Resistance training induces the expression of calpain protease and its inhibitor calpastatin in human

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It is well known that periods of contractile activity result in adaptations to muscle structure and function. Clearly, this type of muscle remodelling must involve changes in the rate of muscle protein turnover. One of the major pathways for muscle protein turnover is the calpain proteolytic system (Goll *et al.* 1998), which comprises several calpain isoenzymes and their specific endogenous inhibitor calpastatin. The calpain system is believed to be responsible for cytoskeletal remodelling in muscle fibres and is also associated with myoblast differentiation and fusion, fibre hypertrophy and growth. However, in human skeletal muscle little is known about the effects of exercise on the calpain system, and how this relates to changes in muscle composition and function.

Five female subjects (age 28 ± 2 years, body mass 69.0 ± 3.9 kg, height 1.69 ± 0.02 m) volunteered to participate in the study, which was approved by the local ethics committee. Supervised resistance training was performed on three occasions each week for a period of 8 weeks, followed by 4 weeks of detraining, where subjects were asked not to perform any strenuous activities. Needle muscle biopsies were obtained from the vastus lateralis (Bergstom, 1962) prior to training, following 8 weeks of training and after 4 weeks of detraining, and immediately frozen in liquid nitrogen. Total RNA was extracted from between 10 and 20 mg tissue, and the expression of calpastatin, μ- and m-calpain and the muscle-specific p94-calpain determined by Taqman real-time PCR.

Calpastatin expression was significantly increased by 300 % following 8 weeks of training and remained upregulated 4 weeks after the cessation of training ($P < 0.05$). This was mirrored by m-calpain expression, which increased 154 % ($P < 0.01$) following training and remained elevated after training was terminated ($P < 0.01$). Training had no effect on either p94 or μ-calpain expression, but both were significantly increased after 4 weeks of detraining (Table 1). Training induced significant increases in resistance workload. Four weeks of training produced a change in resistance from week 0 of 9 ± 2 kg for leg squat ($P < 0.05$) and 9 ± 1 kg for leg rowing ($P < 0.05$) exercises. These changes were magnified after 8 weeks of training: 20 ± 4 kg for leg squat ($P < 0.05$) and 14 ± 2 kg for leg rowing ($P < 0.05$) in addition to a resistance change of 19 ± 3 kg for leg press exercises ($P < 0.05$).

Table 1. Relative expression of the calpain system following 8 weeks of training and a further 4 weeks of detraining (12 weeks)

	0 weeks	8 weeks	12 weeks
Capastatin	4 ± 1	16 ± 1†	18 ± 1†
μ-Calpain	0.93 ± 0.05	0.97 ± 0.10	1.20 ± 0.10†
m-Calpain	1.3 ± 0.18	3.3 ± 0.15‡	4.4 ± 0.25‡
p94	1.5 ± 0.15	2.0 ± 0.11	2.6 ± 0.21†

Different from 0 week: †$P < 0.05$; ‡$P < 0.01$ (one-way ANOVA plus LSD *post-hoc* test). Data values are arbitrary and are normalised to actin expression, and represent means ± S.E.M.

In conclusion, the data show that resistance training markedly increases the expression of m-calpain protease and to an even greater extent the calpain inhibitor, calpastatin. Furthermore, after detraining, m-calpain and calpastatin remained elevated, in addition to a significant upregulation of both muscle-specific p94 calpain and μ-calpain protease. The implication of this induction of the calpain proteolytic system to muscle mass and fibre type is yet to be elucidated.

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Goll, D.E. *et al.* (1998). *Can. J. Anim. Sci.* **78**, 503–512.

All procedures accord with current local guidelines and the Declaration of Helsinki.

Effects of the menopause and hormone replacement therapy on the contractility of isolated human subcutaneous small arteries

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The effects of the menopause and hormone replacement therapy on small artery contractility are poorly understood. In this study we investigated the responsiveness to agonist stimulation of subcutaneous resistance arteries (150–250 μm diameter), obtained from gluteal biopsies taken (under lignocaine anaesthesia) from four volunteer groups of women: pre-menopausal (pre-mw) ($n = 11$), post-menopausal (post-mw) ($n = 17$), post-menopausal receiving oestrogen replacement therapy (post-mw + ERT) ($n = 8$) and post-menopausal women receiving combined oestrogen and progestin replacement therapy (post-mw + HRT) ($n = 7$). Approval was obtained from the Central Manchester Healthcare Trust Ethical Committee.

Isolated arteries were mounted on a pressure myograph (at 30 mmHg) and superfused with physiological salt solution (pH 7.4, 37 °C). All data are expressed as means ± S.E.M. with differences between groups being tested for by Student's unpaired *t* test.

All arteries exhibited concentration-dependent constrictor responses to phenylephrine (PHE), although those obtained from post-mw were significantly more sensitive compared with those from pre-mw (EC_{50} values = $4.16 \times 10^{-8} \pm 4.08 \times 10^{-9}$ M and $8.83 \times 10^{-8} \pm 1.55 \times 10^{-8}$ M, respectively ($P < 0.05$)). Arteries taken from post-mw + ERT had EC_{50} values similar to those observed in vessels from post-mw ($7.91 \times 10^{-8} \pm 1.61 \times 10^{-8}$ M) while those taken from post-mw + HRT had sensitivities which were intermediate between those of pre-and post-menopausal samples: $4.96 \times 10^{-8} \pm 1.16 \times 10^{-8}$ M. Maximal responses to PHE were similar in all groups.

Both maximum responses and sensitivities to acetylcholine (ACh) were similar in arteries from all groups (75 ± 7 μm and $1.45 \times 10^{-8} \pm 4.38 \times 10^{-9}$ M for pre-mw, 79 ± 7 μm and $9.87 \times 10^{-9} \pm 5.88 \times 10^{-9}$ M for post-mw, 82 ± 6 μm and $1.59 \times 10^{-8} \pm 1.06 \times 10^{-8}$ M for post-mw + ERT, and 89 ± 2 μm and $3.89 \times 10^{-9} \pm 1.17 \times 10^{-9}$ M for post-mw + HRT). The presence of L-NNA (to inhibit nitric oxide synthase) reduced the maximal responses in arteries from pre-mw by 66.6 ± 8.9 %, from post-mw + ERT by 70.8 ± 8.0 % and from post-mw + HRT by 66.1 ± 9.6 %. Responses of arteries from post-menopausal women were, however, not altered by the inhibitor (101 ± 10 %). EC_{50} values were not significantly affected by L-NNA. Vasodilatory responses to sodium nitroprusside were similar in tissues from pre- and post-menopausal women.

These results suggest that sensitivity of isolated subcutaneous small arteries to PHE is enhanced in post-mw compared with pre-mw. Responses to ACh are unaffected by the menopause although the relative contribution of nitric oxide appears altered.

These changes may be reversed by ERT and fully (ACh) or partially (PHE) reversed by HRT.

All procedures accord with current local guidelines.

Weightlifting exercise increases the mRNA expression of a mechanosensitive IGF-I (MGF) in the muscles of young, but not old men

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Muscle mass is lost in later life and the mechanisms underlying this phenomenon remain unclear. It is possible that older muscles are less sensitive to mechanical signals. Skeletal muscle is known to express at least two isoforms of IGF-I and it has been reported that in response to overload the muscles of aged rats are less able to upregulate the splice variant of IGF-I termed MGF (Owino *et al.* 2001). In the present study, approved by the local ethics committee, we aimed to determine whether there were any age-related differences in MGF mRNA expression in human muscle shortly after a bout of high resistance exercise.

