive assessment of cardiac output during exercise in healthy young humans: comparison between Modelflow method and Doppler echocardiography method. Acta Physiol Scand 179:361–366

Timmons JA, Gustafsson T, Sundberg CJ, Jansson E, Greenhaff PL (1998) Muscle acetyl group availability is a major determinant of oxygen deficit in humans during submaximal exercise. Am J Physiol 274:E377–E380

Whipp BJ, Rossiter HB, Ward SA (2002) Exertional oxygen uptake kinetics: a stamen of stamina? Biochem Soc Trans 30:237–47

Wilkerson DP, Koppo K, Barstow TJ, Jones AM (2004) Effect of prior multiple-sprint exercise on pulmonary O_2 uptake kinetics following the onset of perimaximal exercise. J Appl Physiol 97:1227–1236

Eur J Appl Physiol (2007) 99:475–483
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 / Published online: 22 December 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 mas (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

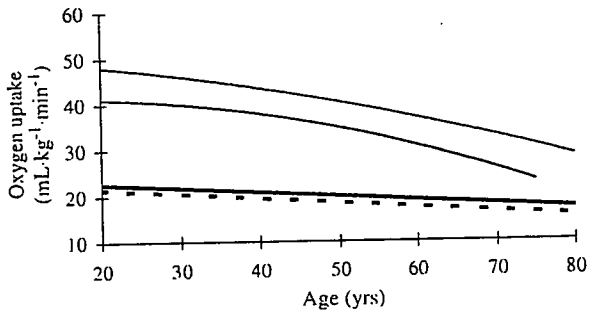

Fig. 1 Relationship between age and cardiorespiratory fitness ($\dot{V}O_2peak$ and VT) are shown for men and women. The *thin line* indicates $\dot{V}_{O_2peak}$ and the *heavy line* VT. The *solid line* indicates men and the *dashed line* women. Significant quadratic age declines were observed in $\dot{V}_{O_2peak}$ 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)

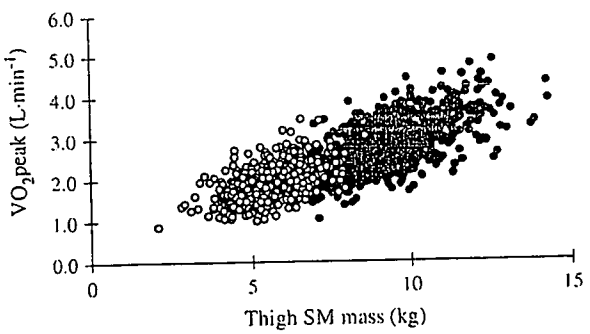

Fig. 2 Relationship between thigh SM mass and $\dot{V}_{O_2peak}$ values in men (*closed circles*) and women (*open circles*). Significant correlations were observed between the thigh SM mass and $\dot{V}_{O_2peak}$. Men; n = 755, y = 0.265x + 0.332, r = 0.63, P < 0.001. Women; n = 620, y = 0.215x +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

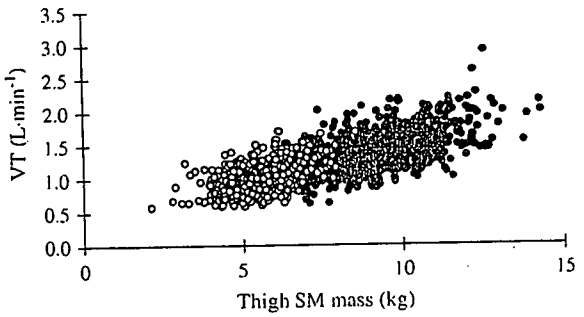

Fig. 3 Relationship between thigh SM mass and VT values in men (closed circles) and women (open circles). Significant correlations were observed between the thigh SM mass and VT. Men; n = 755, y = 0.119x + 0.297, r = 0.58, P < 0.001. Women; n = 612, y = 0.112x + 0.382, r = 0.47, P < 0.001

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