C112

Human brain MRS demonstrates pregnancy-induced phosphate and pH changes

A. Holdcroft*, L. Hall*, S. Counsell†, G. Hamilton†, G. Bydder† and J. Bell†

*Departments of *Anaesthesia and the †Robert Steiner MR Unit, Imperial College, London, UK*

We have demonstrated a reversible decrease in brain size in pregnancy that has a prolonged recovery, taking months to return to normal after delivery (Oatridge *et al.* 2002). The mechanism and functional significance is speculative so this study was designed to investigate the metabolic changes that may be part of this normal adaptation to parturition.

After Ethics Committee approval consenting women were scanned at term, 6 weeks and, if possible, 6 months after delivery. Control women had scans a month apart. All measurements were obtained using a 1.5 T Eclipse™ MR scanner (Philips Medical Systems Inc., Cleveland, Ohio, USA). ^{31}P MR spectra (TR = 10 s, 64 signal averages) were localised on a 70 × 70 × 70 mm voxel in the brain using an ISIS sequence. The MR spectra were analysed by an observer blinded to the clinical status of the subjects using prior knowledge (Hamilton *et al.* 2002) in the AMARES algorithm included in the MRUI software program. The peak areas of phosphomonoester (PME), inorganic phosphate (P_i), phosphodiester (PDE), phosphocreatine (PCr), and nucleoside triphosphate (γ, α and βNTP) resonances were calculated as a percentage of the total peak areas. Further the intracellular pH was calculated using:

$$pH = 6.77 + \log((A - 3.29)/(5.68 - A)),$$

where A = chemical shift difference in p.p.m. between P_i and PCr (see arrow in Fig. 1).

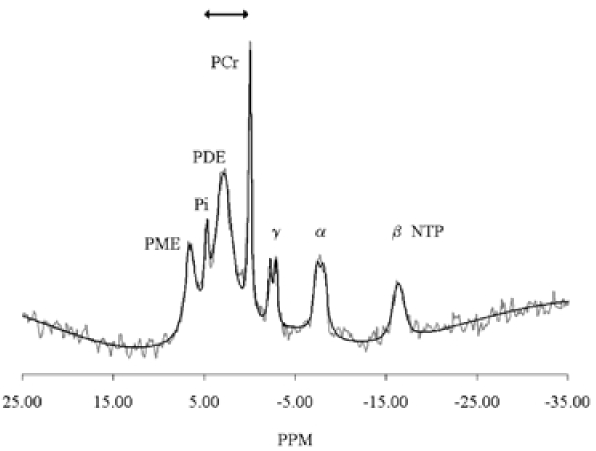

Figure 1. Localised brain ^{31}P MR spectrum and fit. The arrow indicates the chemical shift measured to determine the pH.

Thirteen pregnant (10 at 6 months) women completed the study. The mean (S.E.M.) pH was 7.029 (± 0.010), 7.072 (± 0.017) and 7.017 (± 0.016) at term, 6 weeks and 6 months, respectively, with $P < 0.05$ from term to 6 weeks and 6 weeks to 6 months using ANOVA. In five controls, at both times, pH was similar to 6 months after delivery. The metabolic change in the ^{31}P spectra was between term and 6 weeks, with P_i 4.59 (± 0.27) % and 5.39 (± 0.26) % of the total peak area, respectively.

Pregnancy is associated with a mild arterial alkalaemia secondary to hyperventilation. MR spectrum of erythrocytes has demonstrated an intracellular acidosis (Bardicef *et al.* 1995) to explain this respiratory stimulation. However, CSF is more alkalotic during pregnancy (Hirabayashi *et al.* 1996). We have demonstrated that prolonged metabolic perturbations may be induced that persist after delivery.

Bardicef O *et al.* (1995). *Am J Obstet Gynecol* **173**, 879–880.

Hamilton G *et al.* (2002). *Metab Brain Dis* (in the Press).

Hirabayashi Y *et al.* (1996). *Br J Anaesth* **77**, 352–355.

Oatridge A *et al.* (2002). *Am J Neuroradiology* **23**, 19–26.

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

C113

Overexpression of thromboxane synthase in human colorectal cancer

H. Sakai*, M. Ukai*, T. Suzuki*, K. Tauchi†, T. Minamimura†, N. Horikawa†, K. Tsukada† and N. Takeguchi*

**Departments of Pharmaceutical Physiology and †Surgery II, Toyama Medical and Pharmaceutical University, Toyama 930–0194, Japan*

Overexpression of inducible cyclo-oxygense-2 (COX-2), which catalyses a key step in the conversion of arachidonic acid to prostaglandin H_2, is found to be deeply associated with tumorigenesis of colorectal cancers in humans. More than 80 % of human colorectal cancers show higher expression of COX-2 than accompanying normal mucosa (Williams *et al.* 1999). Thromboxane (TX) synthase, a downstream enzyme of COX-2, catalyses the conversion of prostaglandin H_2 to TXA_2. Recently, the involvement of TXA_2 in endothelial migration, angiogenesis and tumour metastasis has been reported in animal models and cultured cells (Nie *et al.* 2000).

Here we investigated whether TX synthase and TXA_2 are related to human colorectal cancers. The specimens of colorectal well-differentiated adenocarcinomas and adjacent normal mucosa were obtained from surgical resection of patients in accordance with the recommendations of the Declaration of Helsinki. Informed consents were obtained from all patients at Toyama Medical and Pharmaceutical University Hospital. Data are shown as means ± S.E.M. The differences between groups were analysed by one-way ANOVA.

Northern blot analysis showed that TX synthase mRNA overexpressed in 17 of 20 cancers (85 %) compared with the normal mucosa, and average score of its expression in the carcinoma was 7.0 ± 1.5-fold higher than that of the normal mucosa ($n = 20$). The overexpression of TX synthase does not seem to correlate with age, sex, location and size of carcinoma, or clinical stage. COX-2 mRNA overexpressed in the carcinoma as previously reported; however, this overexpression was less consistent among the samples (40%) compared with the case of TX synthase mRNA. TX synthase mRNA also highly expressed in human colonic cancer cell lines such as HT-29, KM12-L4, T-84 and WiDr. In the immunohistochemistry of fresh-frozen tissues using the anti-human TX synthase monoclonal antibody, the cancer cell itself showed clear immunoreactivity of TX synthase. Such immunoreactivity was not observed in epithelial cells in the adjacent normal mucosa. 9,11-Epithio-11,12-methano-TXA_2 (STA_2; 0.1 μM), a stable analogue of TXA_2, accelerated proliferation of HT-29 and KM12-L4 cells ($P < 0.01$, $n = 6$), whereas TXB_2 (0.1 μM), a stable metabolite of TXA_2, was ineffective ($P > 0.05$, $n = 6$).

These results suggest that overexpression of TX synthase and TXA_2-induced cell proliferation are associated with human colorectal cancers.

Nie D *et al.* (2000). *Biochem Biophys Res Commun* **267**, 245–251.
Williams CS *et al.* (1999). *Oncogene* **18**, 7908–7916.

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

C114

Heat strain during a work-in-heat test is greater in a warm-humid than in a hot-dry environment of equal wet bulb globe temperature in men

E.-M. Kellett, A.S. Weller and W.R. Withey

Centre for Human Sciences, QinetiQ Ltd, Farnborough GU14 OLX, UK

Wet bulb globe temperature (WBGT) is a widely used heat stress index (Parsons, 1995). It is used to predict the physiological consequences of heat stress (i.e. heat strain), and to set WBGT threshold limit values to minimise heat illness risk, in many occupational and athletic settings. Implicit in its use is that it is valid in all environmental conditions. This study aimed to establish if heat strain is equivalent in humid and dry heat matched for WBGT, as previous work on this topic is sparse and equivocal (e.g. Keatisuwan *et al.* 1996).

Following local ethics committee approval, ten euhydrated men (mean (1 S.D.): age, 27.4 (3.6) years; height, 1.80 (0.06) m; body mass, 75.2 (9.3) kg; peak oxygen uptake rate, 55.4 (3.8) ml min^{-1} kg^{-1}) completed 60 min of continuous treadmill walking (speed, 1.53 m s^{-1}; grade, 0 %) in two simulated hot environments – warm-humid and hot-dry. Dry-bulb temperature, globe temperature, relative humidity, and air speed were 33.4 °C, 34.1 °C, 88 % and 1.2 m s^{-1}, respectively (WBGT 32.1 °C) for warm-humid, and 45.6 °C, 46.3 °C, 20 % and 1.3 m s^{-1}, respectively (WBGT 32.3 °C), for hot-dry. Subjects wore lightweight clothing (intrinsic clothing insulation, 0.63 clo; Woodcock moisture vapour permeability index, 0.55), and were encouraged to drink 6 ml (kg body mass)$^{-1}$ of water every 30 min. Changes in rectal temperature (ΔT_{re}), mean skin temperature ($\bar{T}_{sk}$) and heart rate (HR) were measured every 5 min. Total (m_{wl}) and evaporative (m_e) water losses were calculated from changes in nude and clothed body mass, and corrected for water consumed. Metabolic rate was measured (Douglas bags) at 25 min.

Table. Indicators of heat strain at 30 and 60 minutes

Variable	Warm-Humid		Hot-Dry	
Time	30	60	30	60
ΔT_{re} (°C)	0.5 (0.2) **	1.1 (0.3) **	0.4 (0.2)	0.8 (0.3)
T_{sk} (°C)	36.6 (0.3) **	36.7 (0.3) *	37.2 (0.4)	37.0 (0.7)
HR (b min^{-1})	121 (13) **	136 (12) **	114 (12.5)	120 (11)
m_{wl} (kg h^{-1})		1.4 (0.4) *		1.2 (0.2)
m_e (kg h^{-1})		0.4 (0.1) **		0.8 (0.1)

Differences (Newman Keuls) between Warm-Humid and Hot-Dry are shown by * = P<0.05; ** = P<0.01.

ANOVA showed that in warm-humid, ΔT_{re} ($P < 0.01$), HR ($P < 0.01$) and m_{wl} ($P < 0.05$) were higher, and $\bar{T}_{sk}$ ($P < 0.01$) and m_e ($P < 0.01$) were lower, than in hot-dry (Table 1). Metabolic rate was the same in both environments (195 (13) and 192 (15) W m^{-2}).