Following prior determination of the maximum one-legged knee extensor lift (1-RM) at least 1 week before the experimental day, eight healthy young (26–36 years) and seven healthy older (70–82 years) men performed ten sets of six repetitions of knee extensor exercise, lifting and lowering a mass equal to 80 % of their 1-RM. Two minutes passive recovery was given between each set. Following local anaesthesia (1 % lignocaine), muscle biopsies were obtained from the vastus lateralis muscle of the exercised (test) and non-exercised (control) legs 2.5 h after the end of the exercise session using the needle technique. Samples were immediately frozen. Subsequent quantification of the mRNA of the two IGF-I isoforms in muscle (MGF and IFG-IEa) was performed using a quantitative reverse transcription-polymerase chain reaction (RT-PCR) method (LightCycler, Roche UK).

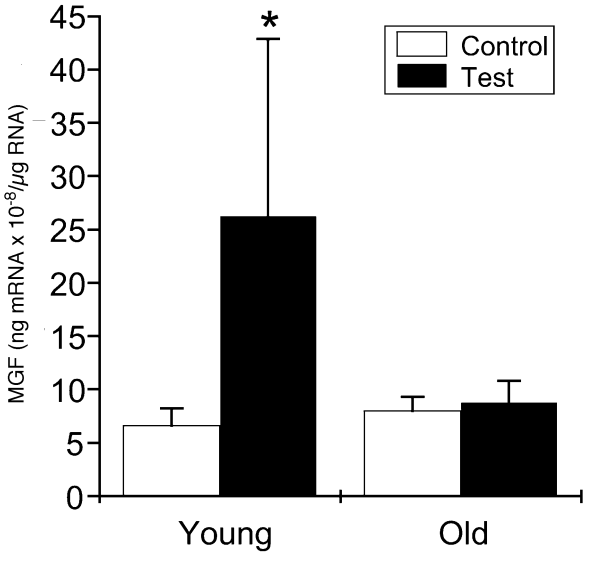

Figure 1. MGF mRNA expression (mean ± S.E.M.) in muscles of young ($n = 8$) and older ($n = 7$) men before and after weight-lifting exercise. *Significant difference between control and exercise samples ($P < 0.05$, Wilcoxon's signed rank test).

The mean (± S.E.M.) 1-RM for the young subjects was 41.9 ± 4.3 *versus* 20.6 ± 1.6 kg for the older subjects ($P < 0.05$). When normalised to total RNA content, levels of IGF-IEa were not different different between the young and old subjects (6.3 ± 0.9 *versus* $6.9 \pm 1.0 \times 10^{-5}$ ng mRNA/μRNA) and no change was observed as a result of the exercise bout. MGF mRNA levels at rest were lower than those of IGF-IEa, but were also not different between the young and old subjects (Fig. 1). In the young subjects, a significantly higher level of MGF was observed in the test leg when compared with the control leg ($P < 0.05$). However, no difference was observed between the test and control legs in the elderly subjects.

These data show that following a standardised bout of high resistance weight-lifting exercise, there is an age-related difference in MGF gene expression. The data in young subjects also suggest that the MGF and IGF-IEa isoforms are differentially regulated in human skeletal muscle.

Owino, V. *et al.* (2001). *FEBS Lett.* **505**, 259–263.

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All procedures accord with current local guidelines and the Declaration of Helsinki.

The influence of fatigued antagonists on net static knee extension torque

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Net knee extension torque is a function of agonist extension torque and opposing antagonistic torque. It has been proposed that antagonistic activity may be functional in stabilizing and/or protecting the joint. The present study has examined the hypothesis that, after fatiguing the antagonist hamstring muscles, a higher net extension torque would be found, which would be related to the degree of fatigue. Moreover, it was investigated whether such an increase was different between knee angles.

Seven, recreationally active males performed isometric MVCs at the following knee angles: 90, 70, 50 and 30 deg knee flexion. Thereafter, subjects performed maximal effort dynamic flexion contractions (180 deg s^{-1}) until torque had declined ~50 %; this was immediately followed by a knee extension MVC. This sequence was repeated four times and randomised for all knee angles. EMG measurements of m. biceps femoris were taken in extension contractions. Antagonist average rectified value (EMG_{ar}) was normalized to the maximal flexion EMG_{ar} at the same knee angle. Voluntary activation (VA) and maximal torque-generating capacity (MTGC) of the m. quadriceps were determined using superimposed electrical stimulation.

Dynamic torque decline after the fatiguing bout of flexion contractions was 48 ± 11 %. The Wilcoxon signed rank test showed no significant effect of fatigue at the different knee angles on EMG_{ar}, MVC torque and MTGC, nor was there a significant effect of knee angle on EMG_{ar} ($P > 0.05$). EMG_{ar} was

(mean ± S.D.) 35 ± 14 and 36 ± 14 % pre- and post-fatigue, respectively. MVC torque was on average 6 ± 4 % lower, MTGC 5 ± 9 % higher and VA 8 ± 6 % lower after the fatiguing exercise. However, these changes were not significant ($P > 0.05$). Only at 50 deg knee angle was VA significantly lower after fatigue ($P < 0.05$).

Antagonistic activity was surprisingly high in the pre-fatigue state but, despite this, a 50 % reduction of antagonistic torque did not increase net extension torque. In fact, MVC torque tended to be slightly lower. This unexpected result could have been due to an inhibition of knee extensors to preserve balance around the joint. However, the change in VA was small compared with the 50 % torque decline. MTGC of the extensor muscle had not changed, thus the dynamic flexion contractions had not fatigued the extensor muscles.

The present results may be due to the recruitment of fatigue resistant motor units during the antagonist activation during MVC which are not, or very little, affected by the dynamic fatiguing protocol (Karatzaferi *et al*. 2001).

Karatzaferi, C. *et al.* (2001). *Exp. Physiol.* **86**, 411–415.

All procedures accord with current local guidelines.

Mechanically evoked long and short latency reflexes in human erector spinae muscles

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Instability of the spinal column is thought to be a contributory factor to the development of low back pain. Local short-latency stretch reflexes have been evoked in the erector spinae (ES) muscles in man (Dimitrijevic *et al.* 1980) and it is known that the supraspinous ligaments contain sensory receptors and are innervated by both spinal and autonomic nerves (Solomonow *et al.* 1998). In this study we have investigated reflexes in ES muscles evoked to mechanical stimulation of either the ES muscle itself or to the adjacent spinal processes along the length of the thoracic spine.

With local ethical approval and informed consent eleven healthy individuals (aged 24–46 years, 3 females) were recruited and seated on a low-backed chair. A Perspex probe (diameter 5 mm) was used to prod the spinal process (PP) at each of thoracic spinal levels T2, T7, T11 and lumbar segment L4 or the ES muscle (PM) adjacent to these segments. An electromagnetic servo applied the probe at a frequency of 1 Hz and a throw of 3 mm. Surface electromyographic (EMG) recordings were made from the right ES 3–6 cm lateral to the same four spinal processes. Trials of 50 mechanical prods were conducted at each probe site and reflex responses in ES were recorded and averaged from each of the four recording sites. Amplitude and latency of the reflex responses were recorded.

Evoked reflexes were seen at short (mean ± S.E.M. 7.54 ± 0.57 ms; $n = 121$) and long (mean 39.21 ± 0.76 ms; $n = 199$) latencies in response to both PP or PM at all four segmental levels. Short latency responses were seen with the following incidence: at prod site PP 100 %, PM 100 %; one recording site away PP 30 %, PM 24 %; two recording sites away PP 7 %, PM 4 %; three recording sites away PP 0 %, PM 0 %. The mean amplitude of the response was smaller (ANOVA on ranks, $P < 0.05$) at recording sites further from the prod site for both PP and PM stimulation. Long-latency responses were seen less frequently to PM than to PP (Student's paired t test, $P < 0.05$) having the following incidence: at prod site PP 75 %, PM 45 %; one recording site away PP 71 %, PM 45 %; two recording sites away PP 64 %, PM 25 %; three recording sites away PP 27 %, PM 14 %. The mean amplitude of the response was smaller (ANOVA, $P < 0.05$) at recording sites further from the prod site for both PP and PM stimulation. There was no consistent change in latency of either reflex with either prod site or recording site.

