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The 'tight-fit' brain; an anatomical risk factor for hypoxic headache?

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Acute exposure to inspiratory hypoxia is an established protocol for the induction of neurovascular headache in the otherwise healthy human brain and is responsible for other vegetative symptoms collectively known as acute mountain sickness (AMS). Though brain swelling may play a role, the lack of any consistent relationship between volumetrics and headache intensity suggests that additional intracranial 'risk' factors may be involved. The inability to accommodate brain swelling subsequent to insufficient intracranial volume reserve (IVR) may predispose to hypoxic headache (HH) and thus by consequence, AMS (Ross, 1985). To examine this, 20 healthy subjects were examined in normoxia (PRE-NORM), following 16h passive exposure to 12% O_2 (HYP) and after 6h recovery in normoxia (POST-NORM). MR-images were acquired on a 1.5T scanner using a standard head coil and T1-weighted gradient-echo sequences were applied across the whole brain to the level of the foramen magnum. Volumetric changes were processed using SIENA software. IVR was calculated as the ratio of brain volume to intracranial volume (BV:ICV). HH was assessed using a visual analogue scale and clinical AMS diagnosed according to established guidelines (Bailey *et al.* 2005).

Ten subjects (50%) were diagnosed with clinical AMS and corresponding headache scores increased more markedly (HYP minus PRE-NORM) compared to the healthier control subgroup (AMS+*ve*: +42 ± 18 *vs.* AMS-*ve*: +15 ± 14mm, $P < 0.05$, independent samples *t* test). While the increase in BV was not selectively different (AMS+*ve*: +8.3 ± 5.1 *vs.* AMS-*ve*: +5.7 ± 4.3mL, $P > 0.05$), IVR was consistently lower in AMS as indicated by an elevated BV:ICV (Fig. 1).

These findings implicate IVR as an anatomical risk factor for HH and AMS. The 'tight-fit' brain may predispose to mechano-chemical activation of pain-sensitive structures during hypoxic brain swelling.

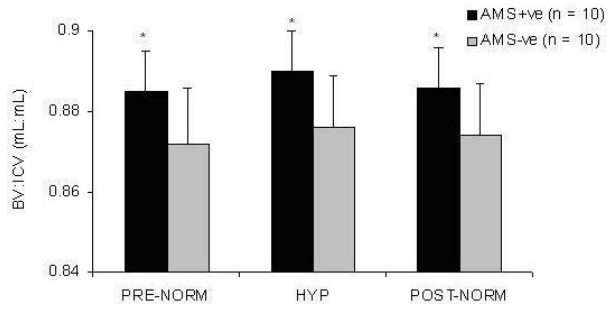

Figure 1. Lower IVR in AMS. Values are mean ± SD; main effect (AMS+ve > AMS-ve, P < 0.05, two-way repeated measures analysis of variance); *different vs. AMS-ve (P < 0.05).

Bailey DM *et al.* (2005). *J Cereb Blood Flow Metab* **26**, 99-111.

Ross RT (1998). *Lancet* **8435**, 990-991.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C38

Psychological skills training improves exercise performance in the heat

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Proposed mechanisms for early fatigue in the heat include changes in dopamine, serotonin and interleukin-6 (IL-6), exceeding critical thresholds in core temperature and impaired voluntary neuromuscular recruitment. Psychological factors leading to an urge to reduce exercise intensity or stop have received less attention: few interventions designed to improve performance in the heat (rehydration drinks, glucose mouth washes) have considered effects of psychological factors. We tested the hypothesis that a 5-day Psychological Skills Training (PST) package (Thelwell & Greenlees, 2003) would increase the distance covered before exhaustion during treadmill running in tropical heat.

Eighteen healthy male participants were studied: a control group (CG; n=8; mean (s.d.) age 28 (5)yrs, height 1.73 (0.04)m, mass 72.83 (6.74)kg; % body fat 16.3 (3.24), $\dot{V}O_2$max 63.49 (6.17) ml kg min^{-1}); an intervention group (PST, n=10; age 23(3) yrs, height 1.77 (0.05)m, mass 69.31 (6.06)kg, % body fat 14.38 (1.96), $\dot{V}O_2$max 67.06 (4.49)ml kg min^{-1}). Subjects completed three 90-min treadmill runs (R) at 30.06°C (0.21) (41.40% (5.01) relative humidity); runs were separated by ≥4 days. During runs, subjects ran as far as possible, without feedback of time or distance.

Subjects were assigned to CG or PST based on matched variability between R1 and R2 (km). PST over 5 days included goal setting, mental imagery, arousal regulation and positive self-talk aimed at improving distance run in R3. CG undertook R3 without any PST. Blood IL-6 and prolactin, an index of serotonergic activity, were measured. Core temperature (aural; T_{au}), mean skin temperature ($T_{skin} = 0.3\,(T_{chest} + T_{arm}) + 0.2\,(T_{thigh} + T_{calf})$ and heart rate (HR) were recorded each minute whilst running. Rating of perceived exertion (RPE) was recorded every 15 min (Table 1).

The hypothesis is supported; PST group ran significantly further (1.15km; 8%) in R3 compared to R2 ($p = 0.002$) or R1 ($P = 0.006$; ANOVA). This occurred despite a high RPE, and not via any of variables measured, which did not differ ($P > 0.05$). CG distance run was unchanged. The improvement in performance in the PST group puts into context results of other studies that do not employ a 'blind' experimental design. Psychological factors may underpin some of the improvement seen with unmasked interventions when subjects exercise in the heat.

Table 1. Physiological responses to exercise in the heat

Variable	R1	R1	R2	R2	R3	R3
Group	CG	PST	CG	PST	CG	PST
T_{tu} (°C)	38.29 [0.57]	38.60 [0.32]	38.38 [0.67]	38.68 [0.48]	38.53 [0.45]	38.77 [0.49]
T_{skin} (°C)	33.83 [1.13]	34.17 [1.00]	34.20 [0.73]	34.69 [0.81]	33.54 [1.40]	34.72 [0.73]
IL-6 (pg/ml) post exercise	14.15 [4.65]	14.96 [5.40]	15.02 [5.82]	17.76 [7.87]	16.88 [5.21]	14.78 [5.17
Prolactin (ng/ml) post exercise	17.50 [8.00]	22.17 [10.52]	12.05 [5.48]	15.40 [8.05]	19.14 [6.57]	13.98 [5.64]
RPE	16.25 [2.12]	16.60 [2.07]	16.81 [2.45]	16.60 [2.50]	17.00 [2.62]	17.20 [2.30]
HR (b.p.m.)	147.15 [11.99]	148.92 [11.03]	146.04 [9.90]	151.72 [12.68]	147.69 [12.41]	154.25 [12.07]
Distance run (km)	16.23 [1.59]	15.35 [1.73]	16.50 [1.58]	15.20 [1.94]	16.63 [1.20]	16.35 [1.46]

Values are mean [SD].

Thelwell RC & Greenlees IA (2003). The Sport Psychologist 17, 318-337.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C39

A new model for describing ventilation during submaximal exercise in healthy men and men with chronic lung disease

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Ventilation during submaximal exercise ($\dot{V}$E,ex) can be represented as the sum of the ventilations per min of (1) anatomical deadspace, (2) any pathological tidal deadspace in continuity with airways and (3) alveoli that participate in gas exchange. The last component is increased by uneven lung function ($\dot{V}$A/$\dot{Q}$ inequality). These causes of increased ventilation have been attributed collectively to a 'physiological deadspace effect' [1] or a distribution of $\dot{V}$A/$\dot{Q}$ ratios [2]. In the present model the ventilation under standard conditions ($\dot{V}$E,ex_{st}) is partitioned into components that are respectively proportional to and independent of respiratory frequency, represented by two coefficient terms in a multiple regression equation.

$\dot{V}$E,ex_{st} = a. standardised CO_2 output + b. standardised respiratory frequency + c. $\dot{V}$E,ex_{st} and standardised CO_2 output were at the O_2 uptake of 1.0 l min^{-1} (45 mmol min^{-1}), and standardised respiratory frequency at ventilation 30 l min^{-1} (designated fR_{30}). The corresponding tidal volume is 30/fR_{30}, hence the fR_{30} reflects the pattern of breathing.

Subjects were 136 working shipyard tradesmen [3] and 69 applicants for respiratory disability benefit who had impaired function.

Lung function and responses to progressive exercise were by standard methods. Indices under standard conditions were by interpolation. Stepwise multiple regression analysis (SPSS) was performed, with terms that were not significant (p>0.05) eliminated progressively.

In the tradesmen, terms for age and smoking did not contribute to the regression. The presence of wheeze did contribute and was allowed for. The values for 'a','b' and 'c' were respectively 17.1, 0.19 l and 4.48 l min^{-1}; 'a' and 'b' were independent of each other (p<0.01). The residual standard deviation (RSD) about this equation (Eqn 1) was 2.02 l min^{-1}. For pooled data from applicants 'a','b','c' and RSD were 40.1, 0.45, -11.4 and 7.14 (Eqn 2). All coefficients were significant (p< 0.01). 'a' differed between the two equations (p< 0.01). For 'b' the probability of a real difference was p=0.10.