Heat strain was greater in the warm-humid than in the hot-dry environment of equal WBGT. Therefore, in the context of this study, the applicability of WBGT as a predictor of heat strain is questioned and warrants further investigation.

Parsons KC (1995). *Ergonomics* **3**, 6–22.
Keatisuwan W *et al.* (1996). *Appl Human Sci* **15**, 249–258.

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

C115

The diuretic effect of caffeine in hydrated and hypohydrated humans

J.B. Leiper, F. McLeod, K. Sommers and R.J. Maughan

Department of Biomedical Sciences, University Medical School, Foresterhill, Aberdeen AB25 2ZD, UK

Caffeine is considered to have a diuretic effect in doses that are commonly ingested in the diet of normal individuals living in the UK (Neuhäuser-Berthold *et al.* 1997). In the present study, which was approved by the local ethics committee, seven individuals, who all consumed daily at least the equivalent of two cups of instant coffee (4 males, 3 females) were dehydrated by a combination of exercise in the heat followed by overnight fluid restriction. Subjects were tested on four separate occasions in the morning at least 12 h after exercising. On two occasions subjects were rehydrated following the dehydrating exercise by ingesting, with their evening meal, a water volume equivalent to twice their exercise-induced sweat loss plus 500 ml before going to bed and 500 ml in the morning 2 h before coming to the laboratory. On both hypohydrated trials, after the dehydrating exercise subjects drank only 100 ml of water with their evening meal and consumed no other liquids before being tested in the morning. The same meals were given to the subjects on all four trials. Each morning, subjects drank, in 30 min, 1000 ml of either decaffeinated coffee or the same coffee to which had been added 250 mg of caffeine; each trial was performed in an euhydrated and hypohydrated state. Urine samples were collected before ingestion and at hourly intervals for 4 h after drinking finished. All subjects were familiarised with the study procedures before undertaking their first experimental trial; the treatment order was carried out using an incomplete block design. Data were found to be normally distributed and statistical significance ($P < 0.05$) was determined using repeated-measures and one-way ANOVA with Tukey's *post-hoc* analysis as appropriated.

Initial mean (± S.D.) body mass (72.5 ± 16.7 kg) was similar ($P = 0.97$) at the beginning of all trials. Immediately before ingesting the test solutions, subjects on both hypohydrated trials were dehydrated to a similar extent (1.9 ± 0.4 %; $P = 0.63$) of initial body mass (mean ± S.D.). Peak urine output was produced 1 h after finishing drinking on all trials. Similar mean (± S.D.) urine volumes were produced at that time on the hypohydrated trials whether subjects drank decaffeinated (HD, 266 ± 158 ml) or caffeine-containing coffee (HC, 148 ± 117 ml); this was less ($P = 0.001$) than on the euhydrated trials following ingestion of the decaffeinated (ED, 766 ± 209 ml) and caffeine-containing coffee (EC, 774 ± 161 ml), which were essentially the same ($P > 0.05$). Cumulative urine output over the 4 h measurement period was similar ($P > 0.05$) on trials HD (518 ± 177 ml) and HC (292 ± 179 ml), but both were less ($P = 0.001$) than that on trial ED (1289 ± 266 ml) and EC (1368 ± 325 ml), which were essentially the same ($P = 0.66$).

The lack of a caffeine-induced increase in urinary output in both well hydrated and hypohydrated subjects in this study clearly demonstrates that moderate intakes of caffeine do not have a significant diuretic effect in individuals who normally consume caffeine in the diet.

Neuhäuser-Berthold M *et al.* (1997). *Ann Nutr Metabol* **41**, 29–36.

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

C116

Biological and analytical variability of biochemical markers specific for bone formation and resorption in healthy men

P. Carroll, A. Hunter, D. Barry, N. Barrett and P.M. Jakeman

Human Science Research Centre and Department of Mathematics and Statistics, University of Limerick and Mid-West Regional Hospital, Limerick, Ireland

In the mature skeleton approximately 2m bone multicellular units (BMUs) are actively remodelling at any one time. This dynamic process reflects bone turnover. Biochemical markers specific for bone formation and resorption have been proposed as a means to study acute changes in bone turnover in response to, for example, physical activity.

Table 1. Mean concentration and individual critical difference (CDI; $P < 0.05$) for biochemical markers of bone turnover obtained over 5 consecutive days

Analyte	Concentration	CDI (%)
OC (ng ml^{-1})	31.63 ± 10.03	9.5 ± 3.5
CrossLaps™ (ng ml^{-1})	0.628 ± 0.247	20.8 ± 7.9
24 h D-Pyr (nmol d^{-1})	161 ± 101	72.6 ± 32.0
FMV D-Pyr (nM mM Cr^{-1})	17 ± 15	61.8 ± 24.7
24 h Pyr (nmol d^{-1})	553 ± 242	54.1 ± 22.9
FMV Pyr (nM mM Cr^{-1})	51 ± 36	62.9 ± 35.1

Values represent means ± S.D., $n = 17$.

This study sought to (i) measure the variability and (ii) determine the critical difference of plasma and urinary markers specific to bone turnover in healthy young adult men.

With ethical approval and informed consent, seventeen healthy males (age 28.2 ± 1.1 years, height 1.71 ± 0.02 m, mass 77.6 ± 2.9 kg, BMI 24.7 ± 0.8 kg m^{-2}; means ± S.E.M.) were screened for bone health and calcium intake. All subjects were non-smokers, not habitually active and maintained normal physical activity and dietary intake for the period of study. For five consecutive days serum samples were obtained following an overnight fast and assayed for N-MID osteocalcin (OC), a specific marker of bone formation, and C-terminal fragment of pyridinium crosslinks (CrossLaps™), specific for bone resorption, by electrochemiluminescence (Roche Diagnostics). In addition 24 h and first morning void (FMV) urinary samples were collected and analysed for pyridinium crosslinks (pyridinoline, Pyr and deoxypyridinoline, D-Pyr) and creatinine (Cr) by high performance liquid chromatography (HPLC). The individual critical difference (CDI; $P < 0.05$), i.e. the minimum significant difference ($P < 0.05$) between two measurements, was calculated for each analyte according to Fraser & Harris (1989). Changes greater than the CD are considered to be representative of a significant modulation of biological activity (Panteghini & Pagani, 1996). Pearson bivariate correlation was used to examine relationships between the dependent variables.

Analyte concentrations were found to be within the normal reference range for young healthy males. The mean critical difference for urinary analytes was found to be approximately 3-fold that of the respective serum measures of bone resorption. A low correlation between serum measures of pyridinium crosslinks and urinary measures of 24 h D-Pyr ($r = 0.280$, $P = 0.009$), 24 h Pyr ($r = 0.229$, $P = 0.025$), FMV D-Pyr ($r = 0.088$, $P = 0.234$) or FMV Pyr ($r = 0.14$, $P = 0.122$) indicates only limited agreement between related measures of bone resorption.

In conclusion, biochemical markers of bone turnover differ in their within-subject biological variance which impacts on their ability to detect change.

Fraser CG & Harris EG (1989). *Crit Rev Clin Lab Sci* **27**, 409–437.

Panteghini M & Pagani F (1996). *Ann Clin Biochem* **33**, 36–42.

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All procedures accord with the Declaration of Helsinki.

C117

Liver mitochondria from female rats exhibit higher antioxidant gene expression and lower oxidative damage than from males: role of oestrogens

Consuelo Borrás, Juan Sastre, David Garcia-Sala, Ana Lloret, Federico V. Pallardó, Giovanni E. Mann* and Jose Viña

*Department of Physiology, School of Medicine, University of Valencia, Spain and *Centre for Cardiovascular Biology & Medicine, King's College London, London SE1 1UL, UK*

Mitochondria are a major source of free radicals in cells, and longevity studies indicate that women have a longer life span than men. Similar findings have also been reported for animal models, including rats and mice.

In the present study, we have investigated mitochondrial oxidative stress in male and female Wistar rats aged between 4–6 months to evaluate the molecular mechanisms enabling females to live longer than males. Animals were sacrificed by decapitation using approved animal handling procedures. As described previously (Sastre *et al.* 1996), mitochondria were isolated from liver homogenates by differential centrifugation (3 × 1000 *g* for 10 min and 3 × 10000 *g* for 10 min). The rate of peroxide production was measured fluorometrically, reduced glutathione (GSH) determined using the glutathione transferease method (Brigelius *et al.* 1983), and mRNA levels for glutathione peroxidase and Mn-superoxide dismutase (Mn-SOD) relative to 26 S rRNA by RT-PCR.

Our results show that the rate of peroxide production was significantly higher in liver mitochondria from male ($n = 13$) compared to female ($n = 14$) rat (0.097 ± 0.028 *vs.* 0.070 ± 0.02 nmol H_2O_2 min^{-1} (mg protein)$^{-1}$, $P < 0.01$, $n = 5$ rats, Student's unpaired t test). In ovariectomized rats ($n = 6$), peroxide levels were similar to those of males (0.112 ± 0.045 nmol H_2O_2 min^{-1} (mg protein)$^{-1}$, $n = 6$). Oestrogen replacement (1 μg kg^{-1} day^{-1} oestradiol administered subcutaneously for 4 weeks) prevented the effect of ovariectomy (0.044 ± 0.017 nmol H_2O_2 min^{-1} (mg protein)$^{-1}$, $n = 7$). In addition, hepatic mitochondria from female rats had higher GSH levels than those from males (9.76 ± 1.8 *vs.*6.41 nmol GSH/mg protein, $n = 4$, $P < 0.01$) or from ovariectomized controls (6.34 ± 1.27 nmol GSH (mg protein)$^{-1}$, $n = 4$). Oestrogen replacement restored GSH levels to values in non-ovariectomized rats (10.29 ± 1.66 nmol GSH (mg protein)$^{-1}$, $n = 6$).

Oxidative damage to hepatic mitochondrial DNA was also higher in males compared to female rats, as assayed by determining levels of 8{nbh}oxodeoxyguanosine. Moreover, mRNA expression and activities of glutathione peroxidase and Mn-SOD were also elevated in mitochondria from female compared to male rats ($n = 5$, $P < 0.01$). In addition, 16S rRNA expression, which is known to decrease significantly with aging, was found to be higher in mitochondria from female compared to age-matched male rats ($n = 5$, $P < 0.01$).