The stronger and more frequently observed long-latency reflex to PP suggests that receptors responsible might lie in the supraspinous ligaments. The rather localised short-latency reflex suggests that mechanical deformation at a site remote from the prod is not evoking the long-latency reflex and that it may be mediated via the motor cortex. Further investigation using conditioning transcranial magnetic stimulation will clarify this.

Dimitrijevic, M.R. *et al.* (1980). *J. Neurol. Neurosurg. Psychiatr.* **43**, 1112–1118.

Solomonow, M. *et al.* (1998). *Spine* **23**, 2552–2562.

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All procedures accord with current local guidelines and the Declaration of Helsinki.

No effect of acute oral glutamine on glutathione status and lipid peroxidation during and in recovery from exercise

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Glutathione (GSH) is the major endogenous intracellular anti-oxidant system, which protects the cell against oxidative damage. GSH is synthesised predominantly in the liver from cysteine, glycine and glutamate (via glutamine); and it has been shown that 4 days intravenous glutamine (gln) elevated plasma GSH in rats (Denno *et al.* 1996). Our aim was to determine whether oral glutamine would alter total GSH status and hence the extent of oxidative damage during and in recovery from exercise.

Seven male untrained subjects (24 ± 1 years, 73.1 ± 3.5 kg, $\dot{V}_{O_2,max}$ 3.17 ± 0.28 l min^{-1}) completed two trials, with Ethical Committee approval, receiving either 3 ml kg^{-1} flavoured water (CON) or 0.125 g kg^{-1} gln in 3 ml kg^{-1} flavoured water (GLN). Overnight fasted subjects consumed a drink, then after 30 min rest cycled at 68.8 ± 1.9 % $\dot{V}_{O_2,max}$ for 45 min, and rested for a further 30 min. Blood and expired air samples were taken at baseline and regular intervals throughout. Whole blood was analysed for total GSH concentration (*OXIS* Research), and plasma was analysed for lipid hydroperoxides (LPO) (*OXIS* Research). Data are presented as means ± S.E.M., and were analysed by two-way repeated measures ANOVA.

Whole blood total GSH concentration did not differ between trials at any time point, although there was a significant elevation at the end of exercise relative to baseline in the CON trial (927.9 ± 46.8 *vs.* 1051.2 ± 50.4 μM, $P < 0.05$) but not GLN trial (969.5 ± 67.7 *vs.* 955.0 ± 90.9 μM). Except at the end of exercise

(6.0 ± 1.8, CON *vs.* 6.7 ± 1.4, GLN; both μM), plasma LPO concentration tended to be higher in the CON than the GLN trial throughout, particularly after 30 min recovery (6.2 ± 1.6, CON *vs.* 4.7 ± 1.7, GLN, μM).

In conclusion, a single bolus of oral glutamine did not enhance blood total GSH status, nor were significant differences in a marker of oxidative damage evident. Possible explanations are that glutamine does not limit biosynthesis of GSH in healthy human beings under conditions of exercise-induced metabolic stress, or that chronic glutamine supplementation may be required to achieve any such benefit.

Denno, R. *et al.* (1996). *J. Surg. Res.* **61**, 35–38.

All procedures accord with current local guidelines and the Declaration of Helsinki.

Post-exercise depression of corticospinal excitability is lateralised in human leg muscles

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Studies on the effects of strenuous exercise have clearly identified both peripheral and central components of fatigue (Bigland-Ritchie & Woods, 1984), which can limit voluntary muscle performance. Stimulation of the motor cortex using transcranial magnetic stimulation (TMS) and electromyographic (EMG) recordings have shown corticospinal excitability to be depressed following exercise (Brasil-Neto *et al.* 1993). We have further investigated central fatigue processes in a leg muscle and whether this fatigue spreads to the homonymous muscle in the contralateral leg.

With local ethical approval and informed consent seven healthy individuals (aged 21–42 years, 5 males) were recruited and seated in a reclining armchair. Surface electromyographic (EMG) recordings were made bilaterally from tibialis anterior (TA). Subjects maximally contracted their dominant TA muscle isometrically for as long as they could sustain (typically for 3–4 min) while keeping the other leg relaxed. TMS was applied using a MagStim 200 stimulator and an angled double-cone stimulating coil with its cross-over located over the vertex. Trials of six stimuli were conducted while relaxed (stimulus strength 1.2 × relaxed motor threshold) and during a 20% maximum voluntary contraction (MVC) in the dominant leg (stimulus strength 1.0 × relaxed motor threshold). Trials were conducted 5 and 10 min before exercise and at regular intervals up to 2 h post-exercise.

While relaxed, the mean (± S.D.) motor-evoked potential (MEP) areas in exercised leg were significantly (ANOVA, $P < 0.05$) depressed, relative to pre-exercise levels, in trials conducted 5 min (63.5 ± 23.7%), 10 min (58.9 ± 32.7%) and 15 min (61.4 ± 23.8%) post-exercise. MEP areas had returned to pre-exercise levels by the trial conducted 20 min post-exercise. No changes in MEP areas were seen in the non-exercised leg. MEPs recorded during voluntary activation showed no depression in either leg at any time after exercise.

Central fatigue processes appear to be lateralised to the exercised leg. When the exercised muscle underwent voluntary activation, post-exercise depression was not observed. Presumably the voluntary drive to produce a 20% MVC contraction countered any effect of post-exercise depression. This confirms that the post-exercise depression was a central rather than peripheral phenomenon.

Bigland-Ritchie, B. & Woods, J.J. (1984). *Muscle and Nerve* **7**, 691–699.

Brasil-Neto, J.P. *et al.* (1993). *Exp. Brain Res.* **93**, 181–184.

All procedures accord with current local guidelines and the Declaration of Helsinki.

High body core temperature does not impair activation of the human calf muscles during sustained maximal voluntary contraction

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Exercise-induced hyperthermia has been linked to failure to activate the exercised muscles during subsequent sustained isometric contractions (Nybo & Nielsen, 2001). We have examined whether hyperthermia is associated with failure of voluntary activation of human calf muscles under properly controlled conditions. We used passive heating in a hot water bath to elevate body core temperature to avoid the confounding effects of prior exercise on muscle function tests. Further, to control for temperature effects on muscle fatigue and afferent feedback we compared responses of both legs which were heated to different temperatures. This was achieved by supporting the lower leg of one limb above water level (WARM) whilst the other limb was immersed in the water (HOT).

Gastocnemius temperatures were measured by indwelling thermocouples and body core temperature by a rectal probe. Muscle function was assessed using standard equipment (Davies & White, 1982) from the decline of force during a 90 s isometric maximal voluntary contraction (MVC). Muscle activation level was assessed from EMG and the size of any additional force evoked by the interpolation of a supramaximal tetanic stimulus (300 ms, 100 Hz) delivered to the triceps surae at 30, 60 and 89 s. With local ethics committee approval, five male subjects were habituated to the procedures prior to entry to the trial. Control measures were performed on each leg at resting core temperatures. On another day subjects were instrumented and placed in a hot water bath to the level of the upper chest wearing a waterproof hooded jacket to cover the head, upper body and arms. This garment was worn throughout the subsequent testing period.

Hot water immersion elevated core temperature (mean ± S.D.) to 39.52 ± 0.27 °C and HOT leg temperature to 40.26 ± 0.30 °C just prior to testing and 39.17 ± 0.16 and 37.11 ± 0.22 °C, respectively, for the WARM leg which was tested last.

