Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C9

Cerebral exchange kinetics of nitrite and calcitonin gene-related peptide in acute mountain sickness

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An increased formation of nitric oxide (NO) and calcitonin gene-related peptide (CGRP) by the human brain in hypoxia may be implicated in the pathophysiology of acute mountain sickness (AMS) through direct activation of the trigemino-vascular system.

To test this hypothesis, we examined eleven healthy males aged 27 (mean) ± 4 (SD) years in normoxia (21.0%O_2) and following 9h passive exposure to normobaric hypoxia (12.9%O_2). Symptoms of AMS were recorded using the Lake Louise (LL) and Environmental Symptoms Questionnaire-Cerebral Symptoms (ESQ-C) scoring systems and headache determined using a visual analogue scale. Blood samples were obtained simultaneously from the radial artery and right internal jugular vein and assayed for plasma nitrite (NO_2^-) using ozone chemiluminescence and CGRP via radio-immunoassay. Global cerebral blood flow (CBF) was determined in the desaturation mode using inhaled nitrous oxide (5%) as the tracer (Kety and Schmidt, 1945). Cerebral plasma flow (CPF) was calculated as CBF x (1-haematocrit) and net flux determined as CPF x arterio-venous concentration difference ($a\text{-}v_{diff}$).

Hypoxia increased the LL (3.0 ± 1.9 *vs.* 0 ± 0 points, $P < 0.05$ *vs.* normoxia), ESQ-C (0.730 ± 0.683 *vs.* 0.000 ± 0.000 points, $P < 0.05$), and headache (20 ± 18 *vs.* 0 ± 0 mm, $P < 0.05$) scores (paired samples *t*-tests). CBF changed from 85 ± 15 to 96 ± 17 mL/100g/min in hypoxia ($P = 0.09$). Normoxia was associated with a positive $a\text{-}v_{diff}$ for NO_2^- (consistent with an influx) that was blunted by hypoxia due primarily to a reduction in arterial inflow (Table 1). In contrast, hypoxia affected neither arterial CGRP nor cerebral exchange and no relationships were observed between the change (hypoxia-normoxia) in the net flux of NO_2^- or CGRP respectively and LL (r = -0.14/-0.10), ESQ-C (r = -0.35/-0.23) or VAS (r = 0.33/-0.11) scores ($P > 0.05$, Pearson Product Moment Correlation).

In conclusion, our findings do not support a role for increased cerebral formation of NO_2^- and CGRP as molecular risk factors for AMS. On the contrary, hypoxia blunted the cerebral uptake of NO_2^-. Whether this reflects decreased consumption subsequent to a free radical-mediated reduction in systemic NO bioavailability and/or PO_2-driven re-apportionment of NO is the focus of current attention.

Table 1. Cerebral exchange

Inspirate:	Normoxia		Hypoxia	
Sample site:	Arterial	Venous	Arterial	Venous
NO_2^- (nmol/L)	474 ± 199	234 ± 85	291 ± 119*	277 ± 105
	Hypoxia < Normoxia (P < 0.05); Venous < Arterial (P < 0.05)			
Net flux (nmol/min/g)	124 ± 90		11 ± 47*	
CGRP (pmol/L)	71 ± 17	70 ± 16	70 ± 20	68 ± 20
Net flux (pmol/min/g)	0 ± 1		1 ± 2	

Values are mean ± SD; two-way repeated measures analysis of variance with paired samples *t*-tests; * different vs. normoxia ($P < 0.05$).

Kety SS and Schmidt CF (1945). *Am J Physiol* **143**, 53-66.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C10

Acute mountain sickness does not alter the coagulation cascade differently in susceptible and non-susceptible individuals

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Introduction: Previous research in our laboratory has identified that the neurological components of AMS, especially headache are a potential consequence of oxidative stress. Since activation of the coagulation cascade is potentially subject to redox regulation in-vivo, the current study aimed to assess the haemostatic differences in AMS (+) and AMS (-) individuals.

Methods: Following local ethical approval, and after gaining written, informed consent, 18 apparently healthy male volunteers were recruited.

Subjects were assigned days to attend the laboratory to be administered 6 hours passive exposure to normobaric hypoxia (Fraction of inspired oxygen [F_IO_2] 12%) in an environmental chamber. After 6 hours exposure, the subjects were asked to perform an incremental cycling test to volitional exhaustion. Blood was sampled at four time points F_IO_2 21% [Normoxia], 6 hours passive hypoxic exposure [Hypoxia (rest)], immediately post exercise in hypoxia [Hypoxia (Exercise)] and at 8 hours exposure: [Hypoxia (Recovery)].

Blood was sampled via an in-dwelling cannula into sodium citrate vaccutainers and tested for plasma levels of Fibrinogen (FB), Prothrombin Time (PT), Thrombin Time (TT) and Activated Thromboplastine Time (aPTT) using a Futura Pluss Coagulometer.

AMS was diagnosed using the Lake Louise (LL) score (clinical + self-assessment scores) of ≥5 points and an Environmental Symptoms Questionnaires (ESQ) cerebral score of ≥0.7 points at Hypoxia (Rest).

Results: Clinical AMS was diagnosed in 50% of subjects. Data were corrected for plasma volume changes where appropriate i.e. fibrinogen data were corrected, but TT, PT and aPTT were not as these data represent times. No differences in plasma levels of FB, PT, TT or aPTT were discovered between sub-groups.
Conclusions: Though exercise activated the coagulation cascade, as seen in significant changes in combined data for TT, PT and aPTT, the difference in response between AMS (+) and AMS (-) was not found to be significant. The alterations in vascular reactivity previously described by our group in AMS (+) cannot be ascribed to haemostatic alterations.

Sub Group	AMS (+)				AMS (-)			
Time	Normoxia	Hypoxia (Rest)	Hypoxia (Exercise)	Hypoxia (Recovery)	Normoxia	Hypoxia (Rest)	Hypoxia (Exercise)	Hypoxia (Recovery)
Fibrinogen (mg/dL)	230.1 ±43.5	227.3 ±41.9	234 ±42.7	239 ±33.0	257.1 ±37.7	247.3 ±94.7	253.9 ±77.5	278.6 ±59.7
TT (s)	14.6 ±1.0	15.1 ±1.2	15 ±1.4	15.1 ±1.2	14 ±0.7	14.3 ±1.3	14.9 ±1.4	13.9 ±0.4
PT (s)	13.4 ±0.8	13.8 ±0.9	13.5 ±0.8	14 ±0.9	13 ±0.7	13.9 ±1.2	13.5 ±0.7	13.7 ±0.8
APTT (s)	27 ±1.7	24.6 ±2.3	22.9 ±1.8	24.2 ±2.0	26.6 ±2.2	25.5 ±3.3	22.9 ±3.0	24.1 ±2.7

Table 1: Data expressed as means ± standard deviations. TT, PT and aPTT showed significant ($p<0.05$) main effect changes for time, but no significant group differences.

Data Table

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C11

Decreased Fischer's ratio and increased cerebral delivery of phenylalanine after LPS infusion in healthy humans

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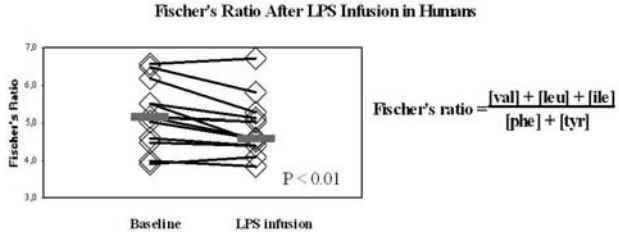
Sepsis is frequently complicated by cerebral dysfunction, so-called sepsis-associated encephalopathy, which may be related to the presence of systemic inflammation. The mechanisms underlying this phenomenon remain unclear; however, a decrease in the plasma ratio between branched-chain and aromatic amino acids (Fischer's ratio) has been speculated to play a role. This may cause a subsequent increase in the cerebral uptake of the aromatic amino acid phenylalanine, which can cause neurological symptoms. We therefore hypothesised that a decrease in Fischer's ratio would occur along with an increased cerebral delivery and exchange of phenylalanine during a human-experimental model of systemic inflammation.
Twelve healthy male volunteers aged 20-33 (median 26) years were enrolled in the study after ethical approval as well as oral and written informed consent. Volunteers underwent a four-hour intravenous infusion of Escherichia coli lipopolysaccharide (LPS) (total dose of 0.3 ng/kg). Global cerebral blood flow (CBF) measurements were performed by means of the Kety-Schmidt technique using inhaled nitrous oxide [1], and arterio-jugular venous differences of aromatic and branched-chain amino acids were determined before and one hour after the cessation of LPS infusion, by use of high-performance liquid chromatography (HPLC). Cerebral delivery and net exchange of amino acids were then calculated by multiplying CBF with the arterial concentrations and arterio-jugular venous differences of amino acids, respectively.
An increase in the circulating levels of phenylalanine ($P < 0.01$, paired samples t-test) with unaltered levels of branched-chain amino acids occurred after LPS infusion, thus causing a decrease in Fischer's ratio ($P < 0.01$, paired-samples t-test). CBF was unaltered after LPS infusion; however, there was an increase in the cerebral delivery of phenylalanine ($P < 0.05$, paired-samples t-test), whereas the cerebral net exchange of phenylalanine remained unchanged ($P = 0.8$, paired-samples t-test).
In conclusion, Fischer's ratio declines and the circulating levels and cerebral delivery of the aromatic amino acid phenylalanine increased after LPS infusion in humans. However, this was not associated with a change in the cerebral net exchange of phenylalanine.



Kety & Schmidt (1948). J Clin Invest 27, 476-83.

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C12

Cerebral blood flow and oxygen metabolism measured with the Kety-Schmidt method using inhaled N_2O in healthy volunteers

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BACKGROUND: The Kety-Schmidt method (1) is the reference method for measuring global cerebral blood flow (CBF), metabolic rate (CMR) and net exchange with the blood in humans in settings where access to a scanner is limited or impractical. The method was originally developed with inhaled N_2O as the

tracer; both the saturation and desaturation mode as well as continuous and discontinuous blood sampling have been used, but the literature on the performance of the method is scarce. Photoacoustic spectrometry is a novel method for measuring N_2O concentration. The aim of the present study was to characterise the Kety-Schmidt method using inhaled N_2O in the washout phase, with continuous or discontinuous blood sampling, and with photoacoustic spectrometry for measuring N_2O concentration.
METHODS: Following ethical approval, a thorough physical examination, and informed consent, twenty-nine healthy male volunteers underwent 61 CBF measurements. Volunteers breathed a normoxic gas mixture containing 5% N_2O until tension equilibrium, following which the N_2O supply was terminated. Paired blood samples were collected simultaneously from an arterial and a jugular bulb catheter during washout, by continuous or discontinuous blood sampling. N_2O concentration in blood samples was measured with photoacoustic spectrometry after equilibration of the samples with air; we calculated a modified coefficient of variation (mCV) for this measurement. CBF was calculated by the Kety-Schmidt equation; CMRO2 was determined according to the Fick principle. Parametric statistical analysis was performed, including the calculation of the repeatability coefficient and limits of agreement (2).
RESULTS: The mean difference in CBF between discontinuous and continuous sampling was 4 ml/100g/min (limits of agreement of -23 to 34 ml/100g/min). CBF measurements based on discontinuous blood sampling provided the most reliable and reproducible values (Table). The mCV for N_2O concentration measurements was 4.0%.
CONCLUSION: The Kety-Schmidt method with inhaled N_2O, discontinuous blood sampling and N2O measurement with photoacoustic spectrometry may be used for measurement of global CBF and CMR.
CBF and CMR Using Continuous and Discontinuous Sampling

Sampling method	CBF rest(ml/100g/min)	CMRO2 rest(mcmol/g/min)	Repeatability coefficient(ml/100g/min)
Discontinuous N=28)	64 (59; 71)	1.9(1.7; 2.0)	11(N=6)
Continuous (N=18)	57 (51; 63)	1.6 (1.4; 1.7)	25(N=3)

Mean (95% C.I.). Repeatability coefficient calculated according to (2).

Kety SS & Schmidt CF. J Clin Invest 1948;27:476-84.

Bland JM & Altman DG. Lancet 1986;1:307-10.