In conclusion, the difference between genders in average life span could be explained, at least in part, by the different rates of oxidant species generated in mitochondria and by differences in mitochondrial antioxidant activity.

Brigelius, R. *et al.* (1983). *Biochem Pharmacol* **32**, 2529–2534.

Sastre, J. *et al.* (1996). *Hepatology* **24**, 1199–1205.

All procedures accord with current National and local guidelines.

C118

The effect of growth hormone administration and strength training on IGF-IEa and MGF mRNA expression in elderly men

M. Hameed*†, K.H.W. Lange‡, J.L. Andersen§, P. Schjerling§, M. Kjaer‡, G. Goldspink† and S.D.R. Harridge*†

**Department of Physiology, †MCR Unit Royal Free and University College Medical School, London, ‡Sports Medicine Research Unit, Bispebjerg Hospital, Copenhagen, Denmark and §Department of Molecular Muscle Biology, Copenhagen Muscle Research Centre, Denmark*

The mRNA of two isoforms of the IGF-I gene has been shown to be expressed in human muscle. One, IGF-IEa, is similar to the systemic, liver-type IGF-I, whilst a second, MGF, is produced in muscle response to mechanical overload or damage. Recent studies of human muscle samples obtained shortly after a single bout of high-resistance exercise suggest that IGF-IEa and MGF mRNA transcripts are differentially regulated (Hameed *et al.* 2003). The present study was aimed at determining the effects of recombinant growth hormone (rhGH) administration with and without resistance training in elderly subjects on the mRNA expression of the two different isoforms of IGF-I.

Healthy elderly men (aged 74 ± 1 years, mean ± S.E.M.) were assigned to either resistance training (3 sessions/week, 3–5 sets of 8–12 repetition maximum per session) with placebo (RT group, $n = 6$), RT combined with rhGH administration (RT + GH group, $n = 6$) or rhGH alone (GH group, $n = 7$) in a randomised, placebo-controlled, double-blinded design (Lange *et al.* 2002). Administration of GH occurred daily through subcutaneous injection in the thigh (0.5 IU m^{-2} rising to 1.5 IU m^{-2}). Following local anaesthesia (1 % lidocaine), muscle biopsies were obtained from the right vastus lateralis muscle at baseline, 5 weeks and 12 weeks. In the two training groups the biopsies were obtained 24 h after completion of the last training session. Samples were immediately frozen in liquid nitrogen. IGF-IEa and MGF mRNA transcripts were analysed using a quantitative reverse transcription-polymerase chain reaction (RT-PCR) method (LightCycler, Roche UK).

After 5 weeks of GH administration without exercise (GH), IGF-IEa had increased, by on average 226 %, in contrast to MGF mRNA levels, which were unchanged (Table 1). However, there was a significant increase in both IGF-1Ea (77 and 145 %) and MGF mRNA levels (200 and 354 %) in the RT and RT + GH groups, respectively, at this time point. A further 7 weeks of GH administration resulted in a significant increase in MGF (63 % relative to baseline), but no other significant changes in MGF or IGF-1Ea mRNA were observed between 5 and 12 weeks.

Table 1. MGF and IGF-1Ea mRNA levels pre, after 5 weeks and after 12 weeks of either resistance training (RT), resistance training and rhGH administration (RT + GH) or rhGH administration only (GH)

	Pre	Post 5 weeks	Post 12 weeks
IGF-1Ea			
RT	5.3 ± 0.7[a]	9.4 ± 2.0†[a]	10.2 ± 1.0†[a]
RT + GH	6.2 ± 0.8[a]	15.2 ± 2.6†[a]	14.8 ± 2.8†[a]
GH	10.6 ± 1.4	34.6 ± 7.4†	23.2 ± 2.2
MGF			
RT	1.6 ± 0.2[a]	4.8 ± 1.2†[b]	4.2 ± 0.4†
RT + GH	2.2 ± 0.6[a]	10 ± 1.4†	7.8 ± 1.2†
GH	4.8 ± 1.2	5.8 ± 1.2[b]	7.8 ± 1.6†‡

Data are expressed as ng mRNA/μg RNA. For IGF-IEa 10^{-5} ng, for MGF 10^{-8} ng. †Significant difference from 0 weeks, ‡ from 5 weeks ($P < 0.05$). [a]Significant difference from GH, [b]from RT + GH at given time point ($P < 0.05$). Data are means ± S.E.M. and analysed using ANOVA followed by Student-Newman-Keuls *post-hoc* tests.

The results suggest that MGF mRNA expression in muscle is less sensitive to GH administration than IGF-IEa, at least in elderly subjects. However, when mechanical loading in the form of resistance training is combined with GH (RT + GH), both MGF and IGF-1Ea mRNA levels are enhanced, which may reflect an overall up-regulation of transcription of the IGF-I gene prior to splicing. Previously reported data from this study showed no greater increase in muscle strength and cross-sectional area with RT + GH compared with RT (Lange *et al.* 2002).

Hameed M *et al.* (2003). *J Physiol* (in the Press).

Lange KHW *et al.* (2002). *J Clin Endocrinol Metab* **87**, 513–523.

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

C119

Strength training increases the stiffness of human tendons in older individuals

N.D. Reeves, C.N. Maganaris and M.V. Narici

Centre for Biophysical and Clinical Research into Human Movement, Manchester Metropolitan University, Alsager Campus, UK

In vitro studies have shown that ageing decreases tendon stiffness (Nachemson & Evans, 1968; Vogel, 1991). However, physical activity has been found to alter the properties and/or the dimensions of tendons, yielding ultimately stiffer structures (Woo *et al.* 1982). The aim of the present study was to investigate the effect of strength training (ST) on the mechanical properties of an elderly tendon *in vivo*: the patella tendon (PT).

After receiving ethical approval and written informed consent, 18 elderly individuals (nine per sex) were randomly assigned to ST (means ± S.D.: age 74.3 ± 3.5 years, body mass 69.7 ± 14.8 kg and height 163.4 ± 9.1 cm) and control (age 67.1 ± 2 years, body mass 73.5 ± 14.9 kg and height 168.3 ± 11.5 cm) groups. Two sets of ~10 leg-extension and leg-press exercises at ~80 % of the five repetition maximum, were performed three times per week for 14 weeks. PT elongation was measured *in vivo* using B-mode ultrasonography (HDI 3000, ATL, USA) during a ramp isometric knee extension at 90 deg (cf. Maganaris & Paul, 1999). PT forces were calculated by dividing joint moments by MRI-measured PT moment arm length, after taking into account antagonist coactivation estimated from EMG activity. Stress and strain were calculated by normalizing forces and elongations to tendon dimensions, measured using ultrasound. Tendon stiffness (gradient of the force–elongation relationship) was multiplied by the ratio of tendon length to cross-sectional area to obtain Young's modulus. All measurements were performed before and after the ST period. Results were analysed using a 2 × 2 ANOVA; level of significance was set at $P < 0.05$. Data are presented as means ± S.D.

Training induced a left shift of the stress–strain relationship indicating a decreased elongation and strain at all levels of force and stress (Fig. 1). Whereas at baseline, PT elongation and strain at maximal tendon load were 4.7 ± 1.1 mm and 9.9 ± 2.2 %, respectively (maximum force: 3346 ± 1168 N; maximum stress: 40 ± 11 MPa), after training these values decreased to 2.9 ± 1.2 mm and 5.9 ± 2.4 % ($P < 0.01$), respectively (maximum force: 3555 ± 1257 N; maximum stress: 42.1 ± 10.5 MPa). As a result PT stiffness increased by 65 % after ST (2187 ± 713 to 3609 ± 1220 N mm^{-1}; $P < 0.05$) and Young's modulus increased by 69 % (1.3 ± 0.3 to 2.2 ± 0.8 GPa; $P < 0.01$). In contrast, no significant changes in elongation, strain, stiffness or Young's modulus occurred in the control group. There was no significant tendon hypertrophy following the training or the control periods. The rate of torque development increased by 27% following training ($P < 0.01$).

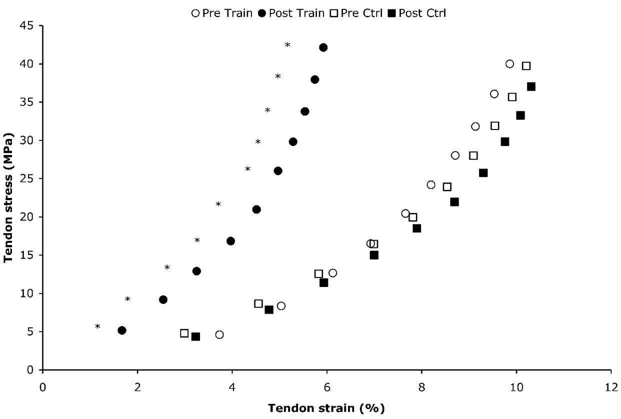

Figure 1. *In vivo* patella tendon stress–strain relationship in elderly humans before and after training and control periods ($n = 18$). *Significantly reduced strain ($P < 0.01$) after training.

In conclusion, this study shows that ST alters the structural and material properties of human elderly tendons. The decreased tendon strain after training may help to reduce the risk of tendon injuries in old age. The increased tendon stiffness after training would increase the rate of force development and may enable the muscle to operate closer to resting length.

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Vogel HG (1991). *Mech Ageing Devel* **1**, 15–24.

Woo SL-Y *et al.* (1982). *Biorheology* **19**, 397–408.

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

C120

Potential mechanisms of the training-induced acceleration of the $\dot{V}_{O_2}$ kinetics at the onset of exercise: theoretical studies

Bernard Korzeniewski* and Jerzy A. Zoladz†

**Institute of Molecular Biology and Biotechnology, Jagiellonian University, ul. Gronostajowa 7, 30–387 Kraków and †Department of Muscle Physiology, AWF-Krakow, al. Jana-Pawla II 78, 31–571 Kraków, Poland*

In the present study we analysed by the means of the computer model of oxidative phosphorylation in intact muscle (Korzeniewski & Zoladz, 2001) two potential mechanisms responsible for the endurance training-induced acceleration of the $\dot{V}_{O_2}$ kinetics at the onset of exercise: (1) increase in the amount of mitochondrial enzymes and (2) increase in the parallel activation of ATP supply and ATP usage during rest → work transition (Korzeniewski, 1998). Within the parallel-activation mechanism it is assumed that, starting from the onset of exercise, some external factor(s) (e.g. Ca^{2+} ions) stimulate(s) directly all oxidative phosphorylation enzymes, in parallel with ATP usage. The computer simulations were performed under the assumption that oxygen delivery does not limit $\dot{V}_{O_2}$ by oxidative phosphorylation.