A two compartment model is realistic, precise and can be used to deconstruct $\dot{V}$ E,ex_{st}. Eqn 1 gives the parameters for working men. They can provide an expected (reference) value for $\dot{V}$ E,ex_{st} at a man's own standardised CO_2 output and fR_{30}. Eqn 2 illustrates the application of the model to data for men with abnormal lung function. The method can be used to estimate the relative contributions of uneven alveolar ventilation, tidal deadspace and pattern of breathing to an increased $\dot{V}$ E,ex_{st} and hence to breathlessness in different chronic respiratory disorders.

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Dr DJ Chinn kindly provided technical support.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C40

Greater PCr utilisation in single human type IIA muscle fibres during the development of maximal power output at higher muscle temperatures

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We have previously shown that the increased power output observed after passive elevation of muscle temperature (Tm) occurs due to a greater rate of anaerobic ATP turnover (Gray et al. 2006). We also hypothesised that this increase in power was due to a greater contribution to power generation from type IIA fibres. The aim of the present study was to investigate this by examining PCr utilisation in single muscle fibres, characterised according to their myosin heavy chain (MHC) content. Six male volunteers (age 25 ± 6 years, height 1.82 ± 0.07 m, mass 77 ± 11 kg; mean ± S.D.) performed a 6 s maximal sprint on a cycle ergometer under control (C, Tm= 34.3 ± 0.6°C) and heated (H, Tm= 37.3 ± 0.2°C) conditions as described previously (Gray et al. 2006). Biopsies were taken from the vastus lateralis prior to and immediately after exercise. Freeze-dried single muscle fibres were dissected and analysed for MHC content (SDS-PAGE) and PCr concentration (Wibom et al. 1991). Fibres were classi-

fied as: type I, I-IIA, IIA, IIAX25 (fibres containing 1-25% type IIX MHC isoform), IIAX50 (26-50% IIX), IIAX75 (51-75% IIX) and IIAX100 (76-100% IIX). Statistical analyses were performed using two-way repeated measures ANOVA and Bonferroni corrected paired t-tests where appropriate.

The warming increased maximal power output by 258 ± 110 W compared to C. Resting PCr content was not affected by the elevation of muscle temperature in any fibre group. There was a 9% greater ($P<0.05$) decrease in PCr content, after exercise, in MHC IIA fibres in H ($\Delta PCr = 57.6 \pm 11.6$ mmol kg $^{-1}$ (dm)) compared to C ($\Delta PCr = 53.3 \pm 13.5$ mmol kg $^{-1}$ (dm)).

We have demonstrated a greater PCr utilisation in single fibres containing 100% MHC IIA during the development of maximal power output when muscle temperature is elevated.

Table 1. PCr concentration in MHC characterised single muscle fibres before and after a 6 s sprint under control and heated muscle temperature conditions

	Control		Heated	
MHC composition	Rest	Post-exercise	Rest	Post-exercise
I	67.6 ± 12.1	34.1 ± 10.0	72.5 ± 16.8	30.7 ± 6.5
I-IIA	70.4 ± 13.8	39.6 ± 16.4	79.2 ± 10.8	24.4 ± 10.0
IIA	84.7 ± 13.5	31.4 ± 13.4	80.4 ± 15.0	22.8 ± 9.4
IIAX25	87.6 ± 18.0	35.2 ± 14.2	88.5 ± 15.8	23.5 ± 12.4
IIAX50	86.0 ± 18.1	31.4 ± 12.8	84.1 ± 18.4	28.4 ± 14.5
IIAX75	84.3 ± 20.5	32.6 ± 8.5	83.2 ± 15.4	24.3 ± 4.0
IIAX100	93.1 ± 9.3	30.6 ± 15.0	82.7 ± 9.1	19.5 ± 13.4

Values are expressed as mean ± S.D. Significant differences between conditions are denoted by * ($P<0.05$). Values are mmol kg^{-1} (dm).

Gray et al. (2006). Am J Physiol 290, R376-R382.

Wibom et al. (1991). J Biol Chemilum 6, 123-129.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C45

The effect of carnitine accumulation on intermediary metabolism in human skeletal muscle

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We have recently demonstrated that insulin stimulates muscle total carnitine (TC) accumulation during hypercarnitinaemia in humans, but hypercarnitinaemia *per se* had no effect (1). Given the essential role of carnitine in the integration of fat and carbohydrate metabolism, the aim of the present study was to investigate the effect of an increase in muscle TC content on skeletal muscle intermediary metabolism in resting humans.

Seven healthy men (age 22.4 ± 1.5 y, BMI 26.1 ± 1.6 kg/m^2) volunteered for the present study. On two randomised occasions, separated by 14 days, subjects underwent a 6 h euglycaemic hyperinsulinaemic clamp (2; 105 mU/m^2/min), aimed at maintaining a physiologically high serum insulin concentration, which indicated the start of a 24 h experimental period. After 1 h, the insulin clamp was accompanied by iv infusion of L-carnitine (CARN; 15 mg/kg bolus followed by 10 mg/kg/h) or the equivalent volume of saline (CON) for 5 h. Thereafter, subjects were fed a standardised, carnitine free meal (approx. 1500 kcal; 55% carbohydrate, 35% fat, and 10% protein). Arterialised-venous blood samples were obtained every hour during each clamp (and the following morning after an overnight fast), and needle biopsy samples were obtained from the vastus lateralis muscle immediately before and after each clamp and the following morning. Statistical analysis was performed using repeated-measures two-way ANOVA and paired Student's t tests. Data are expressed as means ± SEM.

The hyperinsulinaemic clamp produced similar steady state serum insulin concentrations of 160.1 ± 1.9 and 155.8 ± 3.9 mU/l during the CON and CARN infusion visits, respectively. The combination of steady-state hypercarnitinaemia (633.5 ± 10.6 μmol/l) and hyperinsulinaemia increased muscle TC content from 22.5 ± 2.0 to 26.6 ± 1.6 mmol/(kg dm) ($P<0.01$), and was associated with a decrease in pyruvate dehydrogenase complex activity (1.1 ± 0.1 vs. 0.7 ± 0.1 mmol acetyl-CoA/min/(kg wm); $P<0.05$) and muscle lactate content (10.4 ± 2.5 vs. 6.0 ± 1.0 mmol/(kg dm); $P<0.05$), and an overnight increase in muscle glycogen (567 ± 22 vs. 736 ± 24 mmol/(kg dm); $P<0.01$) and long-chain acyl-CoA content (12.1 ± 3.0 vs. 19.8 ± 3.5 mmol/(kg dm); $P<0.05$) compared to CON. Muscle TC content was 25.1 ± 2.2 mmol/(kg dm) following the overnight fast.

This study suggests that an acute increase in skeletal muscle TC content in humans results in an inhibition of carbohydrate oxidation in conditions of high carbohydrate availability, which is probably due to a carnitine-mediated increase in fat oxidation. These novel findings may be of importance to the regulation of muscle fat oxidation, particularly during exercise when carnitine availability may limit fat oxidation and in obesity and type 2 diabetes where it is known to be impaired.

Stephens FB *et al.* (2006). *FASEB J* **20**, 377-379.

DeFronzo RA *et al.* (1979). *Am J Physiol* **237**, E214-223.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C46

Insulin inhibits muscle protein breakdown by down-regulating MAFbx protein expression in humans

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Insulin is known to inhibit muscle protein breakdown in humans (Bennett & Rennie, 1991), but the mechanism by which this is achieved is unknown. Skeletal muscle proteins are predominantly degraded via the ubiquitin-proteasome system. Of particular note, the muscle specific ubiquitin E3 ligases, muscle-specific muscle ring finger 1 (MuRF1) and muscle atrophy box factor (MAFbx), have been shown to be markedly up-regulated in animal models and human wasting conditions in multiple catabolic states, such as sepsis, renal failure and burns (Wray *et al.* 2003; Lecker *et al.* 2004). The aim of this study was to determine whether insulin-mediated inhibition of muscle protein breakdown is associated with a concomitant down-regulation of MAFbx mRNA and protein expression in human skeletal muscle, and whether this response is dose dependent.

Following an overnight fast, 8 healthy young men (20.4 ± 1.2 y, BMI 23.8 ± 2.6 kg/m^2) had leg protein breakdown determined, using D5-phenylalanine, before and during a hyperinsulinaemic-euglycaemic clamp, during which mixed amino acid infusion also occurred (18 g/h, Glamin, Kabi Fresenius). Leg protein breakdown was measured before and during 3 h of insulin infusion on 4 separate occasions with the aim of maintaining steady-state plasma insulin concentrations of ~5 (achieved with octreotide (30 ng/kg/min with replacement glucagon (15 ng/kg/h) and 20% glucose), ~30, ~80 and ~170 mU/l. Needle biopsy samples were obtained from the quadriceps muscle in the basal state and after 3 h infusion to determine MAFbx mRNA and protein expression. Infusion of amino acids when insulin was maintained at ~5 mU/l (equivalent to the fasted state) had no effect on leg protein breakdown. However, when insulin availability was increased to ~30 mU/l protein breakdown was suppressed by ~50% ($P<0.001$), and was maintained at this rate as insulin availability was increased further. MAFbx mRNA expression was unchanged throughout the study. Infusion of amino acids, when insulin was maintained at ~5 mU/l, reduced MAFbx protein expression by 47% from basal ($P<0.01$). Increasing steady-state plasma insulin concentration to ~30, ~80 and ~170 mU/l was accompanied by a decline in MAFbx protein expression from basal by 80% ($P<0.01$), 87% ($P<0.01$) and 90% ($P<0.01$), respectively.