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C13

Effects of hyperoxic exercise on post-exercise haemodynamics and redox regulation of circulating NO bioavailability in man

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The detrimental effects of hyperoxia on haemodynamics have been shown to persist for some time following return to normoxia (Waring et al, 2003; Thomson et al, 2006). The present study sought to determine the role of a redox-mediated regulation of circulating Nitric Oxide (NO) bioavailability as a mediator of the underlying augmented vasoconstriction following hyperoxic exposure.
To examine this hypothesis, 9 pre-hypertensive males, mean arterial pressure (MAP) = 106 (mean) ± 5 (SD) mmHg (50 ± 10 yr), not on medication, were studied following 30-minutes of cycle exercise at 70% normoxic maximal oxygen consumption in hyperoxia (50% O_2) and normoxia (21% O_2). Echocardiography determined cardiac output (Q) and systemic vascular resistance (SVR)was computed by the quotient of MAP & Q. Venous blood was sampled from an antecubital vein pre-, immediately post-, 1-hour post- (P1) and 2-hours post- (P2) exercise and corrected for plasma volume shifts. Plasma nitrate (NO^-_3) and nitrite (NO^-_2) were determined fluorometrically, whilst S-Nitrosothiol (RSNO) concentrations were assayed by the Saville reaction. Indirect markers of oxidative stress were determined spectrophotometrically detecting lipid hydroperoxides(LOOH).
Hyperoxic exercise blunted post-exercise haemodynamics by significantly attenuating the reductions (from normoxic baseline) in SVR (21 ± 20.7 *vs.* 38 ± 19.3 %, 11 ± 8.2 *vs.* 19 ± 5.5 % and 11 ± 8.9 *vs.* 15 ± 9.6%, $P<0.05$ *vs.* normoxic exercise at post, P1 and P2 respectively) and MAP (3 ± 4 [elevation] *vs.* 0.5 ± 5 mmHg, 3 ± 3 *vs.* 6 ± 4 mmHg, 3 ± 3 *vs.* 4 ± 3 mmHg, $P<0.05$ *vs.* normoxic exercise at post, P1 and P2 respectively) (Paired samples T-tests). Indices of NO metabolism, total plasma NO and LOOH failed to differ between conditions ($P>0.05$, Paired samples T-tests; Table 1).
In conclusion, our results indicate that hyperoxic exercise has a deleterious effect on post-exercise haemodynamics and do not support a role for a redox-mediated regulation of circulating NO bioavailability as being a principle governor of the attenuated vasodilatation following hyperoxic exercise. This suggests that the vasoconstriction is resultant from a metabolic pathway independent of NO.
Table 1. Systemic Venous Biomarkers of NO and ROS Metabolism

F_IO_2		0.21			0.50	
	NO^-_3 (µmol/L)	NO^-_2 (µmol/L)	LOOH(µmol/L)	NO^-_3 (µmol/L)	NO^-_2 (µmol/L)	LOOH(µmol/L)
Pre	10.8 ± 3	1.1 ± 1	0.8 ± 0.2	14.6 ± 9	1.8 ± 1	0.6 ± 0.3
Post	11.3 ± 5	1.8 ± 1	0.7 ± 0.1	13.3 ± 8	1.2 ± 1	0.7 ± 0.3
P1	9.9 ± 2	1.4 ± 1	0.9 ± 0.1	13 ± 6	1.1 ± 1	0.9 ± 0.2
P2	8 ± 3	1.1 ± 1	0.8 ± 0.2	10 ± 6	1.8 ± 1	0.9 ± 0.1

F_IO_2, fraction of inspired oxygen. Values are mean ± SD; two-way repeated measures ANOVA. *n*=9 for each group.

Thomson AJ *et al* (2006). *J Appl Physiol* **101**, 809-816.

Waring WS *et al* (2003). *J Cardiovasc pharmacol* **42**, 245-250.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C14

Regulation of skeletal muscle and systemic blood flow in humans: Role of erythrocyte ATP and NO release

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Current models of circulatory control postulate that the red blood cell (RBC) acts as an O2 sensor and regulator of local muscle blood flow at rest and during exercise in humans (González-Alonso et al. 2001) via three signalling mechanisms: 1) ATP release in proportion to the degree of oxyhaemoglobin saturation (FO2Hb) (Ellsworth et al. 1995); 2) S-nitrosohaemoglobin (SNO-Hb)-dependent vasodilatation (Stamler et al. 1996), and 3) reduction of plasma nitrite to vasoactive NO by deoxyhaemoglobin (Cosby et al. 2003). Whether these local signals also mediate the cardiac output (Q) response is presently unknown. Thus, the aim of this study was to examine whether alterations in leg blood flow (LBF) and Q with graded hypoxia and hyperoxia at rest and during exercise are intimately related to changes in FO2Hb and alterations in either plasma ATP, SNO-Hb and/or nitrite. To this aim, we measured leg and systemic haemodynamics, O2 transport and arterial and femoral venous haematological variables together with SNO-Hb, ATP and nitrite at rest and during 6-min constant-load knee-extensor exercises (25±1 W or ~30% of peak power; mean±SEM) in 7 active male subjects (27±2 yr) in normoxia and under 10%, 13%, 16%, 48% and 100% FiO2. Data were analysed by a one-way ANOVA with repeated measures and Pearson product moment correlations. By design, arterial FO2Hb at rest and during exercise was gradually increased from 75 to 99 % and 70 to 99 %, respectively. LBF and Q were linearly related to changes in arterial FO2Hb at rest (r=-0.45 and r=-0.77; P<0.05) and during exercise (r=-0.39 and r=-0.69; P<0.05), such as leg and systemic O2 delivery was kept constant across conditions. Despite the large changes in FO2Hb at rest and during exercise, arterial and venous plasma ATP, SNO-Hb and nitrite remained unchanged. However, when all conditions are taken into account, lower FO2Hb correlated positively with plasma nitrite (r=0.27; P<0.05) and negatively with SNO-Hb (r=-0.29; P<0.05). At rest and during exercise, LBF and Q correlated with arterial plasma nitrite (r=-0.34 to -0.66) whereas during exercise LBF also correlated with venous plasma nitrite (r=-0.60; P<0.05). In conclusion, limb muscle and systemic blood flow are closely tied to blood oxygenation over a wide range of O2 availability. Few changes appeared in plasma ATP, SNO-Hb and nitrite, possibly because any increase in their concentrations might be followed by rapid degradation, utilisation or scavenging. Nevertheless, plasma nitrite were coupled to FO2Hb and LBF suggesting that the consumption of nitrite may be essential to local muscle flow regulation in which SNO could be a marker of nitrite reduction by the erythrocyte.

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C15

***In vitro* quantification of lipid-derived free radical species: relevance to exercise and ascorbate prophylaxis**

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Alkoxyl free radical species have been identified by Electron Paramagnetic Resonance (EPR) spectroscopy following exercise and it is postulated that this radical originates from the phospholipid membrane (Davison et al. 2006). However, no conclusive evidence has been provided to confirm this supposition. We therefore tested the hypothesis that exercise-induced oxidative stress is caused by free radical-mediated damage to polyunsaturated fatty acids (PUFA) which can be prevented following ascorbate prophylaxis.

Hyperfine coupling constants (HCC) of alpha-phenyl-tert-butylnitrone (PBN)-adducts were measured via room temperature EPR spectroscopy in the venous blood of 12 subjects at rest and following maximal exercise and compared to those observed following room-air incubation (2 hr at 37oC) of L-alpha-phosphatidycholine, linoleic acid, alpha-linolenic acid and arachidonic acid. Subjects also received an acute oral bolus dose of either 1000mg of ascorbic acid or placebo (6 placebo and 6 ascorbic acid), 2 hrs prior to the exercise challenge in a randomised, double-blind, placebo-controlled design, while ascorbic acid was also added to the in vitro model. All adducts exhibited similar HCC [aN 13.6 Gauss (G) and abetaH 1.8G] with the exception of L-alpha-phosphatidycholine [aN1= 13.4G, abetaH1 = 1.6G (37%) and aN2 = 14.9G, abetaH2 = 0.3G (63%)] consistent with the trapping of lipid-derived alkoxyl and oleate radicals respectively. Ascorbic acid pre-treatment ablated free radical formation in both model systems. Furthermore, the

decrease in PBN-adduct concentration by ascorbic acid was not an artefact associated with adduct instability and subsequent reduction to an EPR "silent" hydroxylamine.
These findings suggest that PBN-trapped free radicals detected following human exercise are likely derived from the oxidation of polyunsaturated fatty acid membranes. Furthermore, clear ex vivo and in vitro evidence suggests that ascorbic acid is an effective antioxidant when required to terminate lipid peroxidation and inhibit the generation of oxygen-centred alkoxyl radicals.

Davison GW et al. (2006). Clin Sci 110, 133-141.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C16

Heat shock and antioxidant protein adaptations of human skeletal muscle to high intensity interval running training: a comparison of the vastus lateralis and gastrocnemius muscle

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Skeletal muscle adapts to the stress of acute (Morton et al., 2006) and chronic (Morton et al., in press) exercise via increased content of heat shock proteins (HSPs) and antioxidant defences. An increased content of such defences following stress function to restore cellular homeostasis and to protect the cell from further insults. A consistent elevation of 'stress proteins' during training may therefore be a crucial component of the cellular mechanisms by which regular exercise provides protection against exercise-induced muscle damage. Adaptations of HSPs and antioxidants following running exercise have only been studied in the vastus lateralis, despite the gastrocnemius muscle being more metabolically active in this exercise mode(Costill et al., 1974). The aim of this study was to compare HSP and antioxidant adaptations in the vastus lateralis and gastrocnemius following high intensity interval training.
Eight males performed 50 min of high intensity intermittent running exercise, four times per week for six weeks. The protocol consisted of five 3 min bouts at 90% 2max separated by 3 min active recovery periods (1.5 min at 25% 2max followed by 1.5 min at 50% 2max). A 10 min warm-up and cool down period at 70% 2max was also performed. Assessments of 2max, running economy (RE) and performance on a Yo-Yo intermittent recovery 2 test (IRT2) were performed before and after training. Resting muscle biopsies were obtained from the lateral portion of the gastrocnemius and from the mid-portion of the vastus lateralis muscle pre- and post-training. Training induced significant ($P<0.05$) improvements in 2max (10%), RE (4%) and IRT2 (16%). Training resulted in significant increases ($P<0.05$) in HSP60 (22%), αB-crystallin (52%), MnSOD (38%) and a tendency ($P<0.09$) for an increase in HSP70 (20%) content of the gastrocnemius. In contrast, only αB-crystallin (22%) and MnSOD (37%) significantly increased ($P<0.05$) in the vastus lateralis following training. HSP27 was unchanged in either muscle following training.
This study is the first to examine HSP and antioxidant protein adaptations of the gastrocnemius and vastus lateralis muscle to exercise. Data demonstrate that short-term high intensity intermittent exercise induces a more pronounced up-regulation of stress proteins in the gastrocnemius muscle compared with the vastus lateralis. This differential response between muscles is likely due to differences in recruitment patterns (and hence greater activation of associated signalling pathways) during this mode of exercise. We therefore suggest that the gastrocnemius muscle is a more suitable muscle for which to study the exercise-induced stress response of human skeletal muscle during running.

Costill D et al (1974). Acta Physiol Scand 91, 475-481.