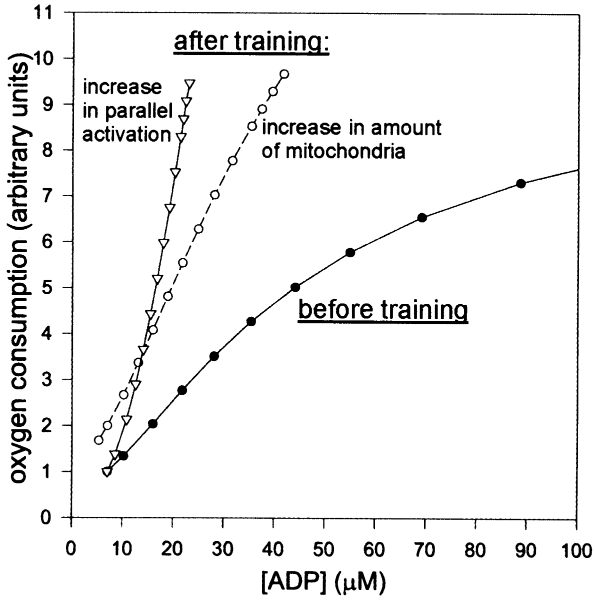

Figure 1. Simulated relationship between $\dot{V}_{O_2}$ (standardized for 1 in resting state in untrained muscle) and [ADP] in untrained muscle without parallel activation (extreme case), trained muscle with a 2-fold increase in mitochondrial enzymes and trained muscle with an induced direct activation of oxidative phosphorylation.

The present theoretical studies show that both mechanisms can markedly shorten the $t_{1/2}$ (half-transition time) for $\dot{V}_{O_2}$ from 88 s (extreme case) to 40 and 26 s, respectively (for the assumed increase in the amount of mitochondria and in the degree of parallel activation), for an exercise causing an ~7-fold increase in $\dot{V}_{O_2}$ in relation to resting state. Therefore, in order to distinguish between these two mechanisms it is necessary to compare their effect on the values of $\dot{V}_{O_2}$ and ADP during rest → work transition. Figure 1 presents the simulated dependence between $\dot{V}_{O_2}$ and [ADP] for three cases: (1) untrained muscle without parallel activation, (2) trained muscle with a 2-fold increase in mitochondrial enzymes and (3) trained muscle with an induced parallel activation. It can be seen from Fig. 1 that an increase in mitochondrial enzymes does not increase by itself the relative sensitivity of oxidative phosphorylation to ADP (the ratio of the relative increase in $\dot{V}_{O_2}$ to the relative increase in ADP) $((\dot{V}_{O_2(work)}/\dot{V}_{O_2(rest)})/([ADP]_{(work)}/[ADP]_{(rest)}))$, while an increase in parallel activation causes large relative changes in $\dot{V}_{O_2}$ that are accompanied by smaller relative changes in ADP.

Comparison of the theoretical results presented in Fig. 1 with experimental data (Constable *et al.* 1987; Dudley *et al.* 1987; Clark *et al.* 1988) suggests that an increase in parallel activation, leading to an increase in the phenomenological 'sensitivity' of oxidative phosphorylation to ADP, can be at least partly responsible for the training/conditioning-induced shortening of the transition time in $\dot{V}_{O_2}$ kinetics.

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C121

The relationship between MyHC II content in vastus lateralis m. quadricipitis femoris and the oxygen uptake during incremental exercise test in humans

Jerzy A. Zoladz*, Krzysztof Duda*†, Janusz Karasiński‡, Joanna Majerczak*, Leszek Kolodziejski† and Bernard Korzeniewski§

**Department of Muscle Physiology, AWF-Kraków, Al. Jana Pawla II 78, 31–571 Kraków, †Cancer Institute, ul. Garnacrska 11, 31–115 Kraków, ‡Institute of Zoology, Jagiellonian University, ul. Ingardena 6, 30–060 Kraków and §Institute of Molecular Biology, Jagiellonian University, ul. Gronostajowa 7, 30–387 Kraków, Poland*

It has been demonstrated that the oxygen cost of work is higher in individuals possessing greater proportion of type II muscle fibres (Coyle *et al.* 1992). In the present study, we examined the relationship between the content of MyHC II in muscle and the oxygen cost of incremental work in humans.

Twenty-one male subjects: aged 24.0 ± 2.5 years (mean ± S.D.), body mass 73.0 ± 7.2 kg, height 179 ± 5 cm, $\dot{V}_{O_2,max}$ 3697 ± 390 ml min^{-1}, participated in this experiment. The exercise test started at a power output of 30 W, followed by an increase amounting to 30 W every 3 min, at 60 rev min^{-1}. Gas exchange variables were measured continuously using a breath-by-breath system (Oxycon-Champion Jaeger). At the end of each step blood samples were taken for lactate concentration.

Muscle biopsy samples taken from the vastus lateralis m. quadricipitis femoris were analysed for the content of different MyHC (I, IIa, IIx) using SDS-PAGE and Western blotting.

The pre-exercise $\dot{V}_{O_2}$, as a mean value of 6 min measurements, expressed both in ml min^{-1}, and in ml kg^{-1} min^{-1}, was positively correlated with the content of MyHC II ($P < 0.01$). We have also found that the pre-exercise values of $\dot{V}_{O_2}$ in the group of subjects with a high proportion of MyHC II (59.9 ± 11.2 %) were significantly higher ($P < 0.02$, when $\dot{V}_{O_2}$ was expressed in ml min^{-1}, and $P < 0.01$ when $\dot{V}_{O_2}$ was expressed in ml kg^{-1} min^{-1}) than in the group with low content of MyHC II (27.5 ± 6.0 %). We, as others (Barstow *et al.* 2000), have also found a significant negative correlation ($r = -0.562$, $P < 0.01$) between the slope in the $\dot{V}_{O_2}$/power output relationship below the lactate threshold (LT) and the content of MyHC IIa. Moreover, the magnitude of the non-linear increase in the $\dot{V}_{O_2}$/power output relationship present above LT (see Zoladz *et al.* 1995) in our study was positively correlated ($r = 0.510$, $P < 0.02$) with the content of MyHC II, opposite to the findings by Barstow *et al.* (2002).

Our results show that individuals with high content of MyHC II consume more oxygen in the pre-exercise conditions and require a smaller increase in $\dot{V}_{O_2}$ for maintaining a linear increase in power output up to LT, but after exceeding the LT they consume more oxygen above that expected from the linear relationship below the LT than subjects with a low content of MyHC II.

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

C122

Effect of pedal rate on oxygen uptake kinetics during submaximal cycle exercise in humans

Jamie S.M. Pringle*†, Jonathan H. Doust‡, Helen Carter*, Keith Tolfrey† and Andrew M. Jones†

**Chelsea School, University of Brighton, Eastbourne BN20 8HG, †Department of Exercise and Sport Science, Manchester Metropolitan University, Alsager ST7 2HL, ‡University of Wales Aberystwyth, Ceredigion SY23 3DA, UK*

During constant-load submaximal exercise above the ventilatory threshold (VT), the fundamental response of pulmonary oxygen uptake ($\dot{V}_{O_2}$) is supplemented by a slow component (SC) that causes it to rise above the anticipated steady-state value. It has been suggested that the $\dot{V}_{O_2}$ SC is related to the recruitment of type II fibres at high-exercise intensities (Barstow *et al.* 1996; Pringle *et al.* 2002). There is evidence that the recruitment of type II motor units is enhanced at high pedal rate (Sargeant, 1994). However, only few studies have examined the $\dot{V}_{O_2}$ kinetic responses to heavy exercise at different pedal rates and these have used a limited range of pedal rates (Barstow *et al.* 1996). In the present study, we manipulated pedal rate during heavy exercise in order to test the hypothesis that the $\dot{V}_{O_2}$ SC was related to the recruitment of type II muscle fibres.

Ten recreationally active subjects (8 male, 2 female, mean ± S.D., age 26 ± 4 years; mass 71.5 ± 7.9 kg) volunteered to participate in this study that was approved by the Manchester Metropolitan University ethics committee. The subjects completed three separate incremental exercise tests at 35, 75 and 115 rev min^{-1}, on an electrically braked cycle ergometer to determine the VT and peak $\dot{V}_{O_2}$ from breath-by-breath pulmonary gas exchange responses. Subsequently, the subjects performed two transitions of 6 min duration at each pedal rate at an intensity equivalent to half way between the pedal rate-specific VT and peak $\dot{V}_{O_2}$. The power output was adjusted during the baseline cycling period at 35 and 75 rev min^{-1} in order that the $\dot{V}_{O_2}$ was equivalent to that during unloaded cycling at 115 rev min^{-1}. For each of the three conditions, breath-by-breath $\dot{V}_{O_2}$ data were interpolated, time-aligned and ensemble averaged, and then modelled using non-linear regression techniques to determine the amplitude of the $\dot{V}_{O_2}$ primary and slow components. ANOVA with Bonferroni adjusted paired *t* tests were used to test for differences across pedal rates. Results are reported as means ± S.E.M.

The temporal aspects of the $\dot{V}_{O_2}$ kinetic responses and the absolute $\dot{V}_{O_2}$ at the end of the primary component were not significantly different across the pedal rates. The gain ($\Delta\dot{V}_{O_2}/\Delta WR$) of the $\dot{V}_{O_2}$ primary component fell as pedal rate increased (10.6 ± 0.3 *vs.* 9.5 ± 0.2 *vs.* 9.0 ± 0.4 ml min^{-1} W^{-1} at 35, 75 and 115 rev min^{-1}, respectively; $P < 0.05$ for 75 and 115 *vs.* 35 rev min^{-1}). The amplitude of the $\dot{V}_{O_2}$ SC increased as pedal rate increased (109 ± 30 *vs.* 202 ± 38 *vs.* 328 ± 29 ml min^{-1} at 35, 75 and 115 rev min^{-1}, respectively; $P < 0.01$ for 115 *vs.* 35 rev min^{-1}).