This study demonstrates, for the first time, that the inhibitory effect of insulin on muscle protein breakdown may be mediated via inhibition of MAFbx protein expression, although this relationship does not appear to happen in a dose-dependant manner. The inhibitory effect of amino acids on MAFbx protein expression warrants further investigation.

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Lecker SH *et al.* (2004). *FASEB J* **18**, 39-51.

Wray CJ *et al.* (2003). *Int J Biochem Cell Biol* **35**, 698-705.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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Growth hormone responses to repeated sprint exercise with and without suppression of lipolysis in men

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Growth hormone (GH) release following the second of two sprints has previously been found to be inhibited (Stokes et al. 2005), possibly via elevated systemic free fatty acids (FFA), which can inhibit GH release at the level of the pituitary (Casanueva et al. 1987). Plasma FFA concentrations can be reduced through suppression of lipolysis using nicotinic acid (NA). The aim of this study was to determine whether plasma FFA can modulate the GH response to exercise. It is hypothesised that when plasma FFA concentrations are low, the GH response to exercise will be augmented.

Following familiarisation, 7 active men (26 ± 3 yr, 1.77 ± 0.05 m, 81 ± 8 kg) performed two trials in a random order, separated by at least 7 days. For 2 days prior to each trial, participants consumed a prescribed high fat diet (isoenergetic with their normal diet; ~20% carbohydrate, ~60% fat, ~20% protein) and refrained from exercise, caffeine and alcohol. Each trial followed an overnight fast and consisted of two 30-s cycle ergometer sprints separated by 4 h of recovery. In one trial (NA), participants ingested a total of 2 g of NA over 3 doses: 1 g ingested 60 min before the sprint 1 and 0.5 g at 60 and 180 min after sprint 1. The other trial was a control (Con) trial. Finger-prick blood samples were taken before the first dose of NA and immediately before and 15, 30, 45 and 60 min after each sprint. Whole blood was centrifuged and plasma stored at -20°C for later determination of GH (DSL Inc., Texas) by routine ELISA and FFA ([NEFA] Wako, Germany) by spectrophotometry (Cobas Mira N; Roche). Repeated measures ANOVA was performed and specific differences were identified using paired t tests with Bonferroni correction for multiple comparisons. Statistical significance was accepted at $P<0.05$; data are presented as mean ± SD.

Plasma FFA was not significantly different between trials prior to sprint 1, but was significantly lower in the NA trial immediately before sprint 2 (NA vs. Con; 0.08 ± 0.05 vs. 0.75 ± 0.34 mmol/l, $P<0.05$). Peak GH and integrated GH were significantly greater following sprint 2 in the NA trial compared to sprint 1 in the NA trial (peak GH, 23.3 ± 7.0 vs. 7.7 ± 11.9 μg/l, $P<0.05$; integrated GH, 1076 ± 350 vs. 316 ± 527 μg/l/60 min, $P<0.05$) and sprint 2 in the Con trial (peak GH, 23.3 ± 7.0 vs. 5.2 ± 2.3 μg/l, $P<0.05$; integrated GH, 1076 ± 350 vs. 206 ± 118 μg/l/60 min, $P<0.05$). However, peak and integrated GH were not different after sprint 1 and sprint 2 during the Con trial (Fig. 1).

In the Con trial, GH release was not inhibited following sprint 2, contrary to previous findings. However, suppression of lipolysis resulted in a significantly greater GH response to sprint 2 in the NA trial. This suggests a role for plasma FFA in modulating GH release following sprint exercise.

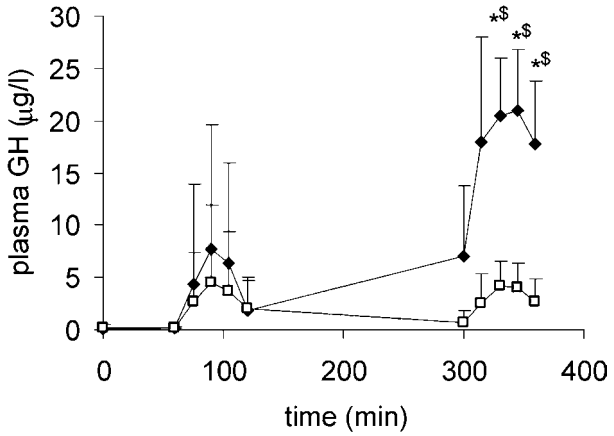

Figure 1. Plasma GH following sprint 1 (60 min) and sprint 2 (300 min) in the NA (closed diamonds) and Con (open squares) trials. * $P<0.05$ vs. same time point in Con, $ $P<0.05$ vs. resting sample in NA.

Casanueva FF et al. (1987). J Clin Endocrinol Metab 65, 634-642.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C48

The effects of two weeks recombinant growth hormone administration on the response of IGF-I and PIIIP to a single bout of high resistance exercise in resistance trained young men

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Recombinant human growth hormone (rhGH) is used by some athletes in an attempt to enhance athletic performance. Insulin like growth factor I (IGF-I) and the N-terminal extension peptide procollagen type III (PIIIP) have been identified as potential markers of rhGH abuse (Dall *et al.* 2000; Wallace *et al.* 2000).

The aim of the present study was was to determine the effects of a single bout of high resistance weight-liftinig exercise with or

without rhGH loading, on these markers in weight trained athletes. Fifteen young male subjects (mean age 25.3±1.6 (SEM) years) were randomly assigned to either placebo or rhGH groups. Subjects were sportsmen already undertaking regular strength training exercise. However, in order to create a more homogenously trained group, the subjects underwent 4 weeks of a standardised and supervised general strength training program prior to entering the study. The subjects were tested on 3 days: T1 following the 4 week training period; T2 following 2 weeks of either rhGH 0.1 IU/kg/day or placebo administration; and T3 following a 1 week washout period. On the test days, subjects performed an acute high resistance exercise test (AHRET) which comprised 6 sets of 10 repetitions at 80% 1-repetition maximum with 2 min rest between sets. The AHRET took place at the same time in the morning for each subject, in the fasted state and following insertion of a cannula in a radial vein. Blood samples were taken at rest, and at 5, 15, 30 and 60 min post exercise. The samples were analysed for IGF-I and PIIIP using radioisotopic assays.

Administration of rhGH resulted in significantly ($p<0.01$, ANOVA with repeated measures) increased levels of both IGF-I and PIIIP at all time points (GH T2 in Fig. 1a and 1b). After a 1 week washout period, IGF-I levels had returned to baseline, but in T3 PIIIP levels remained significantly elevated in the rhGH group($p<0.01$). Exercise alone did not significantly affect levels of IGF-I or PIIIP, although there was a tendency for mean PIIIP values to be elevated in the rhGH treatment group at 5 min in T2 and T3.

The data suggest that in strength trained individuals rhGH adminstration has a marked effect on serum IGF-I and PIIIP, but this is not appreciably effected by high resistance exercise. The results of the study would suggest that elevated levels of these markers are indicative of rhGH abuse independent of exercise in strength trained athletes.

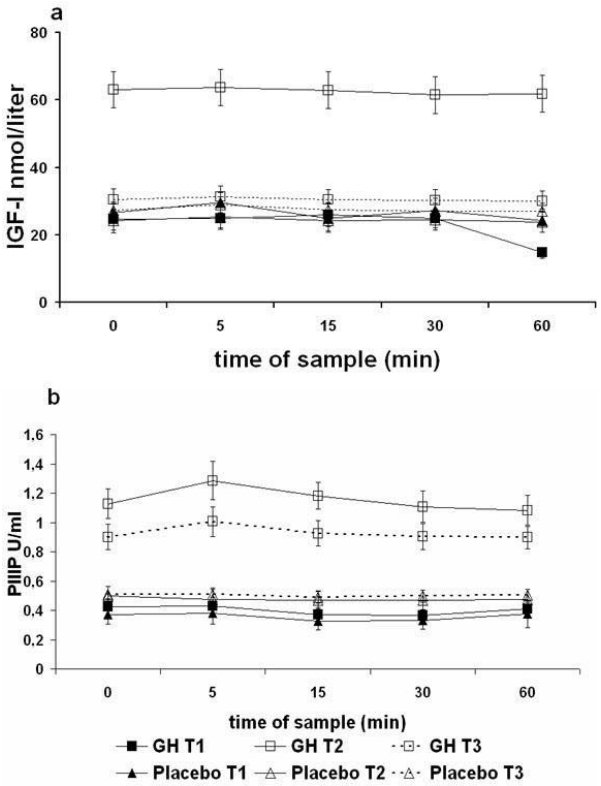

Figure 1. IGF-I and PIIIP levels in serum before (0 min) and after a bout of exercise.

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Wallace *et al.* (2000). *J Clin Endocrinol Metab* **85**, 124-133.

*S. Harridge and G. Goldspink were both principal authors for this work.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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Effects of increasing RR interval on saturation of high frequency spectral power with and without paced breathing

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Harmonic variations in heart period between 0.15 and 0.40 Hz (high frequency spectral power, HF) measure cardiac vagal modulation. Data obtained under autonomic blockade show either a linear (Hayano *et al.*1991) or curvilinear (Goldberger *et al.* 1994) relationship between HF and beat to beat (RR) interval. Kiviniemi *et al.* (2004, 2006) demonstrated a curvilinear relationship between 5-min epochs of HF and RR data taken from 24-h recordings in some subjects. The aim of this study was to determine the magnitude and nature of the HF/RR relationship during wakeful, resting ECG recordings.