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C17

Exercise-induced cardiac stem cell activation and ensuing myocyte hyperplasia contribute to left ventricular remodelling

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Traditionally, it was thought that exercise improved cardiac function by only increasing myocardial mass and contractility through physiological hypertrophy of existing myocytes. Recent data on myocardial cell homeostasis and the identification of cardiac stem cells (CSCs), in the adult mammalian heart, could challenge this concept. We sought to assess if CSC activation and ensuing myocyte formation participates in cardiac remodeling induced by exercise. To this aim, 36 FVB mice underwent a programme of controlled swimming exercise (Ex) and were sacrificed at different time points over 28 days. Ten untrained mice acted as sedentary controls (Sed). To track myocardial proliferation, BrdU was administered (i.p.) twice daily. Hearts were processed for immunohistochemistry and confocal microscopy analysis (n=30) and c-kit-positive CSCs and myocytes were also isolated for molecular analyses (n=16). Results showed that exercise training resulted in increased heart:body weight ratios in Ex (6.1±0.7mg/g, Mean±SD) vs. Sed (4.3±0.2mg/g) mice. Average myocyte volume was greater in

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This study was funded by the BBSRC.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C48

Development and validation of the QPatch medium-throughput patch clamp electrophysiology assay for brain sodium channel inhibitors

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Sodium channel inhibition is an established mechanism that can confer anticonvulsant efficacy across a broad spectrum of seizure types [1-3]. However, a quantitative relationship between sodium channel inhibition and clinical anticonvulsant efficacy has yet to be determined. Traditional patch-clamp electrophysiology is the in vitro gold standard for measurement of compound activity on ion channels. However it has limited use for the evaluation of many drugs due to low throughput. The QPatch automated planar array patch-clamp system has been designed to increase throughput. The aim of this study was to develop and validate a robust electrophysiology assay using QPatch to define the interaction of drugs with recombinant human Nav1.2 sodium channels. In particular, to allow estimation of the affinity of drugs for the inactivated state of the channels (Ki). This value could then be used to explore the relationship between drug potency observed in vitro and brain concentration required for efficacy in vivo.

A first set of experiments was performed to assess assay fidelity and recording quality. A classic voltage step protocol was applied consisting of depolarising voltage steps (10 ms), increasing by 10 mV from -40mV to +40 mV from a holding potential of -90 mV. The I-V plot obtained suggests that the inward current reverses at around +50 to +60 mV, consistent with the reversal potential of sodium ions observed using a manual patch clamp assay. Secondly, the sensitivity of the QPatch assay to DMSO, used to dissolve test compounds, was assessed. The data obtained suggest that a maximum concentration of 0.3% can be reached without affecting recorded currents.

In a further set of experiments the viability of whole cell recordings over time was determined. For these experiments, test pulses to 0 mV were applied at a regular interval. Currents remained stable for at least 20 minutes.

Finally, a steady state inactivation protocol was applied to determine the affinity of compounds for the inactivated state (Ki)[4]. HEK-hNav1.2 cells were held at -120 mV and stepped to different conditioning voltages (-120/-40 mV) for 9 s to induce steady state inactivation. At the end of each conditioning period the cell was stepped to +20 mV for 2 ms to elicit sodium current. For each recording, the peak current was plotted against the conditioning voltage and the data fitted to a Boltzmann equation from which the half-maximal inactivation voltage (Vh) could be determined. Application of drug caused a concentration-dependent leftward shift of the inactivation curve. By plotting the shift in Vh against drug concentration it was possible to estimate the Ki of the compound tested. The results show that the QPatch system provides a robust assay that can be used to evaluate the interaction of compounds with recombinant human Nav1.2 sodium channels.

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Kuo and Lu, Characterization of lamotrigine inhibition of Na+ channels in rat hippocampal neurones1997, B.J.P. 121, 1231-1238

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C49

Uncoupling protein 3 inversely correlates with resting metabolic rate in fit, young men

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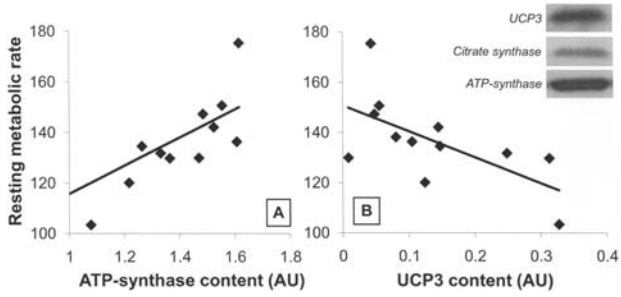
Uncoupling protein 3 (UCP3) is a mitochondrial protein found principally in skeletal and cardiac muscle, and thought to play a role in reducing reactive oxygen species (ROS) production via respiratory uncoupling. We therefore examined the relationships between UCP3, resting metabolism and oxidative modification of myocellular proteins in fit, young men.

METHODS: Twelve male subjects were recruited from the Oxford colleges' rowing crews. Resting metabolic rate (RMR) was estimated from respired gases during a ten-minute period of quiet sitting. Percutaneous needle biopsies were taken from the *m. vastus lateralis* of each subject under local anaesthetic (5ml of 2% Lidocaine), and snap-frozen in liquid nitrogen. UCP3, citrate synthase and ATP-synthase protein content were measured by western blotting. Protein carbonylation was measured using the DNPH-derivitization method of Levine *et al*.[1]. All western blots were performed in duplicate.

RESULTS: We observed an inverse correlation between UCP3 and mass$^{2/3}$-adjusted RMR ($r = -0.63$, $P < 0.05$, $n = 12$, Fig. 1B). This relationship remained after UCP3 was normalised to either citrate synthase or ATP-synthase ($r = -0.66$ and $r = -0.69$, both

$P < 0.05$, $n = 12$). Furthermore, there was a positive correlation between mass$^{2/3}$-adjusted RMR and ATP-synthase content ($r = 0.65$, $P < 0.05$, $n = 12$, Fig. 1A). There was no significant relationship between UCP3 level and protein oxidation.

DISCUSSION: Mitochondrial ROS-production is highly dependent upon the inner membrane potential, or protonmotive force. As UCP3 knock-out mice display increased skeletal muscle ROS-production, it has been suggested that UCP3 may act as an antioxidant by dissipating the protonmotive force under certain conditions. We observed an inverse relationship between UCP3 and RMR, the opposite of what would be expected if UCP3 were a significant uncoupler of oxidative phosphorylation. However, UCP3 is lower in trained than untrained individuals, and RMR may be higher in trained individuals. The observed relationship may therefore be secondary to a range of physical fitness in our cohort. We did not observe a relationship between UCP3 and myocellular protein oxidation. Taken together, our data show that fit, young men with a high metabolic rate have a higher content of the ATP-synthase, but a correspondingly lower content of UCP3, with perhaps all being due to a higher level of physical fitness.



The relationships between mass-adjusted resting metabolic rate (in kcal day^{-1} kg$^{-2/3}$) and (A) ATP-synthase and (B) UCP3 content in whole muscle homogenates ($n = 12$). AU = arbitrary units.

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz A-G, Ahn B-W, Shaltiel S, Stadtman ER, Lester Packer and Alexander NG. (1990) *Methods in Enzymology* **186**, 464-478.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C50

Metabolic responses to intermittent and continuous exercise in Type 1 diabetes patients

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Hypoglycaemia is a frequent occurrence in Type 1 diabetes mellitus (T1DM) patients following endurance exercise (Tsalikian et al., 2005). Interestingly, completion of a short sprint following endurance exercise significantly reduces the degree of post-exercise hypoglycaemia in T1DM patients (Bussau et al., 2006). However, the impact of prolonged intermittent high intensity exercise patterns on the post-exercise glucose responses of T1DM patients is unclear. The aim of this study was to compare the metabolic responses of T1DM patients following intermittent and continuous exercise.

With local ethics committee approval and informed consent 9 non-diabetic (ND) and 9 T1DM patients participated in this study. Physical and physiological characteristics of ND and T1DM respectively were (mean±SD); age: 27±7, 35±12 years; body mass: 79±6, 84±12 kg; HbA1c: 5.3±0.3, 8.1±0.6 %; VO_2max: 48.3±8.8, 41.8±4.9 ml kg min^{-1}. After two preliminary visits participants completed two main 45 min exercise trials in a randomised order, an intermittent running protocol designed to simulate intermittent games play (INT, Nicholls et al., 2000) and a continuous treadmill run (CON) that matched the mean rate of oxygen consumption of INT. Venous blood samples (10 ml) were taken at rest and for 3 h post-exercise to determine blood glucose and lactate concentrations. A continuous glucose monitor measured interstitial glucose concentrations for 24 h post-exercise. Data were analysed using a two-way ANOVA with post-hoc testing where appropriate and significance was established at $P<0.05$.

The rate of oxygen consumption (expressed as a percentage of VO_2max) was similar between conditions (ND: CON 72±5, INT 73±5; T1DM: CON 77±8, INT 77±5 %, NS). Peak blood lactate concentrations were greater in INT compared with CON (INT: ND 6.8±3.0, T1DM 9.5±2.8 mM vs. CON: ND 1.9±0.8, T1DM 3.7±2.1 mM, $P<0.05$). Blood glucose concentration decreased significantly in T1DM compared to ND immediately after CON (ND 0.6±1.0 vs. T1DM -4.1±2.9 mM, $P<0.05$) and INT (CON 1.7±1.1 vs. T1DM -1.5±4.3 mM, $P<0.05$) and remained lower for 3 h post-exercise ($P<0.05$). Interstitial 24 h AUC glucose profiles were greater in T1DM compared to ND (T1DM: CON 2432±922, INT 2542±547 mM.24 h^{-1} vs. ND: CON 1540±262, INT 1598±69 mM.24 h^{-1}, $P<0.05$) but there was no significant difference between INT or CON conditions within each group. The results demonstrate a significant reduction in blood and interstitial glucose concentrations of T1DM patients following both INT and CON exercise. However, there were no significant differences in glucose concentrations between the exercise types when performed at the same physiological intensity, despite a different anaerobic contribution. These data suggest the pattern of exercise does not influence the degree of post-exercise hypoglycaemia when the oxygen demand of exercise is similar.

Bussau VA, et al. (2006). Diabetes Care. 29(3):601-606.

Nicholas CW, Nuttall FE and Williams C. (2000). J Sports Sci. 18:97-104.

Tsalikian E, et al. (2005). J Paediatrics. 147(4):528-534

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C51

Insulin resistance is developed after two weeks of pace reduction in healthy humans

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Background: The Centers for Disease Control and Prevention has designated physical inactivity as an actual cause of chronic disease, although there is little direct physiological evidence proving that reductions in daily physical activity increase the risk of disease. Therefore we wanted to determine the metabolic consequences of a reduced number of daily steps.
Design: Ten healthy young men decreased their daily activity level from a median of 10,053 (range 6,854 -13,218) to 1,381 (range 1,061 –1,658) steps/day for 2 weeks. Pre- and post-test were done during ambulatory care at the research institution. The period with reduced stepping was implemented in the participant's daily living. Dietary records were taken throughout to ensure that habitual dietary intake was maintained. The study was performed according to the Declaration of Helsinki and approved by The Scientific-Ethical Committee of Copenhagen and Frederiksberg Municipalities, and participants provided written informed consent.
Method: The study's primary outcomes were 1) insulin sensitivity measured with a hyperinsulinemic-euglycemic clamp with stable isotopes, 2) insulin signalling (tyrosine (tyr) phosphorylation (p) of the insulin receptor (IR) beta (pTyrIRbeta), pThr308Akt, and pAS160) in skeletal muscle biopsies (Western blotting), and 3) maximal oxygen consumption (VO2max) and body composition (measured by DXA scanning). Statistical analysis was done by analysis of variance followed by Bonferroni-corrected t tests or by paired t-tests.
Results: A reduced number of daily steps induced a decline in peripheral insulin sensitivity (Paired t-test $p < 0.01$) without an effect on endogenous glucose production. Insulin-stimulated pThr308Akt decreased after pace reduction (ANOVA $p<0.01$) with a post hoc analysis revealing the most pronounced effect after 4 h of insulin infusion ($p<0.05$). There was no change in pTyrIRbeta and pAS160 and the protein expression of IR, Akt, and AS160 was not affected by insulin stimulation or intervention. In addition, a 7% decline in VO2max (ml/min) (Paired t-test $p < 0.01$) and a decline in fat-free mass (1.2 kg) (Paired t-test $p < 0.001$) were found.
Conclusion: One biological cause for the enormous public health problem of type 2 diabetes and central obesity has been identified. A reduction in daily physical activity as implemented by a reduction in the number of daily steps in healthy young men for only two weeks induces insulin resistance with a reduction in insulin-stimulated pAktthr308; this effect may represent one link between an inactive lifestyle and increased risk of chronic disease.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C52

Glucose regulates the glucose-6-phosphatase catalytic subunit 3 promoter in HeLa cells