In conclusion, our results demonstrate that both the primary and slow components of $\dot{V}_{O_2}$ are affected by differences in pedal rate during heavy exercise. These effects are presumably mediated by altered motor unit recruitment patterns at the onset of exercise and the associated changes in these and in the rate of fatigue development and efficiency as exercise progresses.

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

C123

Walking performance and cardiorespiratory responses to upper- and lower-limb exercise training in patients with peripheral arterial disease

I. Zwierska, J.M. Saxton, J.S. Male, S. Choksy*, A.G. Pockley* and R.F.M. Wood*

*The Centre for Sport and Exercise Science, Sheffield Hallam University, Collegiate Crescent Campus, Sheffield S10 2BP and *Division of Clinical Sciences (NGH), Northern General Hospital, Herries Road, Sheffield S5 7AU, UK*

Peripheral arterial disease (PAD) is characterised by lower-limb ischaemic pain during walking, which limits peak exercise performance and oxygen consumption during graded treadmill exercise testing (Hiatt *et al.* 1987). Upper-limb aerobic exercise, however, is well tolerated by these patients (Zwierska *et al.* 2002) and an improvement in their cardiorespiratory capacity through a training intervention might also provide an improvement in walking performance.

Following familiarisation with the training and assessment protocols, 27 patients (16 males, 11 females, median age 67 years, range 50–82 years) with stable PAD were randomised into arm- and leg-crank exercise training groups or a non-exercise training control group. Training was performed twice weekly for 24 weeks, with incremental arm- and leg-crank assessments to maximum exercise tolerance being undertaken before and after the intervention period in all groups. A standard electronically braked cycle ergometer (modified for arm-cranking) was used for all incremental assessments, with work rate being increased by 7.5 and 15 W per increment in arm- and leg-crank assessments, respectively. Pulmonary gas exchange variables and blood lactate concentration were recorded at each work increment. Walking performance was assessed at the same time points on flat ground using a shuttle-walk protocol (Walker *et al.* 2000). Approval for this study was obtained from the North Sheffield Local Research Ethics Committee.

Peak oxygen uptake measured during the incremental arm-crank assessment was unchanged following the intervention period in both exercise training groups and in the controls. However, peak oxygen uptake (mean ± S.E.M.) measured during the incremental leg-crank assessment was improved to a similar degree following both arm (1.02 ± 0.14 *versus* 1.29 ± 0.16 l min^{-1}, $P < 0.01$; ANOVA) and leg (1.03 ± 0.08 *versus* 1.32 ± 0.12 l min^{-1}, $P < 0.01$; ANOVA) training, but not in the controls. Maximum walking distance improved by 27 % ($P < 0.01$; ANOVA) and 37 % ($P < 0.01$; ANOVA) following arm and leg training, respectively, and was highly correlated with the change in peak oxygen uptake measured during incremental leg cranking ($r = 0.8$; $P < 0.01$) in the leg training group only. Only moderate correlations (r ~0.4; $P < 0.05$) were observed between blood lactate concentrations measured at the end of the incremental leg-crank assessment and maximum walking distance. Arm-cranking appears to be a good exercise modality for improving walking performance in patients with PAD.

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Walker RD *et al.* (2000). *J Vasc Surg* **31**, 662–669.
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All procedures accord with current local guidelines and the Declaration of Helsinki.

C125

Long-term decrease of force after isometric exercise in humans is muscle length specific

A. Philippou, M. Maridaki and G.C. Bogdanis

Department of Sports Medicine & Biology of Physical Activity, University of Athens, Dafni, 172 37, Greece

Strenuous eccentric exercise has been shown to cause a decrease in maximum voluntary contraction (MVC) that persists for several days after exercise, and is more pronounced when measurements are performed at short muscle lengths (Saxton & Donnelly, 1996). A previous paper by Jones *et al.* (1989) reported that a decrease in force may also occur after maximal isometric exercise when the contracting muscles are fixed at a long muscle length. The purpose of the present study was twofold: (1) to examine whether a protocol of repeated maximal isometric contractions at long muscle length causes long-term decreases in MVC, and (2) to observe whether the magnitude of decrease in MVC following this type of exercise is dependent on muscle length.

Seven male volunteers (age 26.6 ± 2.2 years, height 174.7 ± 1.8 cm, mass 74.3 ± 4.3 kg, means ± S.E.M.) participated in the study that had been approved by the University Committee. Following familiarization, the MVC of the elbow flexors was measured at five different elbow angles: 50, 70, 90, 140 and 160 deg. Three days after the preliminary force measurements, each subject performed 50 maximal voluntary isometric muscle contractions (10 s contraction–20 s rest) of the elbow flexors with the shoulder hyper-extended at 45 deg and the elbow joint fixed at 140 deg. This position was chosen to make the elbow flexors to contract from a lengthened position. Following the exercise protocol the MVC at the above five elbow angles, range of motion (ROM), muscle soreness and plasma creatine kinase activity were measured at 24 h intervals for 4 days. Statistical analyses were performed using a two-way analysis of variance.

The greatest decrease (about 40 %) in MVC, 24 h after isometric exercise, was observed at the more acute elbow angles (50 and 70 deg, $P < 0.01$, Fig. 1). On the same day, smaller decreases of 26 and 16% in MVC were found at the elbow angles of 90 and 140 deg ($P < 0.01$), while no significant decrease in MVC was evident at the elbow angle of 160 deg (Fig. 1). MVC restoration was a function of the elbow angle, with force recovery being less at the smaller angles throughout the 4-day observation period (Fig. 1). This pattern of angle-specific MVC decline may be attributed either to sarcomere overstretching, which caused a subtle increase in muscle length and therefore a shift in the force–length relationship, or to a differential activation of the contracting muscles.

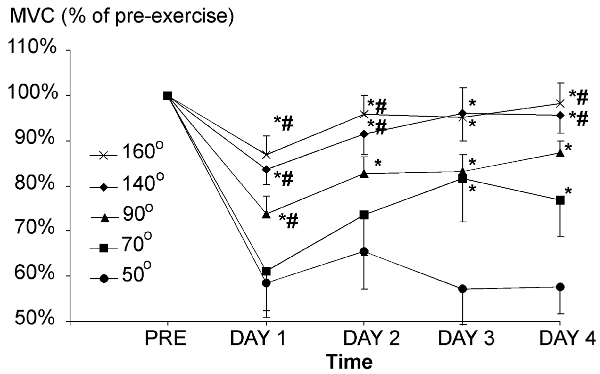

Figure 1. Changes in MVC (% of pre-exercise values) at the elbow angles of 50, 70, 90, 140 and 160 deg after isometric exercise at long muscle length. Values are means (± S.E.M., $n = 7$). *$P < 0.01$ from 50 deg, #$P < 0.01$ from 70 deg.

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

PC3

Saliva flow rate, total protein concentration and osmolality as potential markers of whole body hydration status during progressive acute dehydration and rehydration in humans

N.P. Walsh, J. Montague, N. Callow and A. Rowlands

School of Sport, Health and Exercise Sciences, University of Wales, Bangor LL57 2PX, UK

The predominant constituent of saliva is water (97–99.5%), which enters saliva from plasma across acinar cells (Young & van Lennep, 1979). Dehydration, induced by a 24 h period without food and water, has recently been shown to decrease parotid saliva flow rate in adults (Ship & Fischer, 1999). To identify if saliva flow rate, total protein concentration and osmolality are sensitive markers of whole body hydration status, we compared changes in these parameters with changes in body mass during progressive acute dehydration and rehydration in humans.

With local ethics committee approval, twelve healthy males (age 21 ± 1 years, body mass 76.2 ± 2.3 kg, $\dot{V}_{O_2,max}$ 57.6 ± 2.2 ml kg^{-1} min^{-1}, mean ± S.E.M.) volunteered to participate in the study. To ensure subjects arrived at the laboratory in a euhydrated state they were instructed to drink 30 ml (kg body mass)$^{-1}$ of water the day before the trial. Subjects reported to the laboratory following an overnight fast and cycled on a stationary ergometer at 70% of age-predicted maximal heart rate in an environmental chamber (30 °C and relative humidity 70%) until progressive body mass loss (BML) of 1.0 ± 0.0, 2.0 ± 0.0 and 2.9 ± 0.2%. After exercise, subjects were given a volume of carbohydrate (CHO)-electrolyte solution (6 % CHO and 25 mmol l^{-1} Na^+) equivalent to 150% BML to consume within 1 h. Unstimulated whole saliva samples were collected over a 2 min period into pre-weighed tubes at pre-exercise, 1.0, 2.0 and 2.9% BML and then at 1 h 15 min, 2 h 15 min and 3 h 15 min post-exercise. Saliva samples were stored at −40 °C prior to analysis. Saliva volume was estimated by weighing to the nearest mg and saliva density was assumed to be 1 g ml^{-1}. Saliva flow rate was determined by dividing the volume of saliva by the collection time. Saliva total protein concentration was measured using a kit (No. 610, Sigma, Poole, UK). Saliva osmolality was measured using a freezing point depression osmometer (Advanced Instruments, MA, USA). Correlations between % BML and each saliva parameter were calculated by pooling individual Pearson's correlation coefficients and applying Fisher's Z_r transformation. Adjustments were made to the significance of correlation coefficients according to sample size and number of correlations performed (Shavelson, 1988). Differences between correlation coefficients were determined using an adjusted z score equation (Meng *et al.* 1992). Results were also analysed using repeated measures ANOVA with *post-hoc* Tukey's test where appropriate. Statistical significance was accepted at $P < 0.05$.

Saliva total protein concentration and osmolality increased (main effect of time: $P < 0.01$), and flow rate decreased (main effect of time: $P < 0.01$), during dehydration (Table 1). After consumption of the rehydration solution saliva parameters were not significantly different from pre-exercise. Saliva total protein concentration and osmolality correlated strongly with % BML during dehydration (r = 0.97 and 0.94, respectively: $P < 0.01$). Correlations for saliva total protein concentration and osmolality with % BML were greater ($P < 0.01$) than the correlation for flow rate with % BML (−0.70: $P < 0.05$) during dehydration (Table 1). These data show that changes in saliva total protein concentration and saliva osmolality are strongly associated with changes in body mass during progressive acute dehydration in humans.