Figure 1 shows activation levels in control and hyperthermic contractions for both limbs. ANOVA revealed a significant time effect on activation level in control, HOT and WARM limbs ($P < 0.05$), but no effect of hyperthermia on voluntary activation and no difference between HOT and WARM.

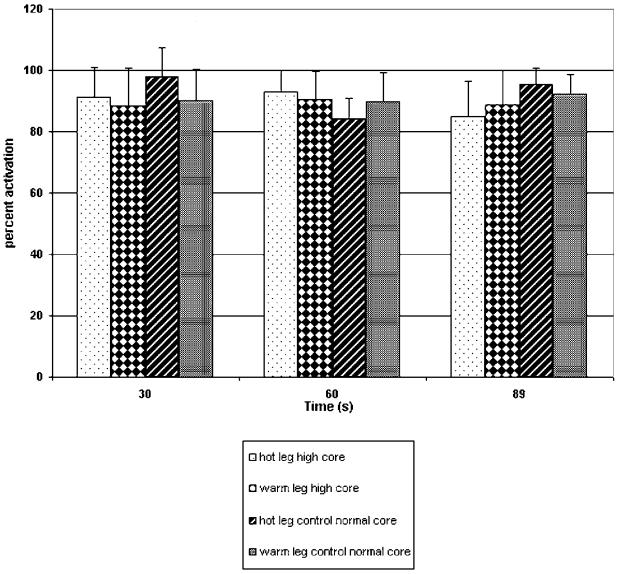

Figure 1. Mean (± S.D.) percentage maximal activation of left (HOT) and right (WARM) legs during MVCs sustained for 90 s. Test contractions performed at resting, core and muscle temperatures (control normal core) and at hyperthermic core and elevated muscle temperatures (HOT and WARM leg high core).

Our results indicate a small progressive decrease in the activation level of the calf muscles during a sustained MVC in both control and hyperthermic conditions, though at worst activation is still in excess of 84 % in control conditions and 85 % in hyperthermia. We conclude that passive elevation of body core temperature to hyperthermic levels and elevation of muscle temperature to above 40 °C has no significant effect on the ability of subjects to activate their previously passive calf muscles.

Davies, C.T.M. & White, M.J. (1982). *E. J. Appl. Physiol.* **49**, 255–269.
Nybo, L. & Nielsen, B. (2001). *J. Appl. Physiol.* **91**, 1055–1060.

All procedures accord with current local guidelines and the Declaration of Helsinki.

Kinetics of oxygen delivery at the onset of exercise in humans

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After local ethical approval, the kinetics of O_2 delivery ($\dot{Q}_{a,O_2}$) at exercise onset was determined on four men (age 25.5 ± 4.2 years, $\dot{V}_{O_2,max}$ 4.42 ± 0.65 l min^{-1}), who performed four rest-to-50 W and rest-to-100 W transitions on a cycle ergometer. Beat-by-beat cardiac output ($\dot{Q}$) was measured by the model flow method (Wesseling *et al.* 1993) from continuously recorded pulse pressure profiles, calibrated against the open circuit acetylene method (Barker *et al.* 1999) at rest and at the exercise steady state. The trials were superimposed and averaged. Arterial O_2 saturation (S_{a,O_2}) was measured by infrared oximetry, and the tracings superimposed and averaged. Blood haemoglobin concentration (Hb) was measured every minute and temporally aligned. Both parameters were interpolated by a 6th degree polynomial. Beat-by-beat $\dot{Q}_{a,O_2}$ was calculated as $\dot{Q} \times S_{a,O_2} \times Hb \times 1.34$ (O_2 binding coefficient). The half-times of the $\dot{Q}_{a,O_2}$ kinetics were 5.52 ± 2.46 and 7.33 ± 2.03 s in the rest-to-50 W and rest-to-100 W transients, respectively. The steady-state $\dot{Q}_{a,O_2}$ was proportional to power, and showed oscillations around its mean value. The time constant of the $\dot{Q}_{a,O_2}$ kinetics, as observed during light exercise, is lower than that reported for the $\dot{V}_{O_2}$ kinetics in conditions of no lactate accumulation during the exercise transient (Binzoni *et al.* 1992; di Prampero & Ferretti, 1999), indicating that the $\dot{Q}_{a,O_2}$ kinetics is faster than the $\dot{V}_{O_2}$ kinetics. This reflects the rapid $\dot{Q}$ response by the Frank-Starling mechanism (De Cort *et al.* 1991) and implies O_2 store changes during the exercise transient. The oscillations at steady state reflect the cardiorespiratory tuning of $\dot{Q}_{a,O_2}$.

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All procedures accord with current local guidelines and the Declaration of Helsinki.

Effect of glutamine and hyperoxia on oxygen uptake kinetics and muscle tissue deoxygenation during exercise

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The expansion of the tricarboxylic acid cycle intermediate (TCAi) pool during the transition from rest to exercise is enhanced by ingestion of glutamine (Bruce *et al.* 2001). However, there was no apparent reduction in substrate-level phosphorylation, and it may be that oxygen or acetyl group delivery is limiting. The aim of the present study was to determine the effect of glutamine upon pulmonary oxygen kinetics under conditions of normoxia and hyperoxia.

Four trained cyclists (aged 36 ± 4 years, $\dot{V}_{O_2,max}$ 5.3 ± 0.3 l min^{-1}; mean ± S.E.M.) completed four trials, each at least 10 days apart, with Ethical Committee approval. Subjects undertook a glycogen-depleting exercise protocol in the evening of day 1 and then consumed a low (30 %) carbohydrate diet (see Bruce *et al.* 2001). On day 2, overnight fasted subjects drank either 5 ml kg^{-1} placebo or 0.125 g kg^{-1} glutamine in 5 ml kg^{-1} water 1 h before exercise in normoxia (CON and GLN trials), or in hyperoxia (50 % F_{i,O_2}; HYP and HPG trials). Subjects cycled for 6 min at 70 ± 2 % $\dot{V}_{O_2,max}$ immediately before completing a performance test (time to complete an individualised set volume of work, equal to 4 min at 95 % W_{max}).

Breath-by-breath oxygen uptake data were obtained from a low inertia turbine and mass spectrometer system from which the time constant (τ) of the oxygen uptake kinetics and the oxygen deficit ($O_{2,def}$) were calculated. Leg muscle tissue deoxygenation (HbH) was assessed using near-infrared spectrometry (NIRS). NIRS data were expressed as a percentage (relative to range induced by exercise or ischaemia), normalised for power output and integrated between 0 and 6 min. Data were analysed using a Friedman test and are presented in Table 1.

Table 1. Measured variables

	CON	GLN	HYP	HPG
τ (s)	31 ± 4	28 ± 2	25 ± 2	28 ± 2
$O_{2,def}$	1.6 ± 0.3	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.2
τ %Hb 0–6 min (% J^{-1})	84 ± 14	95 ± 11	77 ± 9	98 ± 22
Performance time (s)	247 ± 12	243 ± 5	233 ± 5	230 ± 9

Values are means ± S.E.M. ($n = 4$).

Glutamine tended to reduce $O_{2,def}$ and τ (in normoxia) and increase HbH relative to respective controls, but no statistical differences were found between trials for any variable. This suggests that enhanced anaplerosis via glutamine supplementation has no significant bearing on oxidative metabolism either in normoxia or hyperoxia. It may be that acetyl group availability rather than oxygen delivery limits oxidative metabolism in the situation of an expanded TCAi pool.

Bruce, M. *et al.* (2001). *Am. J. Physiol.* **280**, E669–675.

All procedures accord with current local guidelines and the Declaration of Helsinki.