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Extra-hepatic tissues contain G6PC3, a different isoform of the glucose-6-phosphatase catalytic subunit than that found in liver (Guionie et al, 2003). The regulation of G6PC3 is poorly understood. This protein is thought to be involved in local glucose supply. Inability to upregulate its synthesis appropriately may be associated with intra uterine growth retardation, delayed or impaired neural development, sudden infant death, diabetes, or other problems caused by disordered glucose metabolism. A luciferase reporter gene system was used to investigate the regulation of the G6PC3 promoter by physiological concentrations of various metabolites. HeLa cells were transiently transfected with a DNA construct containing one of 4 common polymorphisms of the G6PC3 promoter coupled to the luciferase gene. Tranfected cells were exposed to glucose concentrations representing extreme hypoglycaemia (1mM), cord blood/ fasting blood glucose (3.5mM), postprandial blood glucose (5.5mM) and moderate (15mM) to high (25mM) diabetic levels (n=5). The effects on G6PC3 promoter activity of 18 hours exposure to 3.5-10 mM lactate, pyruvate, acetate and β-hydroxybutyrate (β-HBA) were also investigated. The cells were harvested and luciferase activity and protein concentration were measured.
There was no significant difference between the four promoter polymorphisms in their response to glucose (2-way ANOVA: $F(3, 99) = 1.09$; $p = 0.359$). At 1mM glucose luciferase activity was 17.87 ± 2.13 times higher than that of the empty vector. Promoter activity at all other glucose concentrations was significantly higher than at 1mM glucose (2-way ANOVA: $F(4, 99)$; $p<0.001$). Activity increased 4.8 ± 0.75 fold from 1mM to 5.5mM glucose, then plateaued. This differs markedly from the response of the catalytic subunit isoform found in liver, G6PC1, which is only stimulated by fasting conditions or high (20mM+) glucose concentrations (Massillon et al, 1996; Massillon, 2001). The equivalent number of carbon units of pyruvate to that found in 5.5mM glucose media was added to the two lowest glucose concentrations. This restored G6PC3 promoter activity to levels similar to that seen at 5.5mM glucose (ANOVA: $p>0.05$), suggesting that other metabolic fuels can regulate activity of the gene. However, lactate, β-HBA and acetate were without effect (ANOVA: $p>0.05$). The G6PC3 promoter does not contain any known consensus glucose responsive or carbohydrate responsive sequences and the mode of regulation by glucose and pyruvate is unknown. Studies to further characterise the mechanism of carbohydrate regulation of G6PC3 are underway.

Guionie, O *et al.* (2003). *FEBS Letters* **551**, 159-164.

Massillon, D *et al.* (1996) *J Biol Chem* **271** , 9871-9874.

Massillon, D (2001) *J Biol Chem* **276**, 4055-4062.

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C53

Differential responses to exercise at 40% and 75% of 1 repetition maximum of myofibrillar protein synthesis and anabolic signalling in muscle of postabsorptive young and old men

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Resistance exercise stimulates myofibrillar protein synthesis (MPS) but the influence of exercise intensity and age on MPS is poorly understood. The present aim was to investigate how MPS and activation of anabolic signalling was affected by resistance exercise at two different intensities in postabsorptive healthy men, young (n=3, 25±5 y, body mass index (BMI) 23±2 $kg.m^{-2}$) and elderly (n=4, 69±3 y, BMI 24±2 $kg.m^{-2}$). We hypothesized that phosphorylation of signaling molecules and MPS would be increased by resistance exercise in a dose dependant fashion in young and elderly men but to a lesser extent in the older men. The subjects performed isotonic unilateral leg extension at 40 and 75% of 1 repetition maximum with similar total work outputs. Muscle biopsies were taken from the vastus lateralis of the exercised leg under local anaesthesia (1% lignocaine sc) before, immediately after and 1, 2 and 4 h after exercise. After separation of myofibrillar protein, incorporation of [1,2-^{13}C]leucine was used to measure MPS (by gas chromatography-combustion-mass spectrometry using plasma labeling of plasma α-ketoisocaparoate as surrogate precursor); Western analysis of sarcoplasmic protein, using anti-phosphoantibodies was used to measure the phosphorylation state of a variety of molecules involved in regulation of initiation of protein synthesis.

The results (Figure 1) demonstrate that: (i) exercise at either intensity acutely reduced phosphorylation of 4EBP1, consistent with a decrease in initiation of protein synthesis, but only increased it above basal in the post exercise period in the young men; (ii) in young men, exercise at both intensities increased MPS significantly, with increases at 75% 1RM tending to be larger than those at 40%, but exercise was not associated with any significant increase above basal in elderly men.

The anabolic resistance of MPS to amino acids in the elderly previously reported by us (Cuthbertson et al 2005) appears to have a counterpart in the responses of the processes of muscle protein synthesis after exercise in the absence of additional amino acids. There appears not to be a close relationship between the extent of activation of initiation of protein synthesis and the degree of stimulation of MPS after exercise.

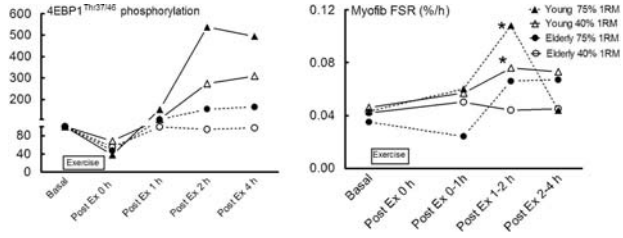

Figure 1. Effects of resistance exercise on phosphorylation of binding protein of eukaryotic initiation factor 4E (4EBP1) (arbitrary units, % basal) and MPS (%/h) * = P<0.05 vs. basal (ANOVA with Bonferroni post hoc adjustment).

Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, Wackerhage H, Taylor P M, and Rennie M J (2005). FASEB J 19:422-424.

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C54

Aged human serum does not determine myogenicity in human primary muscle cells

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Ageing is characterised by a loss of muscle mass (sarcopenia). The mechanisms contributing to this loss are not known. One theory is that sarcopenia results from an impaired response to contraction-induced injury. In rodent muscle, evidence suggests that changes in circulatory factors contribute to the decline in regenerative capacity, since the % of desmin (a muscle cell marker) is lower in primary cells cultured in serum from old animals (1). The aim of this study was to assess the myogenicity of human satellite cells cultured in serum from young or old people.

Needle biopsies were taken (under local anaesthetic: 1% Lignocaine) from the vastus lateralis muscle of a young (aged 23 yrs) and an older (aged 65 yrs) female subject. Serum was obtained from 8 young (aged 23-36 yrs) and 8 elderly (aged 65-82 yrs) subjects (4 male and 4 female per group). The tissue was digested with trypsin and the resulting cell supernatant was cultured in a humidified incubator at 37°C and 6% CO_2 in skeletal muscle cell growth medium (SMCGM, Promocell, Germany) supplemented with 10% foetal calf serum. Cells were plated at a density of 1250/well in 96 well dishes. After 24 hours the SMCGM was replaced with skeletal muscle cell basal medium (Promocell) which was supplemented with 15% human serum. Cells were fixed after 46 hours in 4% paraformaldehyde + 0.2% triton. Immunocytochemistry was performed using mouse monoclonal antibody against desmin (D33, Santa Cruz) and a rabbit anti-mouse secondary antibody conjugated to

Alexa Fluor 488 (Invitrogen). The nuclei were counterstained with Hoechst 33258. Analysis was performed using a Zeiss Axiovert fluorescence microscope. Myogenicity was defined as the percentage of the proliferating cells expressing desmin. Two wells were studied per condition and the mean value used for analysis. At least 500 cells per well were counted.

Satellite cells from the elderly subject showed no difference (unpaired t-test) in myogenicity when cultured in either young serum (24.9±3.9%, mean±SEM) or old serum (26.6±3.6%). Although myogenicity of cultures from the young subject was consistently higher, the age of serum also had no effect (41.6±4.1%, versus 42.3±4.0% for young and old serum respectively).

The data suggest that in contrast to studies on mice, factors in the aged human circulation do not appear to affect the myogenicity of human primary muscle cells.

1. Carlson M, Conboy I (2007). *Ageing Cell* 6: 371-82.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C55

Contractile and fatigue properties of fast-twitch EDL muscle from an α-actinin-3 knockout mouse

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The actin-binding protein α-actinin-3 is one of the two isoforms of α-actinin that are found in the Z-discs of skeletal muscle, and is specifically expressed in fast glycolytic muscle fibres. Homozygosity for a common polymorphism in the ACTN3 gene results in complete deficiency of α-actinin-3 in about 1 billion people worldwide. Although α-actinin-3 deficiency does not cause disease, recent studies suggest that the absence of α-actinin-3 is detrimental to sprint and power performance in elite athletes (Yang et al., 2003). To determine the effect of α-actinin-3 deficiency on the contractile properties of skeletal muscle, we studied isolated extensor digitorum longus muscles (EDL) from a specially developed α-actinin-3 knockout mouse. Animals aged 8 to 10 weeks were sacrificed with an overdose of halothane (ethics approval UNSW). The EDL muscle was dissected from the hindlimb and tied by its tendons to a force transducer at one end and a tissue puller at the other. It was placed in a bath continuously superfused with oxygenated Krebs solution. The muscle was stimulated by two parallel platinum electrodes. At the start of the experiment, the muscle was set to the optimum length L_0 that produced maximum twitch force. All experiments were conducted at room temperature (~ 22°C to 24°C). Each set of experiments was carried out on 10 wild-type and 10 knockout muscles (n=10). All tests were two-tailed t-tests with a significance level of 5%. Data are presented as Mean ±S.E.M. α-Actinin-3-deficient muscles showed similar levels of damage to wild-type muscles following eccentric contractions of 20% strain, 1.6 ± 2.0% in wild-types and 2.6 ± 1.5% in knockouts, suggesting that the presence or absence of α-actinin-3 does not influence the mechanical stability of the sarcomere. α-Actinin-3 deficiency does not result in a loss of fast glycolytic fibres (expressing myosin 2B). However, α-actinin-3-deficient muscles were 9% lighter than α-actinin-3-positive muscles, with a corresponding 9% reduction in cross-sectional area. Knockouts displayed longer twitch half-relaxation times; the half-relaxation time of 15.7 ± 0.6 ms in knockouts was 2.6 ms longer than the half-relaxation time of 13.2 ± 0.6 ms in wild-types ($p = 0.008$). α-actinin-3-deficient muscles showed significantly better recovery from fatigue; 30 minutes following the fatigue protocol knockouts recovered to 86.1 ± 1.1% of their original force, but wild-types recovered to only 78.4 ± 1.9% of original ($p = 0.013$). In combination, these data suggest that α-actinin-3 deficiency results in fast-twitch, glycolytic fibres developing slower-twitch, more oxidative properties while not affecting the mechanical strength of the fibre. This alteration towards a slow contractile profile of the fast muscle would be detrimental to optimal sprint and power performance but beneficial for endurance activities.

Yang, N, MacArthur, DG, Gulbin, JP, Hahn, AG, Beggs, AH, Easteal, S & North, K. (2003). American Journal of Human Genetics **73**, 627-631.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C56

The effects of androstanolone (a synthetic DHT) on maximum isometric force in intact mouse skeletal muscle fibres

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Although the chronic administration of testosterone and its derivatives to both animals and humans has been the subject of numerous previous studies (Kuhn, 2002) little is known about the acute actions of these compounds in isolated intact mammalian skeletal muscle fibres. Therefore, the main aim of this study was to investigate the rapid actions of androstanolone (a synthetic dihydrotestosterone; DHT) in intact mammalian skeletal muscle fibre bundles isolated from adult female CD1 mice. The mice were killed by dislocation of the neck followed by severing of the spinal cord and the extensor digitorum longus (edl, a mainly fast-twitch muscle) and the soleus (a predominantly slow-twitch muscle) were isolated. Small muscle fibre bundles (4-6 fibres – mean cross-sectional diameter ~120μm) were then dissected under dark-field illumination and mounted between two stainless steel hooks in a flow through muscle chamber. The experiments were performed at 20±0.1°C. The results show that treating muscle fibre bundles with physiological concentrations of androstanolone for only 30 minutes increases maximum isometric force (P_o) in fast-twitch fibres but decreases it in slow-twitch fibres. Furthermore, in both fibre types these effects could be reversibly abolished by cycloheximide and cyproterone but they were relatively insensitive to

endocardial region of the left ventricle (LV) (P<0.01) correlated with a decrease in I_{to} current density, recorded under whole-cell patch clamp, in the region (P< 0.05; n= sham, 25; CHF, 16). Western blot analysis of the protein constituents of both *Ito* "fast" and "slow" was performed in the sham and CHF ferret tissue. Kv4.2 and Kv4.3 constitute the "fast" component of I_{to} in this model. Analysis of full thickness LV protein preparations revealed that Kv4.2 expression was unchanged but there was an increase in total Kv4.3 in CHF (P=0.03; n= sham, 8; CHF, 8). Western blot analysis was also performed on sham and CHF ferret endocardial and epicardial protein preparations and densitometry analysis revealed down-regulation Kv4.2 in the endocardial region whereas Kv4.3 expression was unchanged (P=0.006; n= sham, 3-4; CHF, 4). Furthermore, there was no change in the expression KChIP2b, the β- subunits to the Kv4 channels in this model. Analysis of transmural Kv1.4 expression, the "slow" component of I_{to} in the ferret, revealed a decrease in expression in the endocardial region of the CHF ferrets relative to sham controls (P=0.03; n= sham, 4; CHF, 4).
In conclusion, the changes in protein expression for Kv4.2, Kv4.3 and Kv1.4 may contribute to the electrical remodelling described above and provide a substrate for arrhythmias in CHF.