Table 1. Saliva flow rate, total protein concentration and osmolality during progressive acute dehydration and rehydration in humans

	Flow rate (μl min^{-1})	Total protein (mg ml^{-1})	Osmolality (mosmol kg^{-1})
Pre-exercise	491 ± 65	0.75 ± 0.07	51 ± 3
1.05 ± 0.03% BML	395 ± 70	1.16 ± 0.14*	80 ± 11*
2.01 ± 0.02% BML	253 ± 58**	1.71 ± 0.13**##	102 ± 10**#
2.89 ± 0.17% BML	205 ± 44**#	1.79 ± 0.12**##	103 ± 10**#
r dehydration	−0.70^a	0.97^b††	0.94^b††
1 h 15 min post-exercise	554 ± 83	1.02 ± 0.09	54 ± 3
2 h 15 min post-exercise	421 ± 71	0.87 ± 0.08	53 ± 4
3 h 15 min post-exercise	557 ± 87	0.79 ± 0.08	54 ± 4

Values are means ± S.E.M. ($n = 12$). Significantly different from pre-exercise: *$P < 0.05$, **$P < 0.01$. Significantly different from 1.05% BML: #$P < 0.05$, ##$P < 0.01$. Significant correlation coefficient: $^a P < 0.05$, $^b P < 0.01$. Significantly greater than corresponding correlation coefficient for flow rate: ††$P < 0.01$. BML, body mass loss.

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

PC4

The effect of fluid intake on human neutrophil degranulation following prolonged exercise

N.C. Bishop, G.A. Scanlon, N.P. Walsh*, L.J. McCallum and G.J. Walker

*School of Sport and Exercise Sciences, Loughborough University, Leicestershire LE11 3TU and *School of Sport, Health and Exercise Sciences, University of Wales, Bangor LL57 2PX, UK*

Ingesting carbohydrate (CHO) beverages at regular intervals during prolonged strenuous exercise is associated with fewer numbers of circulating neutrophils and attenuated neutrophil functional responses (Bishop *et al.* 2000). These effects are thought to be largely mediated through the influence of CHO in blunting the stress hormone response to exercise, particularly that of cortisol (Gleeson & Bishop, 2000). However, there is little information concerning the effect of fluid intake alone on immune responses to prolonged exercise, yet reports suggest that restricting fluid intake during exercise augments the plasma cortisol response (McGregor *et al.* 1999). Therefore, the aim of the present study was to investigate the influence of regular fluid ingestion compared with no fluid ingestion during a bout of prolonged cycling on plasma cortisol, circulating neutrophil and lipopolysaccharide (LPS)-stimulated neutrophil degranulation responses.

Following Loughborough University Ethical Committee approval, nine recreationally active males (means ± S.E.M.: age 21 ± 0 years, body mass 72.6 ± 1.6 kg, $\dot{V}_{O_2,max}$ 55.5 ± 1.9 ml kg^{-1} min^{-1}) volunteered to participate in the study. On two occasions, separated by 1 week, subjects reported to the laboratory following an overnight fast and were assigned to either the fluid (F) or no fluid (NF) trial. On the F trial subjects consumed 5 ml (kg body mass)$^{-1}$ of artificially sweetened lemon flavoured water 5 min before cycling for 2 h on a stationary ergometer at 65 % $\dot{V}_{O_2,max}$. Subjects consumed a further 2 ml (kg body mass)$^{-1}$ of the flavoured water at 15 min intervals throughout the exercise. On the NF trial no fluid was consumed before or during exercise. On both trials subjects consumed 5 ml (kg body mass)$^{-1}$ of flavoured water at 5 min post-exercise. The order of the trials was randomised. Laboratory conditions were 19 ± 0 °C and 58 ± 2 % humidity. Venous blood samples were obtained from a superficial forearm vein at 10 min pre-exercise, immediately post-exercise and at 1 h post-exercise. Plasma cortisol was measured using ^{125}I radioimmunoassay. Blood neutrophil counts were performed using a Sysmex SE9000 cell counter. The *in vitro* neutrophil degranulation response (elastase release) to bacterial LPS was assessed as described by Blannin *et al.* (1997). Results were analysed using a two-factor (time × trial) repeated measures ANOVA with *post-hoc* Tukey and paired *t* tests applied where appropriate. Statistical significance was accepted at $P < 0.05$.

Mean sweat rate (calculated from net body mass loss) during both exercise trials was 0.76 ± 0.05 l h^{-1}. Immediately post-exercise, plasma cortisol concentration had increased significantly from pre-exercise values on both trials (F: pre-exercise, 453 ± 42 nM, post-exercise, 592 ± 46 nM, $P < 0.01$; NF: pre-exercise, 416 ± 29 nM, post-exercise, 670 ± 63 nM, $P < 0.01$) and was significantly higher on the NF trial compared with the F trial ($P < 0.05$). Numbers of circulating neutrophils increased similarly on both trials over the sample time points to 12.6 ± 0.9 × 10^9 cells l^{-1} and 12.9 ± 0.5 × 10^9 cells l^{-1} at 1 h post-exercise on the F and NF trials, respectively (main effect of time, $P < 0.001$). LPS-stimulated elastase release per neutrophil decreased similarly on both trials in response to the exercise (F: pre-exercise, 167 ± 23 fg cell^{-1}, post-exercise, 100 ± 10 fg cell^{-1}; NF: pre-exercise, 173 ± 13 fg cell^{-1}, post-exercise, 99 ± 12 fg cell^{-1}, main effect of time, $P < 0.001$). Plasma volume changes were −5.0 ± 0.6% (F) and −6.5 ± 1.0% (NF) (n.s.). Adjusting the data for changes in plasma volume did not alter the relationships observed. These data suggest that in ambient environmental conditions fluid ingestion alone has negligible influence on circulating neutrophil and LPS-stimulated neutrophil degranulation responses to prolonged exercise.

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McGregor SJ *et al.* (1999). *J Sports Sci* **17**, 895–903.

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

PC5

The cotton swab method for human saliva collection: effect on measurements of saliva flow rate and concentrations of protein, secretory immunoglobulin A, amylase and cortisol

T.-L. Li and M. Gleeson

School of Sport and Exercise Sciences, University of Birmingham, Birmingham B15 2TT, UK

Salivettes are commonly used to collect human saliva for the estimation of saliva flow rate and concentrations of steroid hormones, total protein, secretory immunoglobulin A (s-IgA) and amylase. Salivettes include an absorbent cotton roll, a plastic roll container with a perforated bottom, and a centrifuge tube. Collection of saliva is usually accomplished by placing the cotton roll under the tongue for a timed period of 1–4 min. The cotton roll is then replaced in the container and back in the centrifuge tube. Centrifugation allows collection of the saliva in the bottom of the tube, which can then be stored frozen prior to analysis. However, the use of an absorbent cotton roll may affect saliva composition and the estimation of saliva flow rate.

In the present study, with local ethics committee approval, eight healthy men (age 29 ± 3 years, body mass 74.2 ± 1.3 kg, mean ± S.E.M.) provided ~15 ml of unstimulated saliva by dribbling into a tube over a 20–30 min period following an overnight fast. The following volumes: 0.2, 0.4, 0.7, 1.0, 2.0, 3.0 and 4.0 ml (with the remaining volume of about 3 ml as a control) were placed in pre-weighed vials. Pre-weighed cotton rolls (diameter 1 cm, length 4 cm) were put into each vial (except for the control sample) and placed on a shaker at 500 r.p.m. for 2 min. After shaking, the swabs were removed and centrifuged at 1500 *g* for 10 min at 18 °C. Saliva volume was estimated by weighing to the nearest mg and saliva density was assumed to be 1 g ml^{-1}. Samples were then stored frozen at −20 °C prior to analysis of total protein, s-IgA, amylase and cortisol by methods previously described (Walsh *et al.* 1999). The cotton rolls were also weighed following centrifugation, so that the amount of saliva retained in the cotton material could be determined. Results were analysed using ANOVA and paired *t* tests applied where appropriate. Statistical significance was accepted at $P < 0.05$.

The cotton roll became saturated when saliva volume exceeded 2 ml. The amount of saliva retained in the cotton roll after centrifugation was not constant and ranged from 0.09 ± 0.01 to 0.28 ± 0.03 ml, for saliva volumes of 0.2 and 4.0 ml, respectively. Thus a higher percentage of the initial saliva volume was retained by the cotton roll at the lower saliva volumes (45, 22, 21, 16, 12, 9 and 7 % for initial saliva volumes of 0.2, 0.4, 0.7, 1.0, 2.0, 3.0 and 4.0 ml, respectively). The saliva levels of total protein, s-IgA, amylase and cortisol (Table 1) were all significantly affected by the presence of the cotton roll, but were not significantly influenced by the volume of saliva. Total protein, s-IgA and amylase were 24, 16 and 12 % lower in the saliva exposed to the cotton roll, respectively, whereas cortisol was 33 % higher than in the control sample.

Table 1. The effect of 2 min exposure to a cotton roll on saliva levels of total protein, s-IgA, amylase and cortisol

	Control	Cotton roll
Total protein (mg l^{-1})	549 ± 53	417 ± 27*
s-IgA (mg l^{-1})	197 ± 14	165 ± 18*
Amylase (U l^{-1})	543 ± 75	479 ± 66*
Cortisol (nmol l^{-1})	7.7 ± 4.1	10.2 ± 4.2**

Values are means ± S.E.M. ($n = 8$). Significantly different from control: *$P < 0.05$; **$P < 0.01$ (Student's paired *t* test). Data for the cotton roll treatment are averaged for the different saliva volumes used.

Our findings indicate that the cotton roll collection method affects the results of total protein, s-IgA, amylase and cortisol. Estimations of saliva flow rate will also be inaccurate when the saliva volume exceeds 2 ml and/or if the volume of saliva retained in the cotton roll after centrifugation is ignored. With regard to previously reported studies in which cotton rolls were used to collect saliva, our findings suggest that the results of such studies may be compromised and need to be reconsidered.

Walsh NP *et al.* (1999). *J Sports Sci.* **17**, 129–134.