Two hundred and fifty volunteers took part in the study (140 men; mean age 35.9 ± 12 years and 110 women; mean age 34 ± 10 years). ECG recordings were made using a two lead ECG with a sampling frequency of 1 ms (Polar Electro Ltd, Oy, Kempele, Finland) while subjects lay supine for 12 min with either free or paced (0.25 Hz) breathing. RR intervals were filtered and a fast Fourier transformation used to calculate HF power. HF was log transformed, HF(ln), and plotted as a function of corresponding mean RR interval. Linear and quadratic regression models were used to evaluate the relationship between RR and HF(ln). The point at which HF(ln) failed to increase for an increase in RR was derived from the differentiated quadratic equation and defined as the deflection point (DP). The criterion used to determine saturation of HF(ln) was that DP lay within the range of measured RR intervals.

With free breathing (n=179 samples), the adjusted R^2 was similar between linear (25.2%) and quadratic (25.0%) fits and DP occurred outside the recorded RR interval range at 2042.0 ms. With paced breathing (n =234), adjusted R^2 values were smaller, but again similar between linear (19.7%) and quadratic (20%) fits. DP was outside the range of recorded RR intervals at 1422.9 ms.

The HF(ln)/RR relationship does not show saturation when the measures are derived from single epochs of ECG data. The HF(ln)/RR relationship is weaker when derived from recordings made between subjects as opposed to 5 min epochs of a single tachogram (Kiviniemi *et al.*2004). Previous data have shown DP within the recorded RR interval range and perhaps, more importantly within the range that is likely to be observed. During free breathing, DP was found at a heart rate of 29 BPM. This is outside the normal range, indicating that HF(ln) shows no potential saturation characteristics. However, during paced breathing, a plateau in HF(ln) was found at 42 BPM. Such low heart rates were not recorded in the present study. However, they do occur and the HF(ln)/RR relationship may, therefore, saturate in elite athletes and beta blocked subjects, particularly if assessed during sleep. Such low heart rates are unlikely during wakeful, short-term ECG recordings.

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Hayano J *et al.* (1991). Am J Cardiol **67**, 199-204.

Kiviniemi AM *et al.* (2004). Am J Physiol Heart Circ Physiol **287**, H1921-1927.

Kiviniemi AM *et al.* (2006). Eur J Appl Physiol 1-7. Epub.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC120

Disability in ambulatory stroke survivors is associated with impaired explosive power in both lower limbs

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Reduced lower limb extensor power (LLEP) is associated with poor performance of functional tasks in healthy people (1). Little is known about LLEP after stroke, other than it is lower than in healthy people when matched for age and gender (2) and that the impairment is bilateral, suggesting the involvement of factors not directly caused by the stroke. We hypothesised that low values of LLEP would be associated with reduced physical function and increased disability after stroke.

LLEP (W kg^{-1}) was determined for each leg in 66 ambulatory stroke survivors (mean (SD): age 72 (10) years, wt 72.6 (15.3) kg, ht 1.67 (8.59) m), using a Nottingham Power Rig (3). We measured physical function (comfortable walking velocity, functional reach, chair rise time and 3-metre timed up-and-go), and disability (Functional Independence Measure, Rivermead Mobility Index and Nottingham Extended ADL). The associations between LLEP and both function and disability were analysed using stepwise multiple linear regression models which included the likely confounding factors age, gender, time since stroke, smoking and use of walking aids.

The median value of LLEP of the affected limb ($LLEP_{aff}$ 0.92 W kg^{-1}) was significantly lower than that of the unaffected limb ($LLEP_{unaff}$ 1.05 W kg^{-1}; p=0.002) but the difference was small (~10%).

Low LLEP of either limb was associated with poor performance in each measure of physical function ($p<0.0001$) and was the exclusive predictor of those which were dynamic (walking, chair rise and timed up-and-go). LLEP showed pronounced curvilinear associations with chair rise time and timed up-and-go, with reductions in performance when LLEP was below 1.0 W kg^{-1} but with no increase in performance above this value (Fig. 1).

Low LLEP was also associated with poor scores in each measure of disability; these associations were strongest for the affected side ($p<0.0001$) with $LLEP_{aff}$ being the only predictor from among the variables included in the regression models.

The ratio of $LLEP_{aff}/LLEP_{unaff}$ had no predictive importance for any measure of function or disability.

In ambulatory stroke survivors reduced performance of physical function and increased disability are associated with deficits in LLEP of both lower limbs, and not the severity of any residual asymmetry. Interventions to increase LLEP in both legs might improve function and reduce disability after stroke.

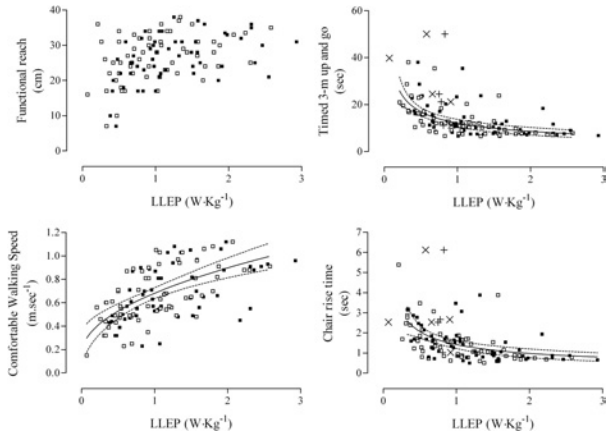

Figure 1. LLEP of the affected (filled symbol) and unaffected (unfilled) lower limbs and specific disabilities. x (affected) and + (unaffected) denote use of arms for chair rise. Where LLEP was the only significant independent variable its regression coefficient (from transformed data) was used to generate a best fit line (and 95% CI) on the untransformed, non-linear data (for clarity shown only on the unaffected side).

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Greig CA et al. (2003). Age & Ageing 32 (Suppl. 1), 34.

Bassey EJ & Short AH (1990). Eur J Appl Physiol Occup Physiol 60, 385-390.

Funded by the Chief Scientist Office of the Scottish Executive (STARTER trial). C.G. is a Research into Ageing Research Fellow.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC121

Laboratory studies and field testing of an aerobic fitness test for use in household surveys

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Very few population surveys of aerobic fitness have been undertaken largely due to the difficulty of carrying out physiological tests in households. Successful surveys in Canada used a graded step test (Shephard et al. 1976) and the present study aimed to develop a suitable test for UK conditions.

Preliminary laboratory studies indicated that a shallow double step (step height 16.5cm) was required for many subjects of average or below average fitness. Under laboratory conditions we looked at the relation between rate of stepping and oxygen consumption ($\dot{V}O_2$) and found it to increase linearly with power ($\dot{V}O_2$ [l/min] = 0.364 + 0.0191*power [W]), very similar to previous studies using steps (e.g. Nagel et al. 1965). Thirty four subjects of varying age, gender and fitness then carried out the step test and also had their maximum oxygen consumption ($\dot{V}O_2$max) determined using a graded treadmill test. During the step test the subjects stepped at an initial cadence of 65, 75 or 85 movements per minute for 3 min and heart rate (HR) was measured using a Polar heart monitor over the last 20 s. Without breaks, subjects continued at 2 increased cadences (+15 and +30 from initial) for 2 min with HR determined during the last 20 s of each. The initial cadence was set by a calculation based on the age, weight, height and gender of each subject with the aim that the final cadence would produce a HR less than 80% of predicted maximum. Heart rate increased linearly with cadence, i.e. with power production, and the HR:power relationship was extrapolated to estimated maximum HR (220-Age). $\dot{V}O_2$max was then determined from the relationship between power and $\dot{V}O_2$ given above and was found to correlate well with $\dot{V}O_2$max from the treadmill test (r=0.80; average difference +0.35 l/min; 95% limits of agreement +1.47, -0.77 l/min).

Research nurses from the Health Survey for England took the step (weight 6 kg) to 29 homes of widely varying type (e.g. top floor flat, terrace houses, isolated farmhouse). They used their survey laptop to run the test, including producing the timing pulses, and for manual data entry. Each visit involved a single nurse running the entire test. The nurses found the test interesting to carry out and the reactions of potential subjects were positive. The test took 20 min, but with extra time for setting up, dismantling and giving explanations it is estimated that under normal survey conditions the test would take 30 min. The equipment performed reliably under field conditions. $\dot{V}O_2$max values agreed on average with those estimated in setting the initial step rate (based on age, gender and height), the average difference being 0.2 l/min (limits of agreement +1.3 and 1.1 l/min). However, the present step needs to be made lighter and simpler to handle before it could be used in a large scale survey.

In conclusion, a simple step test can be used for measuring fitness during a household survey. However, around half an hour is needed per test and the step itself needs to be easily portable and straightforward to assemble and pack away.

Nagle FJ, Balke B & Naughton J (1965). J Appl Physiol 20, 745-748.

Shephard RJ, Bailey DA & Mirwald RL (1976). Can Med Assoc J 114, 675-679.