This work is supported by the British Heart Foundation.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC26A

Overexpression of SUR2A protects against β-adrenergic-mediated Ca^{2+} loading in cardiomyocytes

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TITLE ONLY

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC27

Transcription of the mTOR repressor REDD1 is lower in old women at rest and is downregulated after resistance exercise in young but not old women

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After a latent period ~1 h post resistance exercise (RE), muscle protein synthesis (MPS) is increased markedly. Previous studies have shown that upregulation of mammalian target of rapamycin complex 1 (mTORC1) signalling is associated with RE-induced MPS through activation of upstream kinases and protein complexes such as Akt, p70S6K1 and TSC1/2, probably through growth factor/mechanosensitive-dependent mechanisms. REgulated in Development and DNA damage responses (REDD1) is a recently identified novel mTORC1 repressor whose transcription is rapidly modulated, and is inversely proportional to mTOR signalling activity (Kimball et al 2008). Thus we hypothesized that REDD1 mRNA expression would be downregulated by exercise as part of the facilitation of mTOR-mediated increases in MPS. We further hypothesized that the downregulation of REDD1 mRNA expression would be greater in young than old women thereby possibly providing a more potent anabolic stimulus in young women.
Twenty two healthy women (old n=9 median age 80 y, range 76-82 y; young n=13, median age 26y, range 19-30 y) undertook a single bout of RE (120 maximal voluntary isometric contractions of the knee extensors of one leg as 20 sets of 6 contractions over 90 minutes). Muscle samples were obtained from the lateral mass of the quadriceps under local anaesthesia (1% lignocaine sc) at baseline and at 2.5 h post-exercise using the Bergstrom needle technique. Changes in muscle mRNA concentration for REDD1 were determined by real-time RT-PCR using pre-validated 28s rRNA to correct for variations in preparation. Statistical analysis was by repeated measures (RM) ANOVA.
In old women at rest muscle REDD1 mRNA was ~30 ± 10 % lower than in muscle of young women (P<0.05) and was unchanged by exercise (Figure 1). In young women, exercise led to an ~80 ± 4 % reduction (P<0.01) in muscle REDD1 mRNA, an expression level that was also significantly lower than that in muscle of old women at rest (P<0.05).
We speculate that the potent downregulation of REDD1 mRNA in young women may be paralleled by a similar decrease in REDD1 protein expression which possibly contributes to increased mTOR signalling and greater initiation of MPS after RE. However, the inability to further respond to RE with downregulation of REDD1 mRNA may limit anabolic responses to RE in old women. Our novel results are consistent with the accumulating evidence for dysregulation of MPS in response to anabolic stimuli in old age.

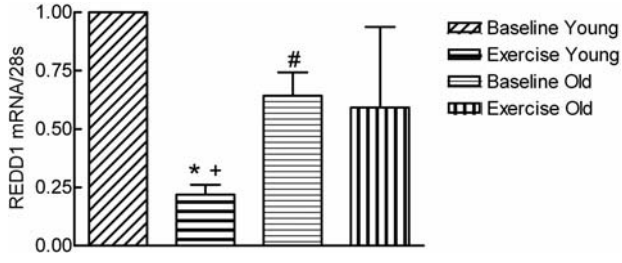

Figure 1: Effect of resistance exercise on mean (SEM) REDD1 mRNA (relative units normalised to baseline for young) for n=9 old and n=10 young women. *+# = P<0.05 (RM ANOVA). * Significantly less than at rest; + significantly less than old women at rest; # significantly less than young women at rest.

Kimball SR, Dang Do AN, Kutzler L, Cavener DR, Jefferson LS, 2008. J Biol Chem 283: 3465-3475.

Funded by Research into Ageing

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC28

Venous cannulation triggers systemic free radical formation; interpretive implications for exercise-induced oxidative stress

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Recognition of free radicals as redox signalling molecules, implicit in the regulation of cellular oxygen homeostasis during physical exercise justifies continued interest into their mechanisms of generation in-vivo. Hypoxia, re-oxygenation or exercises, independently have a profound influence on the level of free radical generation in human beings(1, 2, 3). The present study was designed to test the hypothesis that the physiological trauma associated with venous cannulation may artefactually stimulate systemic free radical formation in the acute phase that if not accounted for may under-estimate the oxidative stress response to exercise.

Six males aged 34 ± 2 years (mean ± SD) participated in Phase I of the study. A resting venous blood sample was obtained from the cephalic vein of the non-dominant forearm using a cuff inflated for 60s to 20mmHg below the measured systolic pressure to avoid ischaemia-reperfusion. Samples were collected immediately following venous cannulation (0min) and at 2, 5, 10, 15, 20 and 30min into recovery. Twelve separate males aged 23 ± 5 years volunteered for Phase II. Samples were obtained at rest after 2 and 30min following venous cannulation and immediately after a cycling test to volitional exhaustion. Plasma was extracted and injected into a high-sensitivity multi-bore aqueous cell prior to X-band electron paramagnetic resonance spectroscopy for direct detection of the ascorbate radical.

Venous cannulation increased ascorbate free radical which remained elevated until 30min into recovery (Fig 1A). The exercise-induced increase in ascorbate radical was subsequently shown to be 48% greater when the 30min as opposed to the 2min post-cannulation resting baseline (1754 ± 361 vs. 1979 ± 375 AU, P < 0.05) was incorporated into the calculation (Fig 1B).

These findings demonstrate that venous cannulation per se stimulates the systemic formation of free radicals which peak at 10min and require approximately 30min to normalise. If this baseline artefact is not taken into account, the "real" magnitude of the exercise-induced oxidative stress response will be under-estimated.

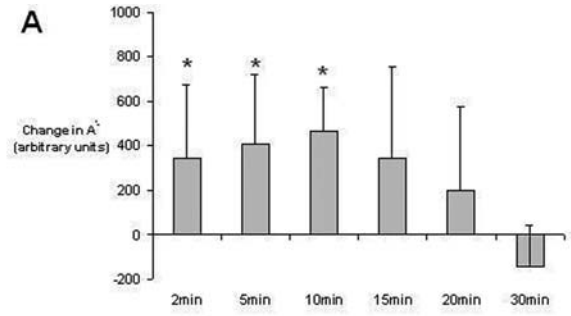

Fig 1A. Systemic oxidative stress response to cannulation (time into recovery minus baseline). *different *vs.* baseline ($P < 0.05$, paired samples *t*-test).

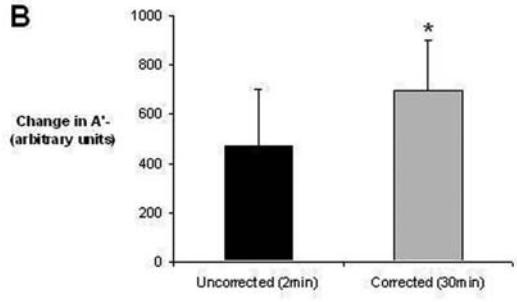

Fig 1B. Exercise-induced oxidative stress response (exercise minus resting baseline obtained at 2 and 30min following cannulation). *different ($P < 0.05$, paired samples *t*-test).

Bailey DM et al. (2007). Free Rad Res 41, 182-190

Zweier JL et al. (1987).Proc.Natl.Acad.Sci.USA, 84, 1404-1407

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC29

Increased vascular nitrite bioavailability in acute mountain sickness; focus on blood-brain barrier function

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Inspiratory hypoxia can cause neurovascular headache which is the primary symptom responsible for acute mountain sickness (AMS). We examined if the more marked arterial hypoxaemia typical of AMS would promote an increase in vascular nitrite (NO_2^-) bioavailability to cause cerebral over-perfusion and mechanical disruption of the blood-brain barrier (BBB) since vasogenic oedema has previously been documented in hypoxia (Bailey *et al*. 2007).

Eighteen males aged 26 (mean) ± 6 (SD) years were examined in normoxia (21% O_2) and after 6h exposure to normobaric

hypoxia (12% O_2). Blood flow velocity in the middle cerebral artery (MCAv) was recorded via trans-cranial Doppler ultrasound and arterial haemoglobin oxygen saturation (SaO_2) measured via pulse-oximetry. Clinical AMS was diagnosed as described previously (Bailey *et al*. 2007). Venous samples were obtained from an antecubital vein and assayed for plasma NO_2^- (ozone chemiluminescence) and serum S100β (radio-immunoassay). Nine subjects developed clinical AMS (AMS+) compared to the remaining 9 who were healthy (AMS-). Hypoxia decreased (SaO_2) more markedly in AMS+ compared to AMS- (-18 ± 5 *vs*. -13 ± 5%, $P < 0.05$). However, while neither AMS nor hypoxia influenced NO_2^- or S100β (Table 1) there was a tendency towards an increase in NO_2^- in AMS+ and decrease in AMS-. MCAv decreased in hypoxia but was not different between groups.

These findings suggest that increased NO_2^-bioavailability may be implicated in the pathphysiology of AMS via mechanisms independent of cerebral over-perfusion and BBB disruption.

Table 1. Metabolic-haemodynamic data

Group: Condition:	AMS-		AMS+	
	NORMOXIA	HYPOXIA	NORMOXIA	HYPOXIA
NO_2^- (nmol/L)	508 ± 178	437 ± 81	479 ± 125	529 ± 121
△ (nmol/L)	-71 ± 214		50 ± 180	
S100β (µg/L)	0.04 ± 0.03	0.05 ± 0.05	0.03 ± 0.04	0.06 ± 0.07
△ (µg/L)	0.01 ± 0.05		0.03 ± 0.04	
MCAv (cm/sec)	60.1 ± 10.6	51.8 ± 9.2	56.6 ± 10.7	53.2 ± 14.1
	Hypoxia < Normoxia ($P < 0.05$)			
△ (cm/sec)	-8.3 ± 6.7		-3.3 ± 14.4	

Values are mean ± SD; Δ, hypoxia minus normoxia. Data analysed using a two factor repeated measures analysis of variance and independent samples *t* test.

Bailey *et al*., (2007). *J Cereb Blood Flow Metab*; **27** 1064-1071.