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

PC6

Effect of high-intensity exercise on stimulated and unstimulated human salivary immunoglobulin A secretion

M. Gleeson, B. Crampton, S. Strachan, T. Mundel and L. Till

School of Sport and Exercise Sciences, University of Birmingham, Birmingham B15 2TT, UK

The quality and quantity of saliva produced at rest and during exercise is thought to be important in defending the body against pathogens affecting the upper respiratory tract (Brandzaeg, 1992; Gleeson, 2000). Exercise has been reported to affect saliva flow rate and decrease the concentration and/or secretion rate of immunoglobulin A (IgA), the major secretory antibody in saliva (Chicarro *et al.* 1998; Gleeson, 2000). Previous studies have investigated the effect of exercise on unstimulated saliva flow rate and composition, but no information is available on the responses when saliva flow rate is stimulated. Since athletes may consume food items or drinks during exercise, it is of interest to know how exercise affects stimulated saliva flow composition.

In the present study, with local ethics committee approval, nine healthy men (age 21 ± 1 years, body mass 75.4 ± 3.0 kg, $\dot{V}_{O_2,max}$ 49.2 ± 3.1 ml kg^{-1} min^{-1}, means ± S.E.M.) volunteered to participate in the study. Resting unstimulated (UNSTIM) and stimulated (STIM) saliva samples were obtained by dribbling into a pre-weighed tube for a 2 min period. For STIM, subjects were given a mint to suck for 1 min prior to saliva collection (Rudney *et al.* 1985). Following an overnight fast, subjects cycled on an ergometer at a work rate equivalent to 85 % $\dot{V}_{O_2,max}$ until volitional fatigue (24 ± 5 min). Timed UNSTIM and STIM samples were taken pre-exercise, after 10 min of exercise and 30 min post-exercise. UNSTIM samples were always collected first. Saliva samples were stored at −20 °C and centrifuged to remove any sediment prior to analysis. Saliva volume was estimated by weighing to the nearest mg and saliva density was assumed to be 1 g ml^{-1}. Saliva flow rate was determined by dividing the volume of saliva by the collection time. The s-IgA concentration was determined using a sandwich type ELISA method (Blannin *et al.* 1998). Saliva IgA secretion rate was calculated by multiplying the saliva flow rate by the IgA concentration. Results were analysed using a two-factor (trial × time) repeated measures ANOVA with *post-hoc* Tukey and paired *t* tests applied where appropriate. Statistical significance was accepted at $P < 0.05$.

Table 1. The effect of high-intensity exercise on unstimulated (UNSTIM) and stimulated (STIM) saliva flow rate, IgA concentration and IgA secretion rate

	Pre-exercise	Exercise	30 min post-exercise
Flow rate (μl min^{-1})			
UNSTIM	265 ± 50	101 ± 34[a]	323 ± 57
STIM	700 ± 111[b]	312 ± 113[ab]	759 ± 172[b]
IgA concentration (mg l^{-1})			
UNSTIM	303 ± 55	354 ± 35[a]	412 ± 49[a]
STIM	170 ± 18[b]	232 ± 24[ab]	204 ± 20[ab]
IgA secretion rate (μg min^{-1})			
UNSTIM	69 ± 12	32 ± 12[a]	124 ± 28[a]
STIM	115 ± 19[b]	69 ± 25[ab]	163 ± 40[ab]

Values are means ± S.E.M. ($n = 9$). [a]Significantly different from pre-exercise, $P < 0.05$. [b]Significantly different from UNSTIM, $P < 0.05$.

STIM saliva flow rate was ~3-fold greater than UNSTIM (Table 1) both at rest and during exercise, though exercising saliva flow rate was significantly lower for both treatments than at rest. IgA concentration was significantly lower in STIM compared with UNSTIM saliva at all time points. IgA concentration increased during exercise in both STIM and UNSTIM saliva. IgA secretion rate was higher in STIM compared with UNSTIM at all time points and IgA secretion rate fell in both treatments during exercise.

Our findings indicate that stimulating saliva flow rate at rest and during high-intensity exercise results in higher rates of IgA secretion, which might be beneficial against oral pathogens. It is possible, of course, that this may only be a temporary phenomenon and may be due to a washout effect.

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Chicarro JL *et al.* (1998). *Sports Med* **26**, 17–27.

Gleeson M (2000). *Exerc Immunol Rev* **6**, 5–42.

Rudney JD *et al.* (1985). *Arch Oral Biol* **1**, 765–771.

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

PC7

The influence of a step-phase-triggered verbal cognitive task on (treadmill) walking (pilot study)

Alexander J. Cran, Dawn A. Skelton, Martin E. Rosenberg, Anthony Christopher and Roger C. Woledge

Institute of Human Performance, University College London, Royal National Orthopaedic Hospital, Stanmore HA4 7LP, UK

Gait disturbances occur in older patients when performing dual cognitive tasks (Bowen *et al.* 2001). At a more extreme level the 'stops walking while talking' test can in a clinical setting, serve as a predictor of future injurious falls in older patient groups (Lundin-Olsson *et al.* 1997). It is our proposition that even some active individuals may manifest subtle changes of gait when faced with a cognitive challenge which, if revealed could serve as warning of increased risk of falling. The aim of this study was to produce a precisely reproducible means of measuring the effects of a cognitive task on walking.

Nine subjects, mean age 34.3 years (S.D. 13.9 years; 5 males) completed the protocol. The study had local ethical committee approval. All subjects were judged healthy on the basis of a medical questionnaire. Eight infrared markers were attached to each lower limb, inside of the left and outside of the right, over the main anatomical points. The subject walked on a treadmill, at a normal pace with markers in view of a CODA *mpx 30* motion analysis system positioned to the walker's right. The cognitive task was to respond while walking to a verbal command presented via radio headphones. On the command 'Red' the subject was to respond with 'Yes' and on 'Green' with 'No'. The Red/Green commands were generated from a sound bank of pre-recorded, digitised words and selected randomly by the control program. Gait was recorded over 20 s epochs with one or other command presented once during each epoch. The timing of a command was triggered via CODA from a marker on the right heel as it elevated in the stride cycle. Forty such epochs overall were recorded for each subject. In the first 20 epochs the subject was required to listen to the commands but not to respond; in the remaining 20 epochs the subject had to respond to the commands.

The difference between the mean and standard error of the amplitude and timing of gait parameters during control and perturbed walking were used to determine significance. Even in a group of healthy and active subjects, on comparing step sequences with and without a verbal response we still found in three of the nine subjects significant perturbations in amplitude and timing of gait parameters, which were synchronized to the command delivery ($P < 0.05$; unpaired t test).

Although our results cannot directly be linked to the elderly we suggest that a method of this kind, which potentially provides a measure of gait instability under dual tasking pressure, might provide a useful method of risk assessment in vulnerable groups.

Bowen A *et al.* (2001). *Age & Ageing* **30**, 319–323.

Lundin-Olsson L *et al.* (1997). *Lancet* **349**, 617.

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

PC8

Effect of glycogen depletion on the oxygen uptake slow component in humans

K. Koppo*, A.M. Jones† and J. Bouckaert*

**Department of Movement and Sports Sciences, Ghent University, Watersportlaan 2, 9000 Ghent, Belgium and †Department of Exercise and Sport Science, Manchester Metropolitan University, Hassall Road, Alsager ST7 2HL, UK*

Constant power output exercise above the lactate threshold is associated with the development of a 'slow component' of oxygen uptake ($\dot{V}_{O_2}$) kinetics that causes $\dot{V}_{O_2}$ to rise to a higher value than would be predicted by extrapolation of the relationship between $\dot{V}_{O_2}$ and power output established during moderate exercise. The cause of this loss of efficiency remains to be elucidated, but the recruitment of type II muscle fibres has been proposed as a likely candidate (Whipp, 1994). Therefore, in the present study, we hypothesised that an exercise and dietary regime designed to deplete type I muscle fibres of glycogen would result in a greater contribution of type II muscle fibres to the exercise power output and a larger amplitude of the $\dot{V}_{O_2}$ slow component.

Eight male subjects (mean ± S.D. age 20.8 ± 1.6 years; body mass 75.7 ± 7.1 kg; $\dot{V}_{O_2,max}$ 3.5 ± 1.1 l min^{-1}) gave written informed consent to participate in this study which was approved by the institutional ethics committee. On day 1, the subjects reported to the laboratory at 08.00 h following an overnight fast, and completed a 9 min exercise bout at a power output calculated to require 85 % $\dot{V}_{O_2,max}$ on an electrically braked cycle ergometer. Pulmonary $\dot{V}_{O_2}$ was determined breath-by-breath and venous and capillary blood samples were drawn immediately before and after exercise for determination of lactate, glucose, glycerol and ammonia concentrations. On day 2, the subjects were fed a 4200 kJ meal (60 % protein, 40 % fat) at 12.00 h and then consumed only water for the rest of the day; at 18.00 h they completed a 2 h exercise bout at 60 % $\dot{V}_{O_2,max}$. This diet and exercise regimen has been shown to result in total depletion of the glycogen content of type I muscle fibres (Gollnick *et al.* 1974). At 08.00 h on day 3, the subjects performed an identical exercise bout to that of day 1. The $\dot{V}_{O_2}$ data were fitted with a mono-exponential function from 20 s to 3 min of exercise to determine the time constant of the fundamental response, and the slow component was quantified as the difference in $\dot{V}_{O_2}$ between 3 and 9 min of exercise. Data were analysed using the signed ranks Wilcoxon test and expressed as means ± S.D.

The respiratory exchange ratio was significantly blunted by the glycogen depletion regimen (from 0.91 ± 0.15 to 0.82 ± 0.12 at rest, and from 1.04 ± 0.05 to 0.98 ± 0.05 during exercise; $P < 0.05$). Furthermore, blood [glucose] and [lactate] were significantly reduced, while blood [glycerol] and [ammonia] were significantly increased, during both rest and exercise following the glycogen depletion regimen. The $\dot{V}_{O_2}$ was significantly higher (by approximately 140 ml min^{-1}) throughout exercise following glycogen depletion. However, glycogen depletion did not appreciably influence $\dot{V}_{O_2}$ kinetics: neither the time constant of the fundamental response (from 35.4 ± 2.5 to 33.2 ± 4.4 s) nor the amplitude of the slow component (from 404 ± 95 to 376 ± 81 ml min^{-1}) was significantly altered.