This study was funded by a contract from the UK Dept of Health.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC122

Bone loss from the tibia during unilateral lower limb suspension in man

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Bone loss is uniformly observed from immobilised limbs, e.g. after spinal cord injury, during space flight, and during bed rest. The latter is an accepted model for ground based microgravity research. Unilateral lower limb suspension (ULLS) is a new alternative to such bed rest studies (1), evoking a muscular atrophy which is comparable to that observed during bed rest. To the

best of our knowledge, bone loss during ULLS has never been demonstrated before. However, there might be reason to assume that bone loss does not occur during ULLS. According to studies both in animals and in humans, an increase in interstitial fluid pressure seems to hamper the bone loss induced by immobilisation. However, we hypothesized that bone loss during ULLS does occur, and that it does so to an extent that is comparable to bed rest.

Eight young healthy volunteers participated in a ULLS study. Their mean age was 19 years (SD 0.76), their height was 179.3 cm (SD 4.7) and their mean body mass 72.4k g (SD 8.6). Their right leg was suspended for 24 days, using a strap to tie it so the knee angle was 10 deg whilst using crutches with both hands and a 7.5 cm sole under their left shoe. Bone scans from both lower legs were obtained by peripheral Quantitative Computed Tomography (pQCT) with an XCT 2000 (Stratec Medizintechnik, Pforzheim, Germany). Measurements were performed during baseline data collection (two times), on days 7, 14 and 21 of the ULS, and on days 4, 9, 35 and 90 of the recovery. Bone mineral content (BMC) of the distal tibia (at 4% of its length) was assessed with the integrated XCT 2000 software version 5.40. A repeated measure ANOVA design with simple a-priori contrasts was used to detect a time effect.

For the distal tibia epiphysis of the suspended leg, a significant time effect was found ($p = 0.001$). The bone loss became significant on day 4 of the recovery ($p = 0.016$). It was largest on day 35 of the recovery, when it amounted to 1.09% ($p = 0.009$, SD 0.68). No significant loss was observed from the left tibia.

For the first time, bone loss has been observed during a limb suspension study in humans. This fills a gap in the scientific literature, as limb suspension is a model which is often used to study the effects of immobilisation on bone in rats.

As the limb suspension in this study was comparatively short for a study of bone, the present data do not allow for a definitive assessment of the rate of bone loss. However, a comparison with data from bed rest studies (2) suggests that the rate of bone loss may be comparable in bed rest and in ULLS. If this were to be substantiated in a future study with limb suspension over a longer period, then that would constitute strong evidence against a decisive role of interstitial fluid pressure for immobilisation induced bone loss.

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The ULLS study was supported by the European Space Agency (ESA).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC123

The relation between heart rate variability and aerobic fitness for subjects of South Asian ethnic origin

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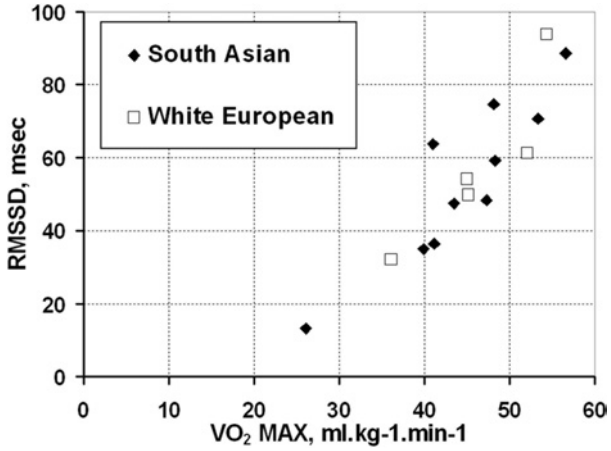
Heart rate variability (HRV) has been used to help estimate aerobic fitness in a recent national survey in Finland (Borodulin, 2006). In order to use such a test in the UK it is important to know whether major ethnic groups differ in the relation between HRV and fitness. There is evidence from studies in the USA that African Americans have higher HRV than Americans of European descent (Gutin et al, 2005). An important ethnic group in the UK is that whose origins are in South Asia (India, Pakistan and Sri Lanka). This study is a first comparison of fitness and HRV in South Indian versus European origin subjects.

Fifteen male subjects, 10 South Asian (mean age 21.3±1.6 years (SD)) and 5 white European (age 21.2±2.7 years) participated. Beat-by-beat heart rates were recorded during the last 6 min of an 8 min period lying supine in a quiet room using a Polar S810 watch and chest strap. Data were analysed using Biosignal v1.1 software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland). Several heart rate parameters were determined and RMSSD (the root mean square of successive differences between intervals) was selected as the most suitable measure. Maximum oxygen consumption ($\dot{V}O_2$max) was determined by standard breath-by-breath pulmonary gas exchange (CPX Ultima, MedGraphics, Minnesota, USA). Subjects performed a graded exercise test on a bicycle ergometer (Lode Corival, Lode BV, Netherlands) with increasing workload (30 W/min) until exhaustion. Mid five of seven smoothing was applied to the breath-by-breath data using associated software before the determination of maximal oxygen uptake (Breeze Suite, MedGraphics, Minnesota, USA).

Resting heart rates averaged 67 for the South Asian group and 63 for the European group. There was a clear approximately linear relation (Runs test, $p>0.5$) between HRV and $\dot{V}O_2$max (Fig. 1) and the data for both ethnic groups were similar (analysis of covariance, $p=0.8$). It therefore appears that at least for young male subjects the relation between HRV and aerobic fitness is similar for South Asian and European white ethnic groups.



Borodulin K (2006). Physical activity, fitness, abdominal obesity, and cardiovascular risk factors in Finnish men and women. The National FINRISK 2002 Study. National Public Health Institute, Helsinki, Finland.

Gutin B et al. (2005) Med Sci Sports Exercise 37, 1856–1863.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC124

EMG spectral moments provide a reliable and highly sensitive index for studying muscle fatigue during dynamic contractions

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This study examined the validity, reliability and sensitivity of a new EMG spectral index in assessing peripheral muscle fatigue during dynamic contractions.

Seven healthy adults (28.7±7 years, 180±10 cm, 78±12 kg, mean±SD) performed metronome guided unilateral knee extension exercise lifting 50% of their one repetition maximum (10 sets x 15 repetitions, 2 min rest between the sets). Knee extensor maximal voluntary contractions (MVC) were performed before and immediately after exercise. Torque (T, Technogym UK Ltd), knee angle (Biometrics system, Gwent, UK) and rectus femoris EMG (bipolar surface electrode, B&L Engineering, CA, USA) were recorded simultaneously throughout the trial (CED, Cambridge, UK). Median (F_{med}) frequency of EMG power spectrum and the ratio between EMG spectral moments of order -1 and 5 (FI_{nsmk}) were calculated for a segment from each repetition of each set. The middle of each segment was selected to coincide with the maximal knee extension angle. Spectral moments (M_k) represent the area under the spectral curve after multiplication with the frequency raised to the power of k (where k is the order of the moment) as the weighting function [1].

$$M_k = \int_{f_{min}}^{f_{max}} f^k . PS(f) . df$$

$$FI_{nsmk} = \frac{M_{-1}}{M_k}$$

where M_k is a spectral moment of order k, PS(f) denotes the EMG power frequency spectrum as a function of frequency f, and f_{min} and f_{max} delineate the bandwidth of the signal.

Peripheral muscle fatigue development was assessed by the normalised relative changes (Δ) of the spectral indices against the first repetition of the corresponding set. The normalized percentage change between pre- and post- exercise MVC was used to evaluate the change in muscle isometric strength. Data were analysed using two-way repeated measures ANOVA (set vs. repetition).

The F_{med} (0.78, P<0.0001) and FI_{nsmk} (0.75, P<0.0001) intraclass correlation coefficients calculated from the first repetition of each set demonstrated fair-to-good reliability (one-way random effects single measure (1,1) model). The maximal ΔFI_{nsmk} observed was approximately 8-fold, while that of ΔF_{med} was only 32%. ΔF_{med} and ΔFI_{nsmk} changed significantly between repetitions (both P<0.0001) but no between sets or set vs repetition interactions were observed for either parameter. ΔFI_{nsmk} variability between subjects was significant (P<0.0001). Hierarchical cluster analysis (average linkage method utilising Eucledian distance as the interval measure of dissimilarity) revealed three distinct subgroups of subjects: high (ΔFI_{nsmk}>400%), medium (200%<ΔFI_{nsmk}<400%), and low (ΔFI_{nsmk}<200%) muscle fatigability (Fig. 1). Linear regression analysis of ΔF_{med} and ΔFI_{nsmk} established different course of fatigue development between clusters. Muscle functional characteristics were significantly different between clusters during- and post- exercise (ΔMVC, P=0.012 and ΔT, P=0.03; one-way ANOVA), thus supporting the functional validity of the grouping.

The new spectral index is a valid and reliable tool for assessment of muscle fatigability during dynamic contractions, and is more sensitive than the traditionally used median frequency of the EMG power spectrum.

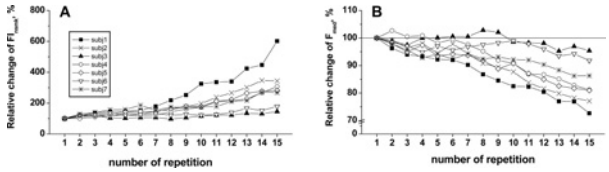

Fig.1. Changes of the new spectral index (FI_{nsmk}, A) and median frequency (F_{med}, B) averaged for all sets (n=10) across repetitions for each subject (n=7).