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PC30

Phosphate loading prolongs post-activation potentiation during twitch and dynamic muscular contractions

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Objective: The purpose of this study was to determine whether 7 days of phosphate loading would augment the post-activation potentiation response of the leg extensors. Research design and methods: 14 individuals (9 males) had their left leg connected to percutaneous stimulation apparatus to evoke a maximum twitch force response. Following 2 minutes recovery subjects performed a 10 second maximum isometric voluntary contraction (MVC). Post-activation twitch responses were then elicited immediately (5 sec) and at 30-sec intervals for 5 minutes thereafter. After 30 minutes recovery subjects performed a 5 second maximal effort cycle test to determine peak power output. A 10 sec 2-leg MVC was then performed, immediately followed by another 5 second sprint and further sprints at one minute intervals for 4 minutes. This procedure was performed prior to any supplementation (CON) and following either a 7 day sodium chloride placebo (6 g.d-1; PLA) or tribasic sodium phosphate (4 g.d-1; P) supplementation period, separated by 14 day. An intravenous blood sample was obtained prior to testing on all conditions for assessment of plasma inorganic phosphate (Pi) concentrations. Results: P supplementation increased mean plasma Pi content 24% above CON (ANOVA $p<0.05$), with no effect following PLA (3% increase; $p>0.05$). Pre-activation peak twitch and peak power were unaffected by either condition ($p>0.05$). The 10 sec MVC resulted in significant twitch force and peak power potentiation immediately post-MVC in all conditions, with no difference between the supplements ($p>0.05$). Post-activation twitch force at the final 5 minute time point remained significantly potentiated in the P group (14%; $p<0.05$), though had returned to within baseline in CON (6%) and PLA (5%). At the final sprint peak power had also fallen to within pre-activation values in CON (-0.6%) and PLA (0.7%) but remained significantly elevated in the P group (1.8%; $p<0.05$). Conclusions: 7 d phosphate supplementation prolongs the effects of post-activation potentiation of both isometric twitch force and peak power output at the 5 minute time point.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC31

Oxidative imbalance in adult attention deficit/hyperactivity disorder

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Objective: There are few studies evaluating the biochemical basis of Adult Attention Deficit /Hyperactivity Disorder (A-ADHD). In the present study, we evaluated whether nitric oxide (NO), an oxidant, level and superoxide dismutase (SOD), an antioxidant, activity are associated with A-ADHD or not. This study also aims to evaluate the NO levels and SOD activities, which were already measured and found to be associated in other psychiatric disorders, in A-ADHD and hopes to find some clues underlining the biological basis of the disease.

Method: Twenty A-ADHD patients from Gaziantep University Sahinbey Research Hospital, Psychiatry Clinic, diagnosed according to The Turkish version of Adult ADD/ADHD DSM IV-Based Diagnostic Screening and Rating Scale by two psychiatrists (H.A.S. and S.S.), and twenty one healthy volunteer controls were included. Blood samples were collected, NO levels and SOD activities were measured.

Results: The mean NO levels in patients (181.39 ± 35.85 µmol/L) were significantly higher than those of controls (40.14± 7.71

μmol/L) and SOD activity of patients (7.00± 1.34 U/L) was significantly lower than controls (11.18 ± 1.31 U/L) (t= 17.64, df= 39, p< 0.01 and t= -10.09, df= 39, p< 0.01 respectively).
Conclusions: Remarkable high levels of oxidant NO, and low SOD activities suggest an oxidative imbalance in A-ADHD. This is the first study evaluating the oxidative metabolism in A-ADHD. Our findings may pioneer the further clinical enzymology and biochemical studies on that disorder.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC32

Skin blood flow responses in exercising and non-exercising limbs in heat-stressed man

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The effect of exercise on skin blood flow (SkBF) in exercising and non-exercising limbs is not well described. SkBF might differ between exercising and non-exercising limbs due to differences in local tissue temperature and/or vasoconstrictor tone. The aim of this study was to examine exercising and non-exercising limb SkBF responses to knee-extensor exercise in normothermic and heat stress conditions. Exercising thigh and non-exercising arm skin blood flow (laser-Doppler flowmetry), blood pressure (radial catheter), rectal temperature (T_{CORE}) and mean skin temperature (T_{SK}) were measured in 5 active males (23±4 yr), dressed in a tube-lined water-perfused suit, at rest and during 6-min of one-legged knee-extensor exercise (25±3 W) in 4 conditions; 1) control (mean ± S.D; T_{CORE} 37.0±0.2 °C, T_{SK} 32.3±0.3 °C), 2) skin hyperthermia (T_{CORE} 37.1±0.2 °C, T_{SK} 36.5±0.5 °C), 3) skin and mild core hyperthermia (T_{CORE} 37.9±0.2 °C, T_{SK} 37.1±0.6 °C) and 4) skin and high core hyperthermia (T_{CORE} 38.5±0.2 °C, T_{SK} 37.6±0.4 °C). Hydration status was maintained throughout. Data were analysed using a one-way repeated measures ANOVA. At rest, leg cutaneous vascular conductance (CVC) increased with T_{SK} hyperthermia and T_{SK} and mild T_{CORE} hyperthermia compared to control (0.48±0.07 and 0.62±0.16, respectively, vs 0.10±0.08 AU.mmHg^{-1}, P=0.001), but did not increase further with T_{SK} and high T_{CORE} hyperthermia (0.63±0.13 AU.mmHg^{-1}, P>0.05). Similarly, arm CVC increased with T_{SK} and T_{SK} and mild T_{CORE} hyperthermia (0.22±0.16 and 0.72±0.61 vs 0.07±0.02 AU.mmHg^{-1}, respectively, P=0.043) and thereafter plateaued (0.80±0.48 AU.mmHg^{-1}, P>0.05). In the transition from rest to exercise, leg CVC increased and the magnitude was significantly lower during each heat stress condition compared to control (278±148 vs 29±31, 34±41, 32±34 %, all P<0.05). Similarly, arm CVC increased with exercise and the magnitude of the increase was reduced during the last 2 heat stress stages (1; 74±51, 2; 160±147, 3; 11±15, 4; 2±7 %, P=0.031). The increase in CVC during control exercise was larger in the leg vs the arm (P=0.043) but there were no limb differences during heating (all P>0.05). Mean arterial blood pressure (MAP) was well maintained throughout heat stress (~100 mm Hg, P=0.19). During exercise, the MAP increase was progressively reduced with heating (35±16 to 10±7 mm Hg, P=0.012) in line with the reductions in exercise-induced CVC increases with increasing heat stress. These findings demonstrate that CVC increases during knee-extensor exercise in exercising and non-exercising limbs and that the magnitude of the increase is larger in the exercising limb under normothermic conditions, possibly through an increased tissue temperature. Furthermore, the size of elevation in CVC is reduced with increasing pre-exercise T_{SK} and T_{CORE} in exercising limbs but only with increasing T_{CORE} in non-exercising limbs.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC33

Systemic inflammatory response during endotoxaemia and acute hypoxia in humans

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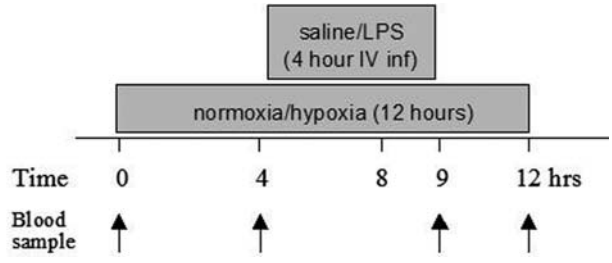
Hypoxia frequently accompanies severe systemic inflammation and has been suggested to modulate the inflammatory response. The present study investigates the effect of acute hypoxia, a systemic inflammatory stimulus, or both on the systemic inflammatory response in healthy volunteers.
36 healthy male volunteers were randomised to one of the following interventions (Figure 1):
1. Normoxia + endotoxin infusion (0.075 ng/kg/hr, total dose 0.3 ng/kg, E; N=12); 2. Hypoxia (12.9%) + saline infusion (H; N=11); 3. Hypoxia (12.9%) + endotoxin infusion (HE; N=13).
Vital signs were monitored throughout the study period, and the study was approved by the local ethical committee. White blood cell and differential counts (WBC), tumour necrosis factor-alpha (TNF) and interleukin (IL)-6 were measured at 0, 4, 9, and 12 hrs. Multivariate analysis of variance and Bonferroni-corrected post hoc tests were used to study the effect of time, intervention and the interaction between time and intervention.
Temperature increased from baseline (E:36.3°C;H:36.2°C;HE:36.3°C) in all groups and peaked at 10 hours in the E (38.4°C) and HE (39.9°C) groups with no difference between groups. Levels were higher in both the E group and the HE group than in the H group from 9 throughout 12 hours (P<0.05). Heart rate increased from baseline (E:68 bpm; HE:69 bpm) and peaked at 10 hours in the E (93 bpm) and HE

(101 bpm) groups. There was no difference between the E and HE groups.
WBC and TNF increased in group E and HE. The increase was more pronounced in group HE than in group E with regard to WBC (P<0.05), and more pronounced in group E than in group HE with regard to TNF (P<0.01). IL-6 increased during all types of interventions, albeit more so in groups receiving endotoxin infusion (E and HE) than during hypoxia alone (group H). No difference was found with respect to increase in IL-6 between groups E and HE (Table 1).
These results suggest, that acute hypoxia may modulate certain aspects of the systemic inflammatory response in humans.



	Intervention	0 hours	4 hours	9 hours	12 hours
WBC (10^9/l)	E	5.0 (4.6;5.5)	6.3 (5.4;7.1)	9.2 (8.2;10)	9.5 (8.2;11)
	H	5.2 (4.4;6.0)	7.2 (6.2;8.2)	8.0 (7.1;9.0)	8.4 (7.5;9.2)
	HE	5.9 (5.1;6.7)	8.7 (6.9;10)	12 (10;14)	12 (9.9;13)
TNF alpha (pg/ml)	E	1.2 (0.6;1.8)	1.1 (0.4;1.7)	11 (9.0;12)	3.4 (2.7;4.0)
	H	1.3 (0.7;1.8)	1.2 (0.7;1.8)	1.1 (0.5;1.8)	1.3 (0.6;1.9)
	HE	1.0 (0.7;1.4)	0.8 (0.6;1.1)	6.6 (5.0;8.3)	1.9 (1.6;2.3)
IL-6 (pg/ml)	E	0.5 (0.2;0.8)	0.9 (0.6;1.2)	79 (40;118)	5.0 (2.2;7.7)
	H	1.0 (0.3;1.8)	1.6 (1.1;2.1)	2.3 (0.9;3.7)	2.1 (1.2;2.9)
	HE	0.9 (0.6;1.2)	1.5 (1.1;1.9)	44 (20;68)	3.6 (2.6;4.7)

Values are mean (lower; upper 95% CI)

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC34

Effect of acclimatization to humid hot environment with transition across five time zones on heart rate variability in elite junior rowers

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TITLE ONLY

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC35

Ontogeny of insulin signalling pathways in ovine fetal skeletal muscle during late gestation

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In man and other animals, the incidence of adult insulin resistance is increased when fetal growth is poor, which suggests that insulin sensitivity is determined, in part, prenatally1. In many species, an increase in fetal glucocorticoid concentrations is responsible for prepartum tissue maturation2. Fetal glucocorticoid levels are also raised earlier in gestation by conditions known to impair fetal growth and adult insulin sensitivity3. Little is known about the ontogeny and developmental control of the insulin signalling pathways in utero. This study determined i) the ontogenic changes in these pathways during late gestation and ii) the glucocorticoid dependence of these changes in sheep.
After maternal and fetal euthanasia (200 mg/kg, Na pentobarbitone), hind limb skeletal muscle was collected from 4 groups of fetal sheep: 1) untreated controls (n=22) at 110, 120, 130 and 140 days (d) of gestation (term ~145d, n≥ 4 per group), 2) adrenalectomised (AX) at 115d and age-matched controls delivered at 145d (n=6 per group), 3) catheterised and infused with cortisol (2-3 mg/kg/day in saline) or saline for 5 days before delivery at 130d (n=6 per group), 4) maternal dexamethasone (2x12 mg in saline im) or saline treatment before delivery at 127d (n=6 per group). All surgical procedures were carried out under halothane anaesthesia (2%, in O2:N2O). After protein extraction and standardization, abundance of the insulin receptor (IR)-b subunit, insulin-like growth factor type I receptor (IGF-1R), protein kinase C zeta (PKC-ζ) and the insulin-sensitive glucose transporter (GLUT4) were measured by SDS-PAGE and Western Blotting using ovine validated antibodies4 (Santa Cruz, CA or Abcam Ltd, UK). Results are mean (± SE) arbitrary units. Statistical significance was assessed by Student's t-Test or ANOVA plus Tukey Test, as appropriate.
From 110d to 120d, there were increases in muscle protein abundance of IGF-1R (10.10±1.30 to 19.15±1.00, P<0.05) and PKC-ζ (5.57±0.20 to 21.25±3.41, P<0.05) but not IRβ in (17.90±3.30 to 27.65±4.69, P>0.05). At 140d, muscle protein abundance of IR-b (13.65±2.58), IGF-1R b (4.47±0.95) and PKC-ζ (7.04±0.76) were significantly less (P<0.05) than the values at 120d but not 110d. Muscle protein GLUT4 abundance was significantly lower at 130 and 140d (18.34±2.75 and 20.63±3.04, respectively) than at 110 and 120d (72.13±6.37 and 73.28±7.32 respectively; P<0.05). Fetal adrenalectomy, intrafetal cortisol and maternal dexamethasone treatment did not significantly alter muscle abundance of any of the proteins, except PKC-ζ, which was higher in AX (10.58±1.24) than control fetuses (7.04±0.76; P<0.05). These data show that ontogenic changes in the insulin-signalling pathway occur in fetal skeletal muscle towards term but that these are unlikely to be glucocorticoid dependent.