The time course of the decrease in maximal oxygen consumption during long-term bed rest in humans

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Maximal oxygen uptake ($\dot{V}_{O_2,max}$) decreases as a consequence of bed rest (Fortney *et al.* 1996; Convertino, 1997). However, the rate at which this decrease occurs has not been established yet. The opportunity of performing $\dot{V}_{O_2,max}$ determinations before and after bed rest periods of varying duration with the same protocols allowed us to make a step forward along this direction.

A total of 17 healthy young men were investigated in three programmes: four (age 22 ± 1.3 years) after a 15-day bed rest, seven (age 28 ± 1.0 years) after a 42-day bed rest and six (age 33 ± 1.8 years) after a 90-day bed rest. All these programmes were organised by the European Space Agency (ESA) either in Köln, Germany (2001) or in Toulouse, France (1994 and 2001). The data obtained in 1994 after a 42-day bed rest were published in a different context (Ferretti *et al.* 1997).

Individual $\dot{V}_{O_2,max}$ was determined during graded exercise on a bicycle ergometer. The oxygen uptake ($\dot{V}_{O_2}$) at the metabolic steady state was measured at rest and during exercises of increasing intensities. Starting from 50 W, power was progressively augmented by steps of 50 W, reduced to 25 W as the expected individual maximum power was approached. The duration of each work load was at least 5 min. Successive work loads were separated by 5 min recovery intervals, during which time blood samples (20 μl) were obtained from an ear lobe at 1, 3 and 5 min for determination of blood lactate concentration ([La]) by means of an electro-enzymatic method. Heart rate (HR) was determined continuously. $\dot{V}_{O_2}$ was measured either by the open circuit method (1994) or on a breath-by-breath basis (2001). Individual $\dot{V}_{O_2,max}$ was established from the plateau attained by the relationship between $\dot{V}_{O_2}$ and power above a given power.

The results are shown in Fig. 1 where $\dot{V}_{O_2,max}$, expressed relative to the values observed before bed rest set equal to 100 %, are plotted as a function of the bed rest duration. The same decrease was observed for maximal power. Maximal HR and maximal [La] were the same after as before bed rest in the three studies. The average rate of the decrease in $\dot{V}_{O_2,max}$ was 1.08 % day^{-1} for 15-day duration bed rest, 0.39 % day^{-1} for the 42-day duration bed rest and 0.32 % day^{-1} for the 90-day duration bed rest.

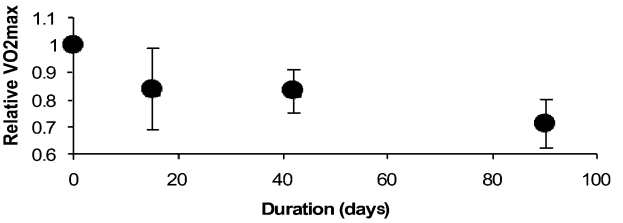

Figure 1. $\dot{V}_{O_2,max}$ evolution as a function of bed rest duration.

These results suggest that the decrease in $\dot{V}_{O_2,max}$ as a consequence of bed rest may tend to an asymptote. We postulate that this tendency may reflect the coupling of a more rapid cardiovascular deterioration with a slower reduction in muscle mass.

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Ferretti G. *et al.* (1997). *J. Physiol.* **501**, 677–686.

Fortney S.M. *et al.* (1996). *Handbook of Physiology. Environmental Physiology*, ed. Fregly, M.J. & Blatteis C.M., pp. 889–939. Oxford University Press, New York.

This study was supported by the Swiss National Science Foundation grant number 32-61780.00 to Guido Ferretti and by the Italian Space Agency to Carlo Capelli. The bed rest programmes were organised by the European Space Agency (ESA) in collaboration with the Centre National d'Etudes Spatiales (CNES) and the Institut de Médecine Spatiale (MEDES), France, the German Institut for Aerospace Medicine and the Japanese Space Agency (NASDA).

All procedures accord with current local guidelines and the Declaration of Helsinki.

EPR spectroscopic evidence for free radical outflow by contracting human skeletal muscle: significance of intracellular oxygenation

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Indirect markers of lipid peroxidation confined to the peripheral venous circulation and a reliance on unsuitable exercise models has previously complicated interpretation of the source and mechanisms associated with exercise-induced free radical generation. Therefore, the present study combined functionally isolated quadriceps exercise with electron paramagnetic resonance (EPR) and ^{1}H magnetic resonance spectroscopy to directly quantify free radical outflow by contracting skeletal muscle and examine implications of altered intracellular P_{O_2} (iP_{O_2}) and O_2 flux. Following ethical approval, five apparently healthy males aged 48 ± 25 years old (mean ± S.D.) performed 3 min of single-leg knee extensor exercise in normoxia at 25, 70 and 100 % of their previously established normoxic maximum work rate (WR_{MAX}). Blood flow ($\dot{Q}$) was assessed using a thermodilution technique (Andersen & Saltin, 1985) and

samples were collected from the femoral artery/vein and immediately mixed *ex vivo* with the spin trap, α-phenyl-*tert*-butylnitrone (PBN). Nuclear hyperfine splittings of resultant nitroxide adducts were consistent with the trapping of oxygen or carbon-centred free radical species ($a^N = 1.38 \pm 0.01$ mT and $a^H_\beta = 0.17 \pm 0.01$ mT). Exercise *per se* resulted in a clear venoarterial difference in PBN adduct concentration ($\Delta_{v\text{-}a}$), thus stimulating a net adduct outflow that was associated with leg $\dot{V}_{O_2}$ ($r^2 = 0.53$, $P < 0.05$, Pearson product moment correlation). However, Table 1 demonstrates that the magnitude of increase in $\Delta_{v\text{-}a}$ expressed in absolute and relative (normalised for $\dot{V}_{O_2}$) terms between 70 and 100 % WR_{MAX} (where iP_{O_2} remained invariant despite an increase in $\dot{V}_{O_2}$) was clearly less marked than that observed between 25 and 70 % WR_{MAX} (where iP_{O_2} was shown to decrease).

Table 1. Exercise intensity-dependent changes in PBN adduct concentration and net outflow

%WR_{MAX}	25	70	100
$\Delta_{v\text{-}a}$ (AU)	76 ± 64	331 ± 128*	370 ± 140
$\Delta_{v\text{-}a}/\dot{V}_{O_2}$ (AU l min^{-1})	376 ± 297	900 ± 673	661 ± 440
Net outflow (AU min^{-1})	142 ± 140	1166 ± 622*	1844 ± 757

Values are means ± S.D.; AU, arbitrary units; net outflow calculated as the product of $\dot{Q}$ and $\Delta_{v\text{-}a}$; *$P < 0.05$ compared with preceding exercise intensity (one-factor repeated measures ANOVA and *a posteriori* Bonferroni-corrected paired samples *t* tests).

These findings provide the first direct, quantitative evidence for free radical outflow from isolated contracting human skeletal muscle. Preliminary indications tentatively suggest that outflow may be regulated by a decrease in intracellular oxygenation and not merely a consequence of 'electron leakage' due to a mass action effect initiated by increased mitochondrial O_2 flux.

Andersen, P. & Saltin, B. (1985). *J. Physiol.* **366**, 233–249.

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All procedures accord with current local guidelines and the Declaration of Helsinki.

Relationships between left ventricular linear dimensions and body size variables: an MRI study in humans

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The ability to determine body size-independent data for linear left ventricular dimensions is important to facilitate group comparisons and construct appropriate normative data. Recently, the adoption of non-linear scaling has been supported both theoretically and empirically (George *et al*. 2001). However, potential inaccuracies in echocardiography and anthropometry have not been addressed. Therefore this study employed MRI as this has become the 'gold-standard' technique for both cardiac and body composition measurements. It is more accurate and reliable than standard echocardiography and has been validated against dissection and chemical analysis (Fowler *et al*. 1992).