Lindström L & Petersén I (1983). Power spectrum analysis of EMG signals and its application. In Computer-aided Electromyography, ed. Desmedt JE. Prog Clin Neurophysiol 10, 1-51.

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PC125

C2C12 muscle cell line as a model to study androgen-induced myofibre hypertrophy

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Muscle hypertrophy is an increase in myofibre size requiring net protein synthesis and the activation and subsequent fusion of satellite cells with the existing myofibres. Anabolic agents act to stimulate these processes. Testosterone is a known anabolic agent and has been shown in human skeletal muscle to invoke dose response increases in muscle fibre size and satellite cell number (Sinha-Hikim *et al.* 2003).

The C2C12 mouse muscle cell line has been widely used to study the signalling pathways involved in insulin like growth factor I-induced hypertrophy (Rommel *et al.* 2001). The purpose of the present study was to determine the suitability of this cell line as an *in vitro* model for studying the hypertrophic response to androgens, by examining changes in myotube size in response to increasing concentrations of testosterone.

Murine C2C12 myoblasts were cultured in a humidified incubator at 37°C and 5% CO_2, in growth medium (GM) containing Dulbecco's Modified Eagle's Medium (DMEM, Gibco, UK), 1000 g/l glucose and 10% fetal calf serum (FCS). Cells were plated onto 35mm

tissue culture dishes at $4x10^5$ cells per dish. After 24 h the cells reached confluency. GM was replaced with differentiation medium (DM, DMEM + 2% horse serum) or serum-free medium (SF) with and without testosterone for 6 days. Media were refreshed every 2 days. The testosterone concentrations were 1nM, 10nM (physiological), 100nM and 1μM. Cells were subsequently fixed in 100% ice cold methanol and digital images obained using a phase contrast microscope (20X). Myotube diameters were quantified according to the method described by Rommel *et al.* (2001). A total of 50 myotubes were chosen per treatment group. The average diameter per myotube was calculated as the mean of 10 measurements taken at regular intervals along the length of the myotube.

The data show that in DM C2C12 myotubes hypertrophy in response to physiological and supraphysiological doses of testosterone ($p<0.05$, ANOVA). In SF medium, myotubes were significantly ($p<0.05$) smaller than those in DM at all concentrations. The myotubes in SF medium were also responsive to testosterone ($p<0.05$), albeit to a lesser extent. The mean increase in myotube size from control to the 1μM testosterone concentration was 36% in DM and 17% in SF medium. This indicates that factors in addition to testosterone are regulating myotube size in DM. Taken together, the data suggest that C2C12 cells may provide an appropriate model for studying androgen-induced mechanisms of muscle hypertophy.

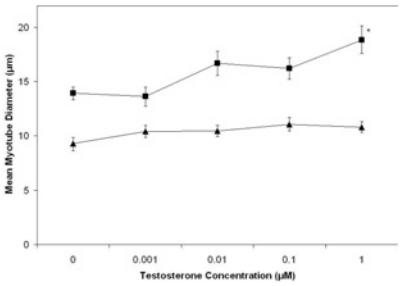

Figure 1. Myotube size in response to increasing concentrations of testosterone in either serum-free medium (triangles) or differentiation medium (squares). * Indicates significant difference from 0 μm testosterone ($p<0.05$, post-hoc t tests corrected for multiple comparisons). Data points are mean ± sem, n=50.

Rommel C *et al.* (2001). *Nat Cell Biol* **3**, 1009-1013.

Sinha-Hikim I *et al.* (2003). *Am J Physiol* **283**, E154-164.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements

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PC126

Post-exercise rehydration in man: role of drink osmolality

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Hypotonic carbohydrate-electrolyte solutions are more effective for rapid rehydration than water (Gonzalez-Alonso *et al.* 1992) due to active co-transport of solutes leading to an increased net efflux of water from the intestinal lumen (Schedl & Clifton, 1963). Hypertonic solutions are thought to be ineffective rehydration solutions due to the net movement of water into the small intestine (Leiper & Maughan, 1986) from the extracellular fluid (Evans *et al.* 2005) that follows their ingestion. However, the reduction in plasma volume that occurs following ingestion of a hypertonic solution may lead to reduced urinary water loss due to an increase in the concentration of vasopressin and aldosterone, thus aiding the rehydration process. Six healthy male volunteers were dehydrated by 1.9 ± 0.1% body mass by intermittent cycle ergometer exercise in a warm, humid environment. Beginning 30 min after the end of exercise, subjects drank, over a period of 1h, a volume that amounted to 150 (130-150)% (median (range)) of the body mass loss. Drinks contained either 0, 2 or 10% glucose with 25 mmol/l NaCl, distilled water and lemon flavouring and had osmolalities of 79 ± 4, 193 ± 5 and 667 ± 12 mosm/kg, respectively. Blood and urine samples were collected prior to and 30 min after exercise and at 0, 1, 2, 3, 4 and 6 h following the rehydration period. Statistical analysis included repeated measures ANOVA and Tukey or Dunnett's pairwise comparisons. Cumulative urine volume was greater on the 0% trial than on the 10% trial ($P < 0.001$). Net fluid balance during the 10% trial was greater than during the 0% trial ($P = 0.002$) and subjects remained euhydrated for 1 h longer on the 10% trial than on the 2% trial (Fig. 1). Plasma volume was elevated from pre-exercise levels immediately after and 1 h after rehydration during the 2% trial ($P < 0.05$).

These results suggest that high-carbohydrate drinks can be effective rehydration solutions. As plasma volume was elevated from pre-exercise levels after rehydration on the 2% glucose trial but not on the 10% trial it would seem that the beneficial effect of hypertonic, energy-dense solutions on net fluid balance may be due to the avoidance of a large acute increase in plasma volume and consequent reduction in plasma water retention hormones that occur following ingestion of a hypotonic solution.

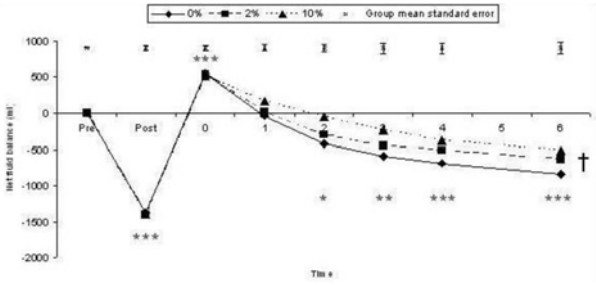

Figure 1. Net fluid balance during all trials. Net fluid balance loss during 0% glucose trial compared to 10% glucose trial ($P = 0.002$). * 0% time point different than pre-exercise value ($P < 0.05$). ** 0 and 2% time point different than pre-exercise value ($P < 0.05$). *** 0, 2 and 10% time points different than pre-exercise value ($P < 0.05$).

Evans GH et al. (2005). J Sports Sci 23, 1189-1190.

Gonzalez-Alonso J et al. (1992). Int J Sports Med 13, 399-406.

Leiper JB & Maughan RJ (1986). J. Physiol 373, 90P.

Schedl HP & Clifton JA (1963). Nature 199, 1264-1267.

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PC127

Thermoregulatory responses to ingesting cold and hot drinks in man at seated rest and during cycling exercise

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The physiological effects of ingesting drinks at different temperatures at rest are unclear (Imms & Lighten, 1989). During exercise at 50% $\dot{V}O_2$peak, the rise in body temperature was similar after ingesting hot or cold drinks (Lee et al. 2004). This study compared thermal responses to ingesting cold and hot drinks at rest and during cycling exercise.

Eight men rested for 75 min (R) or cycled (EX) at 60 ± 1% (Mean ± SD) $\dot{V}O_2$peak at 25.3 ± 0.2°C with relative humidity of 56 ± 5%. Subjects ingested 300 ml of cold (C; 4°C) or hot (H; 50°C) flavoured water at 15, 30, 45 and 60 min during each trial. Rectal temperature (T_{re}), weighted mean skin temperature (T_{sk}; Ramanathan, 1964), heart rate (HR), skin blood flow (SBF), sweat loss and thermal comfort (TC; adapted from Parsons, 2003) were recorded. Differences ($P<0.05$) between trials were assessed using ANOVA followed by Tukey post-hoc and paired t test as appropriate. As there were no differences between trials prior to drinking, the remaining hour was used to assess the effect of drink temperature.

The change in T_{re} over each trial was different between trials (-0.71 ± 0.24, 0.03 ± 0.07, 0.49 ± 0.17 and 0.74 ± 0.28°C for trials R-C, R-H, EX-C and EX-H, respectively; $P<0.05$). T_{re} changed on all trials throughout the 1-h period except on trial R-H. Mean T_{sk} was lower with ingestion of cold drinks than with hot drinks at rest (31.44 ± 0.42 and 31.70 ± 0.30°C for trials R-C and R-H, respectively; $P<0.05$) but not during exercise (32.82 ± 0.64 and 33.05 ± 0.61°C for trials EX-C and EX-H, respectively; $P=0.074$). Ingestion of hot drinks resulted in higher HR at rest (58 ± 8 and 64 ± 7 beats/min for trials R-C and R-H, respectively; $P<0.01$) and during exercise (145 ± 12 and 150 ± 12 beats/min for trials EX-C and EX-H, respectively; $P<0.05$). There was no difference between drinks in mean SBF relative to baseline at rest (-20 ± 17 and -7 ± 13% for trials R-C and R-H, respectively; $P=0.152$) or during exercise (473 ± 184 and 601 ± 283% for trials EX-C and EX-H, respectively; $P=0.242$). Sweat loss during exercise was higher when hot drinks were ingested (EX-C = 1.15 ± 0.22 l; EX-H = 1.31 ± 0.25 l; $P<0.01$). Ratings of TC were affected by drink temperature (-3.5 ± 1.5, 0.7 ± 0.8, 2.5 ± 1.6 and 3.5 ± 1.3 for trials R-C, R-H, EX-C and EX-H, respectively; $P<0.05$). The predicted differential in T_{re} between the cold and hot drinks was 0.99 ± 0.11°C (Nadel & Horvath 1969), but the observed differentials at rest and during exercise were 0.74 ± 0.29 ($P<0.01$) and 0.24 ± 0.17°C ($P<0.01$), respectively.