McMillen IC & Robinson JS (2005) Physiol Rev 85, 571.

Fowden AL Li J & Forhead AJ (1998) Proc Nutr Soc 57, 113.

to STa, making unlikely a local neural component to the action of STa.
Luminal carbachol (1mM) reduced net fluid absorption to 31.9 ± 7.6 (6) ul/cm/hr that was significantly lower than control values and comparable to absorption rates after exposure to STa. Isotonic choline chloride perfused in combination with STa suppressed sodium ion dependent fluid absorption. Additionally perfusing with 1 mM carbachol gave no further decrease in fluid absorption, indicating that no secretion process was detected that could worsen inhibited absorption. This indicated that a cholinergically mediated secretory process was unlikely to be present in the proximal jejunum. The reduction in fluid absorption after STa exposure and after pressure distension is likely to be the result of a final convergence of both pathways on sodium: hydrogen ion exchange with the pressure distension mediated by the internal release of acetylcholine through an initiation of the intestino-intestinal stretch reflex.

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C88

Mouse duodenal iron transport is decreased following chronic exposure to hepcidin

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Hepcidin, the main circulating iron regulatory hormone exerts its actions by binding to the iron efflux protein ferroportin, inducing transporter degradation and thereby inhibiting iron release from cells [1]. We have shown previously that injection of hepcidin into mice results in a rapid (within 4h) decrease in serum iron and a concomitant decrease in ferroportin expression in splenic macrophages [2]. Interestingly, duodenal ferroportin expression in the same animals was not altered within this time frame [2]. The aim of this study was to examine the effects of longer-term exposure to hepcidin on duodenal iron transport.
Male C57BL/6 mice (aged 4 weeks) were given injections of hepcidin (10μg/mouse, i.p.) or an equivalent volume of saline at 24h intervals. A final injection of hepcidin was administered 4h prior to experimentation. In anaesthetised animals (sodium pentobarbitone, 60 mg/kg, i.p.), tied-off duodenal segments were washed with saline, followed by air, filled with Hepes-buffered saline (pH6.5) containing 0.2mM ^{59}Fe, complexed with 4mM ascorbate and incubated for 10 min. At the end of the exposure period, the amount of ^{59}Fe in the duodenal mucosa and the animal carcass were determined by gamma counting. Data are presented as mean ± SEM, and were analysed using one-way ANOVA and Tukey's post hoc test with differences considered significant at P<0.05.
Iron transfer from the duodenal mucosa to the animal was significantly decreased in both 24h and 72h hepcidin treated mice compared with the control group (Table 1). Interestingly, iron retention within the mucosal tissue was significantly elevated in 24h hepcidin treated mice compared with the other two experimental groups, suggesting that enterocytes were still able to take up iron despite the inhibition of the efflux pathway. Taken together with our previous data [2], we propose that duodenal enterocytes are less sensitive than splenic macrophages to a hepcidin challenge, and that this is consistent with the relative importance of these two cell types in maintaining body iron homeostasis.

Table 1. *In vivo* duodenal iron transport (n = 4-6 mice in each group)

	saline	24h hepcidin	72h hepcidin	P values
Mucosal retention (pmol/g mucosa)	158.4 ± 11.8	347.0 ± 79.8*	124.1 ± 14.1	*P<0.05 c.f. saline group
Mucosal transfer (pmol/g mucosa)	433.2 ± 115.9	8.8 ± 3.3*	19.3 ± 2.5*	*P<0.05 c.f. saline group

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C89

Factors contributing to an increase in quadriceps specific tension following resistance training in young men

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BACKGROUND: The maximal force a muscle can generate depends on the number of sarcomeres in parallel and thus its physiological cross sectional area (PCSA). However, the increase in muscle strength with training is widely reported to be greater than expected from the increase in size (1). The aim of the present investigation was to systematically address potential problems that may be caused by changes in voluntary activation and coactivation and changes in muscle architecture during maximum voluntary contraction (MVC). To our knowledge, this is the first study to investigate the effect of resistance training on specific tension taking into account the structural differences between the four quadriceps muscles.
METHODS: Fourteen healthy male volunteers aged 21 ± 3 yrs performed unilateral leg-extension (4 sets of 10 repetitions at 80% 1RM), 3 times/wk for 9 weeks. Quadriceps tendon force (F_t) was calculated by correcting maximum isometric torque obtained at the optimum knee angle for antagonist coactivation (estimated from electromyographic activity), voluntary activation (using the interpolated twitch technique), patella tendon moment arm length and the ratio of quadriceps tendon force to patella tendon force (2). The PCSA of each quadriceps muscle was calculated by dividing the volume measured

using magnetic resonance imaging by ultrasound measurements of fascicle length at MVC. The effective PCSA of the whole quadriceps was determined as the sum of the PCSAs of the four constituent muscles, each multiplied by the cosine of the appropriate pennation angle. The specific force of the quadriceps femoris muscle was obtained by dividing F_t by the sum of the effective PCSAs.
RESULTS: Isometric torque increased by 33 ± 12%, ($p<0.05$) and total quadriceps ACSA increased by 5 ± 4% ($p<0.05$); quadriceps volume increased by 6 ± 3% ($p<0.05$); quadriceps PCSA did not change significantly (+5 ± 11%); F_t increased by 23 ± 18% ($p<0.05$). Specific tension of the complete quadriceps femoris increased by 22 ± 18%, from 53 ± 9 N/cm2 to 64 ± 10 N/cm2 ($p<0.05$).
DISCUSSION: The increase in specific tension confirms previous reports (see reference 1) and was not explicable by changes in muscle architecture, voluntary activation and/or coactivation. It is still not clear what causes the large specific force values or increase in specific force with training but differences or changes in myofibrillar packing and lateral transmission of force remain possibilities to be examined.

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C90

The effects of isometric strength training on stretch reflex-induced tremor in humans

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Isometric strength training (IST) can reduce anisometric tremor in healthy individuals and in patients with Essential Tremor (Bilodeau *et al.* 2000; Tracy *et al.* 2004). One explanation for this might be a reduction in stretch reflex instability (SRi), a mechanism associated with tremor (Durbaba *et al.* 2005), as IST is known to alter SR properties (Zehr 2006). Here we investigate the effects of short term IST on SRi in healthy individuals.
Fourteen individuals (aged 20–39 years) took part in the study. Ethical approval was obtained and subjects gave written, informed consent. Subjects were seated on a dynamometer (Biodex Medical Systems Inc., New York, USA) with their knee and hip angles at 90 degrees. Contraction force from knee extensors of the dominant leg was recorded to assess maximum voluntary contraction (MVC) and SRi at baseline and after 4 weeks. Following baseline recordings, 7 subjects acted as controls and continued their normal daily activity, whilst 7 undertook a 4-week period of IST involving the knee extensor muscles of the dominant leg; 3 times a week, 45 minutes per session. The IST protocol used was designed by Macaluso *et al.* (2000) and produces an increase in muscle strength. Tremor was induced with anisometric contractions, at 30% baseline MVC, against spring loading that preferentially activated the long or short latency components of the SR (long=5.35 N mm^{-1}; short=11.06 N mm^{-1}). Subjects had visual feedback to help maintain the appropriate force level for the recording and training sessions. Autospectra of force fluctuations were computed for analysis. Statistical significance within and across the two groups was accessed by paired and unpaired t-test as appropriate.
The training group showed a significant increase in MVC as compared to baseline (28.6±6.4%, mean±SEM; $p<0.01$), which was significantly greater ($p<0.001$) than the minimal change observed for controls (0.7±1.9%). Tremor amplitude was significantly decreased ($p<0.01$) in the training group (long=-43.2±8.2%; short=-34.8±8.4%) as compared to controls (long=-1.8±4.4%; short=-8.1±3.9%). In each group, tremor frequency was not significantly altered for either spring. The results are consistent with a decrease in SR gain, without altering the system dynamics. Also, they support the idea that reductions in anisometric tremor observed in previous studies may be due to changes in SRi. Thus, isometric strength training could be a useful rehabilitation tool in individuals with exaggerated forms of tremor.

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C91

The effect of recovery work-rate on the parameters of the power-duration relationship following exercise to exhaustion in humans

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In order to sustain exercise immediately following exhaustive cycle ergometry, the external power output (P) needs to be reduced below the subject's critical power (CP) (Coats *et al.*, 2003): CP being the asymptote of the hyperbolic power-duration relationship (P-t_{lim}), which is also defined by a curvature constant, W′, equivalent to a fixed amount of work that can be performed above CP (Poole *et al.*, 1988). This is consistent with a W′ 'depletion' mediated intolerance – supra-CP exercise is then only feasible with, at least partial, W′ replenishment. That some subjects cannot sustain even sub-CP exercise following exhaustion (Coats *et al.*, 2003) suggests that the preceding exercise may reduce CP and constrain W′ repletion. We therefore aimed to estimate the P-t_{lim} relationship, together with pulmonary O_2 uptake (VO_2), across a range of recovery work-rates following exhaustion. We hypothesised that CP

would be reduced following recovery at high (95% CP) but not low work-rates. Six healthy men (23 ± 5 yrs; mean ± SD) gave informed consent to perform: 1) incremental-ramp cycle ergometry for estimation of the lactate threshold (LT); 2) 4 different high-intensity constant-P tests (CON), each on separate days and taken to the tolerable limit, to estimate VO_{2max} and establish the P-t_{lim} parameters (CP and W′); 3) a 10 min constant-P test at CP. CP and W′ were also estimated after each of three 6 min recovery bouts (REC) from exhaustive exercise (~6 min): 20 W, 105% LT and 95% CP. Gas-exchange was measured breath-by-breath by mass-spectrometry and turbinometry (MSX, Morgan Medical, UK). At the tolerable limit of all exercise bouts, VO_2 attained maximum (4.13 ± 0.43 l.min^{-1}). Whereas CP (CON: 242 ± 27 W) was unchanged in REC (240 ± 28, 239 ± 25, 243 ± 31 W at 20 W, 105% LT, and 95% CP, respectively), W′ (CON: 20.1 ± 4.9 kJ) was progressively reduced the higher the recovery P (REC: 10.9 ± 2.6, 8.2 ± 1.5, and 2.8 ± 2.1 kJ). VO_2 recovery during REC was also dependent on recovery P (reaching 1.13 ± 0.13, 2.45 ± 0.35, and 3.68 ± 0.39 l.min^{-1}, respectively), but was not well correlated with W′: the repletion of which was appreciably attenuated during high P recovery. Therefore, these data suggest that neither prior exhaustive exercise nor recovery P affect CP. However, because VO_2 during REC 95% CP remained above ($p < 0.05$; t-test) the 10 min CP value (3.48 ± 0.38 l.min^{-1}) this suggests that the metabolic equivalent of CP may be increased during high P recovery, and limit W′ restoration. Exercise tolerance following exhaustion therefore, presumably depends on the degree to which the external power requirement and its metabolic equivalent are reduced at the tolerable limit.