After local ethical approval data were collected in 172 young adult male military recruits (age range 17–28 years) who were free of known cardiovascular disease. Multiple ECG-gated transverse slices through the heart were obtained. Maximal LV septal (LVSWT) and posterior wall thickness (LVPWT) as well as LV internal dimension at end-diastole (LVIDd) were obtained at the level of the mitral valve by digitisation. Forty transaxial spin echo image slices (TE40, TR500, field of view 45 × 45 cm, slice thickness 10 mm) were obtained of the whole body. Adipose tissue and thus fat mass was measured by an automated technique and was then subdivided into various fat compartments (e.g. subcutaneous fat mass). This allowed the calculation of fat-free mass (FFM). Height (HT), body mass (BM) and body surface area (BSA) were determined anthropometrically. Log–log least-squares linear regression analyses were performed to determine the slope exponents (b) for all relationships between linear dimensions and body size/composition variables. All data are means ± 95 % confidence intervals.

Geometric consistency was confirmed for LVSWT-FFM ($b = 0.29 \pm 0.07$), LVPWT-FFM ($b = 0.27 \pm 0.09$) and LVSWT-BM ($b = 0.27 \pm 0.07$), whereas LVIDd-FFM ($b = 0.19 \pm 0.09$), LVPWT-BM ($b = 0.24 \pm 0.08$) and LVIDd-BM ($b = 0.22 \pm 0.07$) did not quite reach the geometrically consistent value of 0.33. Exponents for HT were not geometrically consistent (1.0) and demonstrated greater variability ($b = 0.03 \pm 0.03$ to 0.34 ± 0.24). Consequently, exponents for BSA were not geometrically consistent. Exponents for all indices of adiposity were small but positive ($b = 0.03 \pm 0.02$ to 0.07 ± 0.02).

This study lends support to previous echocardiographic data that $FFM^{0.33}$ can be used in scaling procedures to determine a body size-independent index for LV wall thickness. The use of $HT^{1.0}$ and $BSA^{0.5}$ for scaling of LV linear dimensions is not supported by the current data. These data should be substantiated by further research.

Fowler, P. *et al.* (1992). *Am. J. Clin. Nutr.* **56**, 7–13.

George, K.P. *et al.* (2001). *Clin. Sci.* **100**, 47–54.

S. Myerson was supported by a British Heart Foundation Fellowship.

All procedures accord with current local guidelines.

Immunolocalisation of the $P2Y_1$, $P2Y_2$ and $P2Y_4$ receptor subtypes in human eccrine sweat glands

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Thermoregulatory sweating in humans is known to be primarily a function of human eccrine sweat glands within the skin. The purinoceptor agonist ATP has been shown to induce sweating (Sato *et al*. 1991) and increase intracellular calcium in both freshly dissociated cells (Clunes *et al*. 1999) and primary culture cells from human glands (Bovell *et al*. 2000). These effects have been ascribed to the presence of purinoceptors of the P2Y class. There are five subtypes of the P2Y family (Burnstock, 1997); however, to our knowledge the subtype(s) present in human sweat glands has never been investigated. Therefore, immunohistochemistry was employed to investigate the presence and localisation of the $P2Y_1$, $P2Y_2$ and $P2Y_4$ receptor subtypes in normal eccrine sweat glands.

Axillary skin biopsies were obtained, with informed consent and local medical ethical committee approval, from both male and female patients with no apparent skin disease. Samples were fixed and processed using standard techniques. Immunohistochemical staining was performed using rabbit antibodies raised against $P2Y_1$, $P2Y_2$ and $P2Y_4$ (Alomone Labs, Israel) employing the avidin-biotin complex (ABC) procedure (Vector Labs, UK). Sections were counterstained with haematoxylin, dehydrated, cleared, mounted and viewed using light microscopy.

In all samples analysed, $P2Y_1$, $P2Y_2$ and $P2Y_4$-like immunoreactivity elicited the same pattern of apical membrane staining in the reabsorptive duct of the eccrine gland ($n = 5$). Citrate buffer antigen retrieval in addition revealed nuclear staining of both layers of ductal cells with $P2Y_2$ antibody ($n = 6$), which was not found with $P2Y_1$ and $P2Y_4$. The secretory coil was shown to contain highly specific staining of the outer layer of myoepithelial cells with both $P2Y_2$ and $P2Y_1$ ($n = 11$ and $n = 6$, respectively), whereas $P2Y_4$ staining was localised to a single cell type resembling that of the clear cells of the secretory coil ($n = 5$). Pre-absorption of antibodies with the appropriate control peptide abolished all staining.

Regulation of sweat secretion has always been attributed to the appropriate receptor occupancy on the basolateral membrane; however, the presence of apical purinoceptors in the eccrine sweat gland is suggestive of apical regulation of sweat secretion and absorption, as has been shown to be important in colonic epithelia (Cliff & Frizzell, 1990). These results imply that apical purinoceptors in the duct may play a role in the control of sweat reabsorption. The clear cells of the secretory coil are thought to be predominantly involved in sweat secretion and the localisation of the $P2Y_4$ subtype in these cells would suggest that this receptor is implicated in sweat secretion. The myoepithelial cells are not regarded as being involved in either secretion or reabsorption of sweat but are instead thought to provide support for the secretory coil during secretion. The presence of purinoceptors suggests that the support these cells provide may also be activated by purinergic agonists.

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Burnstock, G. (1997). *Neuropharmacol.* **36**, 1127–1139.

Cliff, W.H. & Frizzell, R.A. (1990). *Proc. Natl Acad. Sci. USA* **87**, 4956–4960.

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Sato, K. *et al.* (1991). *J. Am. Acad. Dermatol.* **24**, 1010–1014.

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All procedures accord with current local guidelines.

The effect of head-up and head-down tilt on lung function

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Body posture affects lung function. Supine values have been shown to be lower in comparison with sitting or standing (Lalloo *et al.* 1991; Meysman & Vincken, 1998). Few studies have examined postures between these positions (Manning *et al.* 1999). The aim of this study is to see the effect of head-up (HU) and head-down (HD) postures on lung function.

Eight healthy Caucasian subjects (7 males, 1 female) participated in a study approved by the local ethics committee and had their lung function measured in six postures: −40 and −20 deg HD, 0 deg supine, and 20, 40 and 60 deg HU). Their mean ± S.D. age was 22.9 ± 3.52 years, height 1.76 ± 0.48 m and weight 76.7 ± 11.15 kg.

A motorized tilt table was used to alter postures and an electronic spirometer (Microlab 3300) was used to record the respiratory indices. The forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and peak expiratory flow (PEF) were measured. Subjects were placed for 10 min at each posture and performed a maximal inspiration followed by maximal expiration three times and the highest values of the indices were used for analysis. Differences in lung function between postures were examined by one-factor repeated measures ANOVA and Bonferroni-corrected paired samples *t* tests ($P < 0.05$).

We found an increase in lung function indices from supine (mean ± S.E.M.; $FEV_1 = 3.97 \pm 0.14$ l, FVC = 4.83 ± 0.23 l, PEF = 8.47 ± 0.62 l s^{-1}) to HU postures (PEF, FVC and FEV_1 increased by 11.9, 7.7 and 6.8 %, respectively). A decrease in lung function was found from supine to HD (PEF, FVC and FEV_1 decreased by 11.6, 4.8 and 6.5 %, respectively). Angle of tilt significantly affected FEV_1, FVC and PEF ($P < 0.005$). Comparison of supine values with the other five postures showed a significant difference for FEV_1, FVC (except for 20 deg HU) and for PEF (except for 20 and 40 deg HU and 20 deg HD). There was a strong correlation between posture and FVC (individual *r* range = 0.84–0.98, mean 0.96), FEV_1 (individual *r* range = 0.84–0.98, mean 0.98) and PEF (*r* range = 0.27–0.97, mean 0.98).