These results show significant thermoregulatory effects of drink temperature. Cold drinks ingested at rest caused a fall in T_{re} whereas T_{re} remained stable with hot drinks. The unaccounted differential from the predicted T_{re} during exercise may be due to a higher evaporative heat loss.

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PC128

Physical activity energy expenditure, haem oxygenase-1 protein expression and cell death in human mononuclear cells

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The induction of haem oxygenase-1 (HO-1) is a general response to oxidant stress in mammalian cells (Keyse & Tyrrell, 1989) and has been shown to inhibit apoptosis in lymphocytes (Choi *et al.* 2004). Since exercise is a form of oxidative stress, cells taken from individuals who take part in regular exercise may respond differently to an exogenous oxidant challenge in comparison to cells taken from less active individuals. The aim of this investigation was to examine the relationship between HO-1 protein induction and cell death in response to hydrogen peroxide (H_2O_2) treatment and habitual physical activity.

Twenty-four males (age 26 ± 4 years, height 180 ± 10 cm and body mass 77 ± 11 kg; mean ± S.D.) reported to the laboratory following an overnight fast. Mononuclear cells were isolated from peripheral blood and exposed to 50 μM H_2O_2 for 30 min at 37°C. HO-1 protein was analysed by flow cytometry at baseline and 48 h after recovery from treatment (Markovitch *et al.* 2005). Cell death was measured by flow cytometry at baseline and 18 h after H_2O_2 treatment using annexin/propidium iodide staining.

Physical activity energy expenditure was estimated using a combined movement and heart rate (HR) monitor (Actiheart, CNT, Cambridge) which was worn for seven representative consecutive days (Brage *et al.* 2005; Thompson *et al.* 2006). Briefly, energy expenditure (EE) in low, moderate and vigorous intensity physical activity (< 3, 3-6 and > 6 Metabolic Equivalents; METs, respectively) was determined using a branched equation model for HR and accelerometer counts. Spearman's rank order correlation was used to determine whether relationships existed between habitual physical activity (PA), HO-1 protein expression and cell death (basal and following H_2O_2 treatment).

Weekly EE at moderate intensity or above (> 3 METs in bouts of 10 min or more) ranged between 373 and 7187 kilocalories. There were no relationships between EE and responses to H_2O_2, however, there was a modest positive relationship between basal HO-1 protein and EE (> 3 METs) in both lym-

phocytes ($r = 0.397$, $P = 0.055$) and monocytes ($r = 0.443$, $P = 0.030$). Furthermore, energy expended in moderate intensity physical activity was inversely related to basal apoptosis ($r = -0.535$, $P = 0.007$). These findings indicate that those individuals who expended a greater amount of energy through at least moderate intensity PA had higher levels of basal HO-1 expression and a lower level of apoptosis. The non-causal nature of these results does not allow firm conclusions. However, it is tempting to suggest that these results show that regular physical activity is associated with an improved profile at rest without compromising the ability of mononuclear cells to respond to an oxidant challenge. Further work is clearly required.

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PC129

Exercise-induced muscle damage impairs endurance running performance in humans

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Exercise-induced muscle damage (EIMD) is known to decrease muscle strength and power, but its effects on endurance performance and running economy are unclear (1-4). The main aim of this study was to determine whether EIMD impairs running performance in a 30 min time trial.

Thirty runners (24 men and 6 women, age 31 ± 9 years, $\dot{V}O_{2max}$ 54.1 ± 6.0 ml/min/kg) were matched for gender and randomly assigned to an EIMD or control group. Subjects in the EIMD group jumped 100 times from a 35 cm bench while controls did not perform any muscle-damaging exercise. Before and 48 hours after experimental treatment, subjects were tested on (1) markers of muscle damage, (2) steady-state cardiorespiratory, metabolic and perceptual responses during a constant speed run at 70% of $\dot{V}O_{2max}$, and (3) distance ran in 30 min on a treadmill. Two-way (group x test) mixed ANOVAs were used for group analyses. Individual changes in performance were analysed using the Reliable Change Index (5).

All markers of EIMD were significantly affected by experimental treatment: delayed-onset muscle soreness increased 4 points on a 0-6 scale, serum CK increased from 159 to 332 IU/l, knee extensors strength was reduced by 12% (all $P < 0.01$). EIMD significantly reduced 30 min time trial performance by 4% ($P < 0.01$) with 7 subjects in the EIMD group showing a reliable decrement in performance compared to only 1 in the control group (Fig. 1). Running economy and other physiological responses to submaximal running were not affected by EIMD (Table 1). However, a trend for increased perceived exertion was found. EIMD has a negative impact on endurance running performance in humans despite no changes in running economy. Further studies on the central and peripheral factors mediating fatigue in subjects with EIMD are warranted.

Table 1. Effects of experimental treatment on responses to running at 70% of $\dot{V}O_{2max}$ (EIMD = 11.6 ± 1.4 km/h, Control = 11.2 ± 1.4 km/h)

Variable	Group	Pre-test	Post-test	*P*
Heart rate (bpm)	EIMD Control	151 ± 18 149 ± 14	152 ± 18 148 ± 13	0.262
Ventilation (l/min)	EIMD Control	67.3 ± 15.2 67.0 ± 15.9	70.5 ± 13.8 68.8 ± 13.3	0.526
[vdot]O_2 (l/min)	EIMD Control	2.72 ± 0.46 2.69 ± 0.61	2.71 ± 0.41 2.68 ± 0.47	0.963
Respiratory exchange ratio	EIMD Control	0.982 ± 0.050 0.965 ± 0.036	0.997 ± 0.048 0.981 ± 0.027	0.950
Post-exercise La (mmol/l)	EIMD Control	2.02 ± 1.36 1.86 ± 0.79	2.12 ± 1.16 1.73 ± 0.71	0.306
Perceived exertion (6-20)	EIMD Control	12.5 ± 0.9 11.8 ± 1.8	13.2 ± 1.2 11.7 ± 2.1	0.076

La, blood lactate.

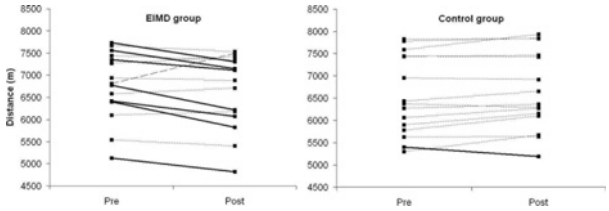

Figure 1. Individual changes in time trial performance. The bold lines represents a reliable decrease, the dashed lines represents a reliable increase, and the dotted lines represents a non-reliable change.

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PC130

The neutrophil responses during recovery period after a 2-h cycling at 55% peak power

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Failure to fully recovery between training sessions has been suggested to evoke chronic fatigue, underperformance and greater

immunodepression (Gleeson, 1998). It has been suggested that the alterations in neutrophil function and trafficking do not recover within 3 h (Li & Gleeson, 2004), but appear to be fully recovered within 18 h after prolonged exercise (Li & Gleeson, 2005). The aim of the present study was to determine the time course of recovery of neutrophil responses following 2 h cycling at 55% peak power. Ten healthy men (age 21.6 ± 0.9 years, height 1.77 ± 0.01 m, body mass 66.9 ± 1.8 kg, VO2max 54.2 ± 2.0 ml kg^{-1} min^{-1}; means ± SEM) performed 2 h cycling (started at 09:00) at 55% peak power (143 ± 4 W) or a separate resting control trial in a counterbalanced order after an overnight fast, separated by at least 6 days. No food was consumed, though water ingestion was allowed ad libitum, until the trials finished at 20:00. Venous blood samples were collected at pre-exercise (pre-EX), immediately post-exercise, and at 3, 6 and 9 h post-exercise. Haematological analysis was performed using an automated cell counter. Plasma hormone concentrations were determined using ELISA kits. fMLP (N-formyl-MET-LEU-PHE)-induced oxidative burst activity was measured using a chemiluminescence (CL) assay (Knight Scientific Limited, Plymouth) and bacteria-stimulated neutrophil degranulation (elastase release) was determined as described by Li & Gleeson (2005). Results were analysed using a two-factor (trial × time) repeated measures ANOVA with post hoc Tukey tests and paired t tests applied where appropriate.