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C92

Heat stress increases leg muscle and skin blood flow in resting and exercising humans

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In humans, heat stress increases cardiac output (Q) at rest and during exercise, in response to an enlarged thermoregulatory demand from the skin circulation (González-Alonso et al. 2008). It remains unclear whether heat stress causes muscle vasodilation and ergo whether muscle perfusion increases. This study tested the hypothesis that local leg and systemic hyperthermia increases leg muscle and systemic perfusion at rest and during exercise. We measured leg and systemic hemodynamics, O_2 transport and VO_2 at rest and during 6-min of one-legged knee-extensor exercise (25±3 W) in 7 active males (21±2 yr) in 4 conditions, in which participants' hydration status was maintained: 1) control (Tcore ~37°C, Tskin ~33°C), 2) skin hyperthermia (Tc ~37°C, Tsk ~36°C), 3) skin and mild core hyperthermia (Tc ~38°C, Tsk ~37°C), and 4) high skin and core hyperthermia (Tc ~39°C, Tsk ~37°C). Femoral artery blood flow (LBF; Doppler ultrasound), thigh skin blood flow (SkBF; laser Doppler flowmetry) and blood gas and haematological variables (ABL 825, Radiometer) were measured in each condition. Data were analysed using a one-way ANOVA with repeated measures and appropriate post hoc analysis with significance accepted at $P \leq 0.05$. Data represent mean±SEM. At rest and during exercise, LBF and Q increased with each elevation in heat stress compared to control (peak delta LBF= 1.1±0.1 and 0.9±0.2 l min^{-1} from baselines of 0.5±0.1 and 2.4±0.2 l min^{-1}, respectively; peak delta Q= 4.0±0.2 and 3.1±0.3 l min^{-1} from baselines of 5.1±0.2 and 7.4±0.4 l min^{-1}, respectively). However, the increase in LBF and Q due to exercise (exercise hyperemia) was the same (~1.6 l min^{-1}) in all heat stress conditions. As expected SkBF increased with skin hyperthermia and the skin and mild core hyperthermia conditions (8.5±1.4-fold) but did not show a further increase with the high skin and core hyperthermia condition. The further increase in LBF was accounted for by an increased muscle perfusion. Additionally, the magnitude of the increase in SkBF with exercise during heat stress was the same in all conditions. Despite leg vascular conductance increasing with heat stress, MAP and perfusion pressure declined thereby suggesting that local vasodilation was responsible for the increase in leg perfusion. The elevation in leg muscle and skin temperature alone accounted for >50% of the increase in LBF and SkBF with high skin and core hyperthermia. The increase in LBF with each level of heat stress was followed by a decline in leg O_2 extraction. In line with this, leg VO_2 remained unchanged at rest and during exercise. The results suggest that leg muscle and skin blood flow increase with heat stress in resting and exercising humans. Furthermore, increases in muscle tissue temperature per se may contribute to the regulation of muscle blood flow and exercise hyperemia.

González-Alonso J, Crandall CG & Johnson JM (2008). J Physiol 586, 45-53.

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C93

Cardiac changes during maximal exercise in varsity athletes using digital ballistocardiography

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Cardiovascular changes during maximal incremental exercise in humans are well known. Maximal aerobic power has been used almost exclusively to assess physiological function and to characterise endurance training adaptations. However, because of technological limitations it is not possible to monitor specific cardiac events during maximal exercise. Time and ampli-

tude of the opening and closing of heart valves could provide new insights on the physiological mechanisms during exercise training, detraining, and in the diseased state.

Recent technological advances are available where micro-accelerometers can be used to monitor the cardiac cycle. Digital ballistocardiography (dBG) measures the seismic activity of the heart, and acceleration forces during systole and diastole produce very-low-frequency compression waves which can be recorded and matched with the EKG. We hypothesised that dBG could be used to detect these cardiac functional changes in response to maximal exercise.
Six male university athletes (Age=20±1.8yrs; Ht=194±4.4cm; Wt=99.7±9.4kg) performed maximal incremental exercise (10 km/h, 2 degree/min incline) on a motor-driven treadmill. Pulmonary gas exchange, heart rate and dBG recordings were monitored at rest, during and immediately following maximal exercise. Paired t-tests were used pre- to post-exercise, with significance reported at $p<0.05$.
Maximal VO2 = 53.3±6.5 mL/kg/min; HR = 193±4bpm, ventilation = 122.6±17.4 L/min, and O2 pulse = 27.6±3.2 mL O2/beat. On average 10 dBG waveforms showed that atrial contraction force increased significantly from 11.9±5.8 mg at rest to 29.4±19.6 mg after exercise, while ventricular contraction force increased from 22.4±12.2 to 53.2±12.7 mg. We also report for the first time during maximal exercise the timing events of the athletic heart (significant changes reported in %): Q-wave to rapid ejection period (-11.8%), Q-wave to aortic valve closure (-30.1%), Q-wave to mitral valve open (-27.4%), Q-wave to early diastole (-27.2%), Q-wave to aortic valve open (-10.5%). Day-to-day reliability for dBG variables is r=0.94.
Based on our results, it appears that: (1) dBG has the ability to determine all timing of the cardiac cycle, including the velocity and amplitude of each of these events (atrial contraction, mitral valve open and close, aortic valve open and close, rapid ejection period), and (2) the athletic heart has an individual response to exercise as reflected by the timing changes between subjects. This is the first study to our knowledge that has examined the mechanical changes in cardiac function in varsity athletes after maximal incremental exercise.

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Mental fatigue impairs physical performance in humans

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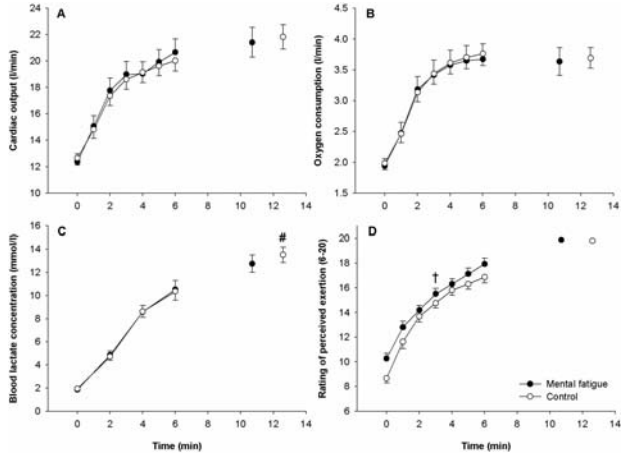
Mental fatigue refers to the subjective (e.g. tiredness) and neurophysiological effects of prolonged and demanding cognitive activity (1). Although the negative consequences of mental fatigue on cognitive performance are well known, its effects on physical performance are unclear. In the present randomised, counterbalanced, cross-over trial, we induced mental fatigue in 16 healthy, fit young adults with a prolonged (90 min) and demanding cognitive task (the AX-continuous performance task) known to activate the anterior cingulate cortex (ACC) (2). The control treatment consisted of watching documentaries about cars and trains for 90 min. Manipulation check with a mood questionnaire confirmed a state of mental fatigue after experimental treatment compared to control ($P = 0.005$). Fifteen minutes after each treatment, subjects warmed-up for 3 min and then cycled at 80% of peak power output on a stationary ergometer until exhaustion. Performance in this physical task (i.e. endurance performance) was measured as time to exhaustion (mean ± SD) which was reduced in the mental fatigue state (640 ± 316 s) compared to control (754 ± 339 s) ($P = 0.003$). There were no significant differences between treatments in cardiac output (Fig. 1A), oxygen consumption (Fig. 1B), and blood lactate concentration (Fig. 1C) during cycling. Lactate at exhaustion was actually higher after control treatment ($P = 0.032$). Therefore, the physiological factors commonly assumed to limit endurance performance (3) could have not mediated the negative effect of experimental treatment on time to exhaustion. Similarly, self-reported success motivation and intrinsic motivation related to the physical task did not differ significantly between treatments. The negative effect mental fatigue on endurance performance seems to be mediated by the significant increase in perceived effort required by the physical task ($P = 0.007$ at isotime) (Fig. 1D). Ratings of perceived exertion at exhaustion were similar between treatments. These findings are in accordance with Brehm's motivational intensity theory (4) which predicts that people disengage from a task when the effort required surpasses the maximum level of effort they are willing to invest for succeeding in that task. Neuroimaging studies in humans are necessary to investigate whether this effort-related decision-making process is associated with altered ACC activity (5). In conclusion, the present study provides experimental evidence in favour of the hypothesis that brain function can limit endurance performance independently of cardiovascular and metabolic alterations.



Effects of mental fatigue on physiological (A, B, C) and perceptual (D) responses to intense cycling exercise to exhaustion. Data are presented as mean ± SE. # significant ($P < 0.05$) paired t-test; significant main effect of treatment at isotime by repeated measure ANOVA; all main effects of time are significant.

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C95

Organisation of microtubules in mpkCCD$_{c14}$ cells

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Microtubules (MT) are dynamic, polar structures which mediate a number of essential cellular functions, including vesicle transport. In Madin-Darby Canine Kidney cells, MT networks reorganise as the cells become polarized (Bacallao et al, 1989), with their minus ends in the apical pole. Consistent with this, vesicles carrying aquaporin 2 (AQP2) to the apical surface of collecting duct cells have dynein, a minus-end directed motor, attached. We investigated MT organisation in mpkCCDc14 cells, a cell line derived from murine cortical collecting duct principal cells (Bens et al 1999).

Cells were seeded onto both coverslips and polycarbonate filters, and grown in modified DMEM medium. In some experiments cells were treated with 1nM desmopressin (dDAVP) for 4-5 days to induce AQP2 expression. After three hours washout, half the cells were treated with dDAVP for 30 minutes to induce shuttling. Cells were fixed with either ice cold methanol (-20 °C) or 4% paraformaldehyde, fluorescently labelled using antibodies against α tubulin, γ tubulin, acetylated tubulin, AQP2 and end binding protein 1 (EB1) in various combinations, and viewed using deconvolution and confocal microscopy. All findings described below were confirmed on at least 2 independent preparations.

As expected, cells grown on coverslips were not polarised, did not express AQP2, and displayed a fibroblastic appearance. Labelling for α tubulin revealed a basket weave pattern with the MT organised around the nucleus. Polarisation was seen in the filter-grown cells, although a proportion of fibroblastic cells were also present. Polarised cells were considerably taller than the fibroblastic cells. Labelling for α tubulin revealed a meshwork of MT in the apical part of the cell; microtubules could also be seen running down the lateral borders of the cell alongside the nucleus. Gamma tubulin, found in the centrioles and microtubule organising centre (MTOC), was located apically, usually as two foci. Co-labelling for γ and acetylated α tubulin (characteristic of cilia) confirmed the presence of primary cilia, with an associated basal body. After acute dDAVP treatment, labelling with EB1, which associates with microtubule plus ends, was most abundant in an apical meshwork, but was also seen at the bottom of the cell. Cells not acutely treated with dDAVP had a similar pattern, but a less prominent apical network, suggesting fewer new MTs forming in this region. In fibroblastic cells plus ends were located throughout, with no clear focus. AQP2 labelling was predominantly apical in cells acutely treated with dDAVP.

Our results are consistent with the hypothesis that microtubule assembly is initiated primarily in the apical part of the cells, with plus ends projecting towards the basolateral membrane. Hence dynein will move AQP2-containing vesicles towards the apical surface.

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C96

The regulation of Na^+ transport in H441 cells by AMPK and PI(4,5)P_2

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The amiloride-sensitive epithelial Na^+ channel, ENaC, composed of α, β and γ subunits controls the fluid lining the airways. Overexpression of ENaC is linked to the pathogenesis associated with cystic fibrosis (Mall *et al*., 2004). We have shown that the AMP mimetic, AICAR, activates the metabolic sensor AMP-activated protein kinase (AMPK) and inhibits amiloride-sensitive Na^+ transport in H441 human lung epithelial cells (Woollhead *et al*., 2005). Its mechanism of action is unknown. As PI(4,5)P_2 is reported to be required for ENaC activation (Kunzelmann *et al*., 2005), we have investigated the hypothesis that AMPK converges on PI(4,5)P_2 and alters ENaC-PI(4,5)P_2 interactions.

Monolayers were mounted in Ussing chambers in physiological salt solution. AICAR (2 mM) rapidly reduced amiloride-sensitive short circuit currents ($I_{SC\text{-}Amil}$) by 49.0 ± 7.6 % ($P < 0.05$, $n = 8$). Compound C, an inhibitor of AMPK, rescued $I_{SC\text{-}Amil}$. Purinergic stimulation by UTP (100 μM) inhibited $I_{SC\text{-}Amil}$ by 49.2 ± 7.5 % ($P < 0.05$, $n = 8$) via depletion of membrane PI(4,5)P_2. Neomycin (5 mM), which sequesters PI(4,5)P_2, inhibited $I_{SC\text{-}Amil}$ by 59.7 ± 12.6 % ($P < 0.05$, $n = 8$) and there was no further inhibition with UTP. Western blotting showed that there