Our results suggest that changes in lung function are directly proportional to posture (angle of tilt) in degrees and that these changes may be due to changes in intrathoracic blood volume produced by tilting.

Lalloo, U.G. *et al.* (1991). *Respiration* **58**, 122–125.

Manning, F. *et al.* (1999). *Physic. Ther.* **79**, 456–466.

Meysman, M. & Vincken, W. (1998). *Chest* **114**, 1042–1047.

All procedures accord with current local guidelines.

Responses of forearm blood flow to neck suction and neck pressure in humans

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The application of positive and negative pressures to the neck has been used to study reflex changes in forearm blood flow following stimulation of the carotid sinus baroreceptors. However, the results of such investigations have been equivocal. Increases (Lindblad *et al.* 1982), decreases (Ernsting & Parry, 1957; Duprez *et al.* 1987; Ebert, 1982), or no change (Bevegard *et al.* 1977; Escourrou *et al.* 1993) in forearm blood flow or forearm vascular resistance have been reported during carotid sinus baroreceptor stimulation. The present study assessed forearm blood flow responses to a wide range of positive and negative pressures applied to the carotid sinus regions of the neck.

With ethical approval (DMU, Bedford), twelve normotensive subjects (5 male, 7 female) with mean (± S.D.) systolic blood pressure (SBP) 118 ± 15 mmHg and diastolic blood pressure (DBP) 64 ± 8 mmHg, had suction at −12, −24, −36, −48 and −60 mmHg, and pressure at 10, 20 and 30 mmHg applied to the carotid sinus region of the neck. Each pressure level was repeated

five times, with approximately 15 s between each stimulus. During an end expiration breath hold, five ECG R waves were recorded, after which an electronic solenoid was activated to initiate the pressure change within the neck chamber and the simultaneous inflation of an upper arm venous occlusion cuff. Pressure change was sustained while a further three ECG R waves were recorded, for a total of 8 s. Forearm blood flow (FBF) was estimated from changes in forearm circumference during venous drainage occlusion. experiments were carried out at a consistent lab temperature of 22°C.

Mean (± S.D.) FBF (in ml dl^{-1} min^{-1}), did not change when the carotid sinus baroreceptors were stimulated by negative pressures (2.68 ± 0.77 at −12 mmHg *vs.* 2.60 ± 0.69 at −24 mmHg *vs.* 2.69 ± 0.69 at −36 mmHg *vs.* 2.54 ± 0.82 at −48 mmHg *vs.* 2.68 ± 0.89 at −60 mmHg), or by positive pressures (2.74 ± 1.55 at +10 mmHg *vs.* 2.66 ± 0.71 at +20 mmHg *vs.* 2.43 ± 0.60 at +30 mmHg) when compared with a control value of 2.57 ± 0.61 ml dl^{-1} min^{-1} ($P > 0.05$ with repeated measures ANOVA). Negative neck pressures induced increases in the change in R–R interval (72 ± 94 ms at −12 mmHg *vs.* 130 ± 104 ms at −24 mmHg *vs.* 177 ± 106 ms at −36 mmHg *vs.* 218 ± 102 ms at −48 mmHg *vs.* 254 ± 115 ms at −60 mmHg), whereas positive neck pressures did not affect the change in R–R interval.

Given the expected response in R–R interval following application of positive and negative pressures to the carotid sinus region, the lack of any changes in FBF may suggest that these baroreceptors do not influence forearm vascular resistance during this type of stimulation.

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Lindbland, L. *et al.* (1982). *J. Auton. Nerv. Syst.* **5**, 373–379.

All procedures accord with current local guidelines and the Declaration of Helsinki.

The effect of active warm-up on surface EMG power spectrum and muscle performance in healthy humans

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An increase in muscle conduction velocity (CV), which has been shown to increase with muscle temperature (Jarcho *et al.* 1954), could be one of the factors determining the improvement in muscle performance following a rise in temperature. CV has been shown to correlate to the median frequency (MDF) of the surface EMG (sEMG) power spectrum (Lindstrom *et al.* 1970). The aim of this study was to observe the effect of increasing quadriceps muscle temperature on maximum voluntary contraction (MVC), peak power output and sEMG variables.

With ethics committee approval, eight volunteers (two female) (mean ± S.D.; age 22 ± 4 years; mass 72.5 ± 9.9 kg; height 1.73 ± 0.06 m) completed two trials (counter-balanced) on the same day, one control (CO), and the other preceded by an active cycling warm-up (WU) at 70 % ventilatory threshold (ventilatory equivalent method). Quadriceps muscle temperature, measured continuously from vastus lateralis with a flexible thermistor (Ellab, UK), was 33.8 ± 0.4 °C in CO compared with 36.8 ± 0.5 °C in WU ($P < 0.05$). Aural temperature, measured by an infrared tympanic thermistor (Braun, type 6013, Germany), was not different between conditions. Experimental trials consisted of three knee-extension MVCs (Kin-Com, USA) at a 90 deg angle with simultaneous recording of sEMG from the vastus lateralis, followed by three squat jumps performed on a force platform (Kistler, Switzerland). sEMGs were analysed in the frequency domain as MDF and in the time domain as root mean square (RMS). All data were compared using a Student's paired *t* test, with an α level of 0.05.

MDF was 59.2 ± 14.1 Hz in CO compared with 67.2 ± 11.8 Hz in WU ($P < 0.05$), while RMS was lower in CO compared with WU (0.65 ± 0.28 *vs.* 0.56 ± 0.19 V, respectively; $P < 0.05$). MVC was not different (465.7 ± 107.6 *vs.* 490.1 ± 117.2 N), whilst peak power output during the squat jump was significantly higher in the WU trial (3324 ± 866 *vs.* 3569 ± 919 W; $P < 0.05$).

These data show MDF to be altered with increased temperature, which would relate to a greater CV. This may translate into faster activation of individual muscle fibres, thus partly explaining the increase in power output. The increased MDF and the decreased RMS in WU may reflect a smaller amplitude and shorter duration of the action potential, possibly because of a decreased time for Na^+ diffusion into the cell (Rutkove, 2001).

Jarcho, L.W. *et al.* (1954). *Am. J. Physiol.* **178**, 129–134.

Lindstrom, L. *et al.* (1970). *Electromyography* **4**, 341–356.

Rutkove, S.B. (2001). *Muscle Nerve* **24**, 867–882.

All procedures accord with current local guidelines.

Stress response to voluntary and involuntary breath-holding in humans

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A number of stressors (e.g. cold pressor, mental arithmetic, breath holding) have been used to investigate the cardiovascular response to stress (Herd, 1991). Blood pressure (BP) has been found to increase but heart rate (HR) responses have been found to vary with the stressor type (commonly a decrease).

Voluntary breath-holding (VBH) has been used as a stressor but the effect of involuntary breath-holding (IBH), requiring a mechanical shutter to occlude breathing, has not been reported.

The aim of this study was to investigate the effect of normal inspiratory VBH and IBH on BP and HR. We expected that IBH would produce a bigger stress response than VBH.

With local ethics committee approval, twenty-four healthy Caucasian student volunteers (12 males, 12 females), mean ± S.D. age 21 ± 2.57 years, height 1.68 ± 0.08 m, weight 69 ± 0.03 kg were tested wearing a noseclip in the seated position. Each completed three VBH and three IBH testings, with a minute's rest after each breath hold. Basal measurements of BP using an automatic sphygmomanometer and HR using an electro-cardiograph were taken before each breath hold. Measurements were taken in the first 30 s of breath holding.