The 2-h cycling bout significantly altered circulating neutrophil count ($P<0.01$) and functions (both $P<0.01$) and plasma stress hormone concentrations ($P<0.01$) as shown in Table 1. The impact of the cycling on neutrophil oxidative burst and degranulation capacities on a per cell basis and plasma adrenaline and cortisol, compared with resting control, recovered within 6 h, 9 h, 3 h, and 9 h post-exercise, respectively; whereas neutrophil count did not recover within 9 h post-exercise. These findings suggest that the recovery interval between exercise sessions should be at least 9 h to allow recovery of neutrophil functions in the fasted state after a bout of prolonged strenuous exercise.

Table 1. The plasma glucose, adrenaline and cortisol concentrations, and blood neutrophil responses during the recovery period (0, 3, 6 and 9 h post-exercise).

		Pre-EX	0 h	3 h	6 h	9 h
Neutrophils	EX	2.56±0.34	9.60±0.58**¶¶	10.70±0.99**¶¶	7.85±0.61**¶¶	6.20±0.50**¶¶
(10^9 L^{-1})	RE	2.51±0.20	2.97±0.25	3.14±0.24	3.48±0.37	3.40±0.27
Elastase release	EX	293±19	180±10**¶¶	163±17**¶¶	204±13**¶	227±19**
(fg·cell^{-1})	RE	256±18	249±20	243±6	240±16	245±20
CL per neutrophil	EX	1.00	0.56±0.06**¶	0.66±0.06**¶	0.69±0.09**	0.55±0.06**
(fold of Pre-EX)	RE	1.00	0.95±0.12	0.98±0.12	0.84±0.12*	0.67±0.9**
Glucose	EX	5.26±0.08	4.36±0.23**	4.41±0.13**¶	4.35±0.11**¶¶	4.30±0.67**¶
(mM)	RE	4.87±0.22	4.81±0.17	4.92±0.24	5.03±0.18	4.73±0.15
Adrenaline	EX	1.32±0.06	3.15±0.31**¶¶	1.30±0.16		
(nM)	RE	1.50±0.13	1.51±0.12	1.68±0.26		
Cortisol	EX	343±31	580±53¶¶	347±30¶¶	293±42¶	177±32
(nM)	RE	342±44	201±26	220±30	186±16	149±20

Values are mean ± SEM (10=n). Significantly different from pre-EX (*$P<0.05$, **$P<0.01$); significantly different from resting control (RE) trial (¶$P<0.05$, ¶¶$P<0.01$)

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PC131

Comparison of exponential and linear incremental work rate protocols in cardiopulmonary exercise testing in healthy volunteers

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An exponentially increasing incremental work rate protocol can be used to accommodate a wide range of peak exercise capacities. (Northridge *et al.* 1990; Riley *et al.* 1992). Theoretical analysis suggests that peak gas exchange would not differ between exponential and linear protocols, but that the more complex kinetics with exponential increments might possibly affect the determination of the gas exchange anaerobic threshold (θ). (Fukuba et al. 2000). This study aims to compare exponential and linear protocols for peak $\dot{V}O_2$ and θ measurements using bicycle ergometer and treadmill.

Cardiopulmonary exercise tests were carried out using breath by breath gas analysis (Sensormedics Vmax 229) on six healthy male subjects aged 20-24 years. We used a standardised exponential exercise protocol (Northridge *et al.* 1990) in which the work rate was incremented each minute by 15% of the previous work rate on a bicycle ergometer (Lode Corival) and treadmill (Marquette Series 2000) and compared with linear protocols (25 W increments each minute on the ergometer) or equal grade increments at constant speed on the treadmill (modified Balke protocol). The modes of exercise were also compared. Subjects were blind to the protocol and randomly allocated to protocol in an orthogonal crossover design. An interval of at least 24 hours was allowed between tests. Peak $\dot{V}O_2$ was taken as the average $\dot{V}O_2$ in the last 30 s of exercise and θ from the accelerated $\dot{V}CO_2$ by the V-slope method, determined blind to both the protocol and mode of exercise. Intraclass correlation and analysis of variance were used to determine the reliability and significance of the results.

All subjects exercised to exhaustion on every test. There was no significant carry-over effect between tests. The mean [SD] peak $\dot{V}O_2$ was 3.2 [0.6] l min^{-1} with the linear protocol and 3.3 [0.7] with the exponential protocol (95% CL of difference -0.1 to +0.3; p=0.5). The mean [SD] θ was 1.61 [0.43] l min^{-1} with the linear protocol and 1.71 [0.44] with the exponential protocol (95% CL of difference -0.04 to +.24) (p=0.3). Intraclass correlation coefficients between protocols were 0.83 (95% CL 0.2 to 0.97) (p=0.01) for peak $\dot{V}O_2$ and 0.90 (95% CL 0.57 to 0.99) (p=0.001) for θ with a scatter close to the line of identity (Fig. 1).

Comparing modes of exercise, the mean peak $\dot{V}O_2$ was higher on the treadmill, 3.4 l min^{-1}, than the ergometer, 3.0 (95%CL of difference +0.2 to +0.6; p=0.01). The mean θ was also higher on the treadmill (1.76 l min^{-1}) than the ergometer (1.56; 95% CL of difference +0.06 to +0.34; P=0.05).
Thus there was agreement within confidence limits between exponential and linear incremental work protocols for both peak $\dot{V}O_2$ and θ measurements. Differences between the two modes of exercise confirm results from previous studies. (Shimizu et al. 1991; Riley et al. 1992)

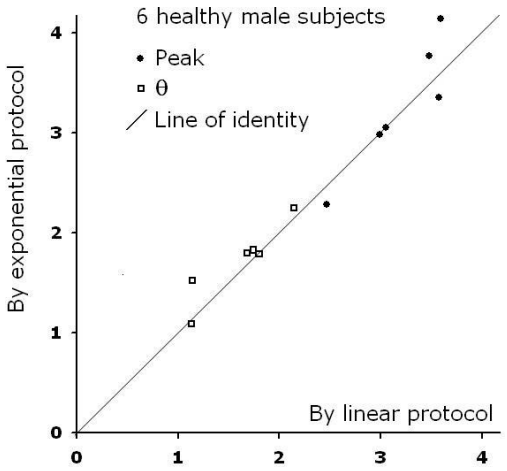

Figure 1. $\dot{v}\,O_2$ (l min^{-1}) means of treadmill and bicycle in 6 healthy male subjects

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Six male subjects (age 21 ± 2 years, height 176 ± 6 cm, body mass 81.9 ± 16.1 kg, $\dot{v}\,O_{2max}$ 48.6 ± 9.2 ml/min/kg) were tested twice in a randomised counterbalanced order (AB/BA design). Treatment A consisted of 100 intermittent jumps (one every 20 s) from a 40 cm high bench. This protocol is known to induce a long-lasting reduction in knee extensor strength (KES) (3). Treatment B was 33 min of rest. Before and 2 min after treatment, KES was measured with an isometric dynamometer chair. Twenty minutes after treatment, subjects exercised on a Lode cycle ergometer at 80% of their peak power output (281 ± 52 W) until exhaustion. Cardio-pulmonary measures at rest, during the first 6 min of exercise (isotime), and at exhaustion are presented. Blood lactate (La) was measured before and 1 min after exercise. A wash-out period of 10-14 days was prescribed to control for muscle damage. Muscle soreness and blood creatine kinase (CK) were measured before treatment. Values are mean ± SD. Paired t tests were used to compare treatments.
The wash-out period was effective as no evidence of muscle damage was found before treatment (Table 1). As expected, Treatment A significantly reduced KES compared to Treatment B with minimal metabolic disturbances (Table 1). This muscle fatigue significantly reduced time to exhaustion by 13% (Treatment A, 633 ± 238 s: Treatment B, 724 ± 274 s, P = 0.034). The only cardio-pulmonary response to exercise significantly affected by locomotor muscle fatigue was heart rate (Fig. 1). Post-exercise La was not significantly different between treatments (Treatment A, 9.3 ± 1.1 mmol/l; Treatment B, 10.5 ± 1.6 mmol/l, P = 0.137).
This experimental study demonstrates for the first time that locomotor muscle fatigue is an important determinant of exercise tolerance in humans. Compared to previous studies using pharmacologically induced muscle weakness (4,5), the effects of exercise-induced muscle weakness on the cardio-pulmonary and metabolic responses to exercise seem less significant. However, locomotor muscle fatigue might contribute to the cardiac drift observed during prolonged exercise.

Table 1. Pre-exercise conditions

Variable	Treatment A	Treatment B	*P*
Muscle soreness (0-6)	0.3 ± 0.5	0.7 ± 0.8	0.363
CK (IU/l)	146 ± 72	166 ± 72	0.640
Pre-treatment KES (N)	695 ± 145	681 ± 149	0.696
Post-treatment KES (N)	593 ± 163	678 ± 128	0.042
Pre-exercise La (mmol/l)	1.2 ± 0.3	1.0 ± 0.2	0.033

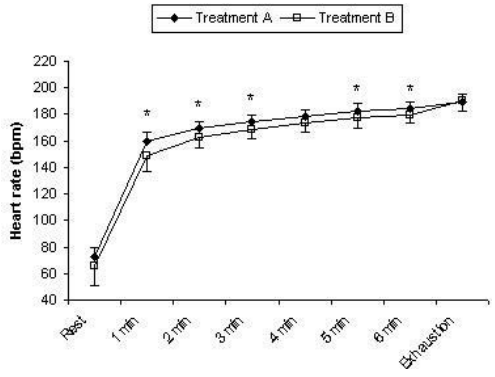

Figure 1. Heart rate response to exercise. * $P < 0.05$

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