Differences in mean data (basal *versus* VBH or IBH) were analysed at the 95 % significance level using a Student's paired *t* test where $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Basal measurements (mean ± S.E.M.) of systolic blood pressure (SBP), diastolic blood pressure (DBP) and HR were 112.2 ± 2.7 mmHg, 73.8 ± 1.4 mmHg and 71.7 ± 2.6 beats min^{-1}, respectively, and these increased during VBH to 119.6 ± 3.2 mmHg***, 78.4 ± 2.3 mmHg** and 74.0 ± 2.9 beats min^{-1}, respectively.

Basal measurements of SBP, DBP and HR were 112.1 ± 2.5 mmHg, 73.2 ± 1.4 mmHg and 72.0 ± 2.7 beats min^{-1}, respectively, and these increased during IBH to 115.9 ± 3.2 mmHg*, 78.4 ± 1.9 mmHg*** and 72.9 ± 2.7 beats min^{-1}, respectively.

Both VBH and IBH increased blood pressure but not heart rate. The change in SBP and DBP between VBH and IBH did not significantly differ ($P = 0.07$ and 0.68, respectively).

Our findings suggest that blood pressure and heart rate responses were not affected by stressor type.

Herd, J.A. (1991). *Physiol. Rev.* **71**, 305–330.

All procedures accord with current local guidelines.

Age-related differences in the response of humans to walking speed descriptors

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Brisk walking is one of the recommended forms of exercise for older adults to achieve physiological benefit (NHS Scotland, 2001). Although the proportion of those reporting their walking speed as 'brisk' declines with increasing age (Skelton *et al.* 1999), it is not clear whether old age alters the response to this walking speed descriptor. The purpose of the study was to test this hypothesis.

The Lothian Research Ethics Committee approved the study. Healthy elderly (75–83 years, $n = 9$) and healthy young (20–23 years, $n = 9$) female volunteers walked 150 m around an indoor 50 m circuit, in response to standardised walking speed instructions which required the subject to complete two separate walks at each of a 'slow', 'comfortable', 'brisk', and 'fast' pace. Each of the eight walks was timed and absolute ($m\ s^{-1}$) and relative (% maximum) walking speeds were calculated. Maximal walking speed was measured separately over 10 m, from a walking start. Absolute oxygen cost ($ml\ kg^{-1}\ min^{-1}$) was measured by an ambulatory system (Metamax 3B, Cortex Biophysik). Relative oxygen cost ($\%\dot{V}_{O_2,max}$) was calculated from $\dot{V}_{O_2,max}$ measured during progressive treadmill walking on two separate occasions (mean $\dot{V}_{O_2,max}$: young volunteers, 38.55 ± 2.19 $ml\ kg^{-1}\ min^{-1}$; elderly volunteers, 20.40 ± 4.37 $ml\ kg^{-1}\ min^{-1}$; means ± S.D.). A test was defined as maximum upon the investigators' subjective assessment that the individual could not continue, plus a respiratory exchange ratio of ⩾1.10 (young volunteers) or ⩾ 1.00 (elderly volunteers). The responses to 'brisk' walking are reported below. Data were analysed using Student's unpaired *t* tests.

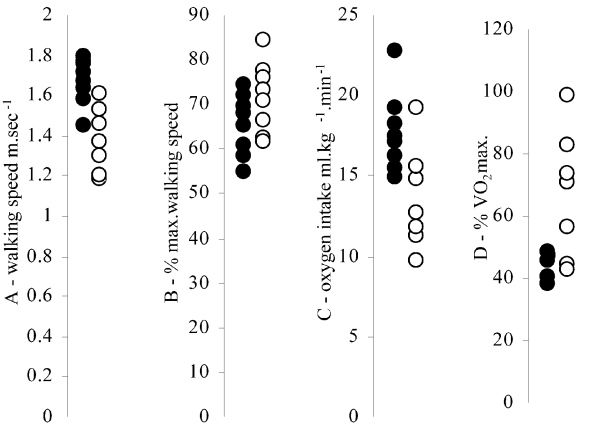

Figure 1. Responses to 'brisk' walking in young (●) and elderly (○) volunteers.

The elderly volunteers walked at a significantly slower mean absolute walking speed ($1.37\ m\ s^{-1}$ (S.D. = 0.156) elderly, $1.68\ m\ s^{-1}$ (0.11) young; $P < 0.001$, Fig. 1A) but at a similar proportion of maximal walking speed (70.48% (8.04) elderly, 66.29% (6.76) young; $P = 0.248$, Fig.1B). Although the mean absolute $\dot{V}_{O_2}$ was significantly lower in the elderly group ($13.6\ ml\ kg^{-1}\ min^{-1}$ (2.94) elderly, $17.48\ ml\ kg^{-1}\ min^{-1}$ (2.41) young; $P = 0.008$, Fig. 1C), $\%\dot{V}_{O_2,max}$ was significantly higher (67.15% (20.58) elderly, 45.05% (4.48) young; $P = 0.026$ ($n = 7$ elderly, $n = 6$ young), Fig. 1D).

The elderly group walked at a similar (mean) proportion of maximal speed (70 %) when compared with the young group (66 %), but at a higher (mean) relative cost (67 % $\dot{V}_{O_2,max}$ elderly, 45 % $\dot{V}_{O_2,max}$ young). This 'cost' difference should be acknowledged when choosing walking speed instructions for training and rehabilitation programmes for elderly people.

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All procedures accord with current local guidelines and the Declaration of Helsinki.

Stability of human corticospinal excitability and grip force over a 24 h period

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A number of studies suggest that the maximum voluntary grip force produced in the hand varies throughout the day; typically being low in the morning and high in the evening (see, for example, Callard *et al.* 2000). In this study we have measured maximum voluntary contraction (MVC) and several indices of corticospinal excitability over a 24 h period.

With local ethical approval and informed consent, six healthy subjects (mean ± S.E.M. age 47.3 ± 6.1 years; 5 males; 2 left handed) took part in this study. Subjects were investigated every 3 h between 09.00 h and 06.00 h the following morning and were not permitted to sleep. In each session electromyographic (EMG) recordings were made from the thenar muscles using self-adhesive surface electrodes; the skin was marked so that the electrodes could be placed accurately in subsequent sessions. Transcranial magnetic stimulation (TMS) of the motor cortex was achieved using a MagStim 200 stimulator connected to a 9 cm circular stimulating coil centred on the vertex. Ten stimuli were delivered at a strength of 1.2 × threshold (T) with the muscle relaxed and further trials of ten stimuli were delivered at strengths of 1.2 T, 1.0 T and 0.8 T whilst the subject maintained a 10% MVC contraction. Latency, duration and area of the motor-evoked potentials (MEPs) were measured. In trials with the muscle contracted, the duration and extent of inhibition during the silent period were measured. MVC was measured in the thenar muscles (highest of 3 readings) of the dominant hand.

Data from the recording sessions were compared over time (ANOVA). MVC of the thenar muscle did not change over the 24 h ($P > 0.05$). The mean areas, latencies and durations of MEPs did not change ($P > 0.05$) over the 24 h test period with the muscle relaxed or contracted. Furthermore, the extent and duration of the silent period seen after the MEP in the contracted muscle did not change ($P > 0.05$) over the 24 h of the experiment at any stimulus intensity.

Previous work (Manganotti *et al.* 2001) during sleep deprivation found no differences in MEP amplitude, or duration of the silent period over the 24 h test period, except at a stimulus strength 1.3 T when they found a small increase in the duration of the silent period at 03.00 h. We have further shown that the magnitude of the silent period (relative to background EMG) does not change over 24 h. This study eliminates time of day as a significant variable to scientists planning future electrophysiological investigations of corticospinal function.

Callard, D. *et al.* (2000). *Chronobiol. Int.* **17**, 693–704.

Manganotti, P. *et al.* (2001). *Neurosci Lett.* **304**, 153–156.

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