The increased $\dot{V}_{O_2}$ throughout exercise following glycogen depletion can be explained by changes in substrate utilisation and/or changes in muscle recruitment. The fact that the amplitude of the $\dot{V}_{O_2}$ slow component was unaltered by a protocol designed to cause glycogen depletion of the type I fibre population indicates either that the slow component is not related to the recruitment of type II fibres during heavy exercise, or that glycogen depletion of the type I fibre pool does not significantly alter the pattern of motor unit recruitment during subsequent exercise.

Gollnick PD *et al.* (1974). *J Physiol* **241**, 45–57.
Whipp BJ (1994). *Med Sci Sports Exerc* **26**, 1319–1326.

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

PC9

Role of transforming growth factor-β in relation to exercise induced local type I collagen synthesis in human tendinous tissue

Katja M. Heinemeier, Henning Langberg, Jens L. Olesen and Michael Kjaer

Sports Medicine Research Unit, Bispebjerg Hospital, Copenhagen, Denmark

Several tissue types react to mechanical stress by increasing the synthesis of type I collagen, and recent microdialysis studies indicate that mechanical loading during exercise can similarly influence type I collagen production in tendon tissue (Langberg *et al.* 1999). However, the link between mechanical loading and type I collagen synthesis in tendon is yet unknown.

Studies indicate that transforming growth factor-β (TGF-β), which potently induces type I collagen synthesis in fibroblasts, could connect mechanical loading to type I collagen production. Cultured tendon fibroblasts increase the expression of TGF-β in response to mechanical stress and mechanically induced type I collagen synthesis has been found to be dependent on TGF-β activity in cardiac fibroblasts and intestinal smooth muscle cells (Gutierrez *et al.* 1999; Lindahl *et al.* 2001). Thus TGF-β could connect mechanical loading to type I collagen synthesis in tendinous tissue *in vivo*. The aim of the present study was to investigate whether exercise increases TGF-β levels both locally, in mechanically loaded tendon, and systemically (plasma).

The six male volunteers, who were included in the study (approved by the Ethical Committee of Copenhagen, KF) 11-088/01)) performed 1 h of uphill (3 %) treadmill running. Before and at several time points after exercise, levels of TGF-β were measured in plasma, and in the peritendinous tissue of the Achilles tendon by the microdialysis method (as described by Langberg *et al.* 1999). Before insertion of microdialysis catheters, an appropriate area of the skin was anaesthetised with lidocaine. Likewise, peritendon tissue levels of pro-collagen I C-terminal pro-peptide (PICP) and C-terminal telopeptide of type I collagen (ICTP), which indicate synthesis and breakdown of type I collagen, respectively, were measured to evaluate the local turnover of type I collagen.

After exercise, a rise in tissue levels of PICP was seen at 68 h post-exercise (from 0 μg l^{-1} to 52 ± 12.6 μg l^{-1}; $P < 0.05$ *vs.* pre) (Wilcoxon signed ranks test). Tissue levels of TGF-β were 30 % higher 3 h post- *vs.* pre-exercise (423 ± 86 pg ml^{-1} post-exercise *vs.* 303 ± 46 pg ml^{-1} at rest) without reaching significance (n.s.) and also plasma concentrations of TGF-β rose 30 % in response to exercise (from 992 ± 49 pg ml^{-1} to 1301 ± 39 pg ml^{-1}; $P < 0.05$ *vs.* pre) (Wilcoxon signed ranks test).

The changes seen after acute exercise are consistent with increased local synthesis of type I collagen in human peritendinous tissue. Although not conclusive, changes in circulating and local (though insignificant) TGF-β demonstrate a release of this cytokine in response to mechanical loading *in vivo*, and the time pattern is suggestive for a role of TGF-β in regulation of local collagen type I synthesis in tendon-related connective tissue subjected to mechanical loading.

Gutierrez JA *et al.* (1999). *Am J Physiol* **277**, G1074–1080.
Langberg H *et al.* (1999). *J Physiol* **521**, 299–306.
Lindahl GE *et al.* (2001). *J Biol Chem* **277**, 6153–6161.

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

PC10

Effect of superimposed ‘vibration’ during leg strength training using a new method high-frequency braking force as the vibrational stimulus

D. Paul Sumners, John Almy, Rod Gordon, Marsha Evans, Wendy Tang, Duncan L. Turner and Joanna L. Bowtell

**Sport and Exercise Research Centre, Faculty of Engineering, Science and Technology, South Bank University, London and †University of Surrey Roehampton, Whitelands College, West Hill, UK*

Near-maximal contractions with superimposed vibration have been shown to cause greater increases in strength in the acute and chronic time domains than non-vibrated contractions (Issurin *et al.* 1994; Issurin & Tenenbaum, 1999). Due to the impressive results observed in those studies we wondered whether we could replicate those findings with an alternate but comparable vibrational stimulus. Twelve recreationally active subjects (7 strength + vibration training, 5 strength training only) trained for 5 weeks, three times per week on a leg extension machine. Strength was assessed PRE and POST training by determining the maximum weight the subject could lift for one repetition (1-RM). Each training session involved a warm-up set of eight repetitions at ~50 % 1-RM, followed by three sets of eight repetitions at ~75 % 1-RM. Vibration was applied to the cable of a leg extension machine by a new method which produced a high-frequency braking force driven by the energy produced by the subject lifting the weight. The vibration frequency was dependent upon the rate of contraction (30–45 Hz) in this study, as opposed to Issurin and colleagues’ experiment where the frequency was contraction rate independent (44 Hz). The experiments had ethical approval and subjects gave written informed consent.

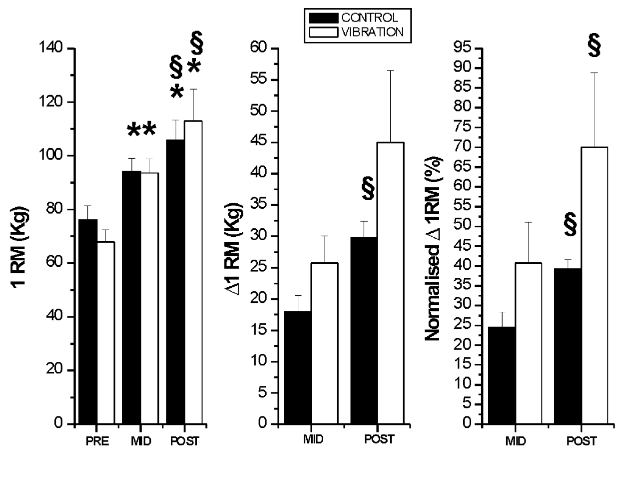

Figure 1. Force expressed as absolute values (left panel), increases in force (middle panel) and increases in force normalised to PRE 1-RM. Data are means ± S.E.M.

■, strength training group; □, strength training with additional vibration. *Difference from PRE values; §difference from MID values ($P < 0.05$; Wilcoxon signed rank test).

Results suggest that strength training with and without additional vibration cause significant increases in strength, but the superimposed vibrations cause trends towards greater strength gains than strength training alone (see Fig. 1). These results provide moderate support for previous studies in which improvement in strength (1-RM) was significantly greater after strength training with superimposed vibration than strength training alone (Issurin *et al.* 1994). Although we found similar increases in strength after strength training with superimposed vibration to those observed in earlier studies (~40 *vs.* 49 %; Issurin *et al.* 1994), the increases in strength after conventional strength training in our experiments were larger than those reported earlier (~25 *vs.* 16 %; Issurin *et al.* 1994). The results are probably related to subject difference as our subjects were only recreationally active and not strength trained as in the previous experiments, also different muscle groups were used in the previous two studies. Further work is required to test alternate frequencies and amplitudes of vibration during training in different subject groups and in different muscles.

Issurin VB *et al.* (1994). *J Sports Sci* **12**, 562.

Issurin VB & Tenenbaum G (1999). *J Sports Sci* **17**, 177.

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PC11

Effects of thromboxane A_2 and prostaglandin E_2 on the short-circuit current across human gall bladder mucosa

N. Horikawa*, H. Sakai†, T. Suzuki†, T. Minamimura*, K. Tsukada* and N. Takeguchi†

**Department of Surgery II and †Pharmaceutical Physiology, Toyama Medical and Pharmaceutical University, Toyama 930–0194, Japan*

In the prairie dog gall bladder set between Ussing chambers, the serosal addition of prostaglandin E_2 (PGE_2) increased Cl^- secretion, while the mucosal addition of PGE_2 had almost no effect (Moser *et al.* 2000). In isolated guinea-pig gallbladder, the serosal addition of PGE_2 exerted triphasic influence on the fluid transport, while the mucosal addition of PGE_2 induced only small changes in fluid reabsorption (Heintze *et al.* 1975). Increases of prostaglandins and thromboxane (TX) synthesis were suggested to be associated with an early stage of cholesterol gallstone formation in the prairie dog model (LaMorte *et al.* 1986).

In this study, we investigated the effects of 9,11-epithio-11,12-methano-TXA_2 (STA_2), a stable analogue of TXA_2, and PGE_2 on the short-circuit current (I_{sc}) across the human gall bladder mucosa. The specimens of normal gall bladder mucosa were obtained at cholecystectomy and set between modified Ussing chambers. All procedures are followed by the recommendations of the Declaration of Helsinki. Informed consents were obtained from all patients at Toyama Medical and Pharmaceutical University Hospital. Data are shown as means ± S.E.M. Differences between groups were analysed by one-way ANOVA. Comparison between the two groups was made by paired *t* test.

The serosal addition of STA_2 (0.3 μM) did not significantly affect the I_{sc} ($P > 0.05$, $n = 4$). However, the mucosal addition of STA_2 (0.3 μM) increased the I_{sc} by 9.7 ± 5.1 μA cm^{-2} ($P < 0.05$, $n = 4$). The mucosal STA_2 (0.3 μM) also changed the transepithelial potential difference by −0.4 ± 0.2 mV (lumen negative) and the tissue conductance by 0.54 ± 0.13 mS cm^{-2} ($P < 0.05$, $n = 4$). In RT-PCR experiments, expression of human TXA_2 receptor in the gall bladder mucosa was confirmed. Interestingly, the serosal addition of PGE_2 (0.5 μM) had no effect ($P > 0.05$, $n = 4$), while the mucosal addition of PGE_2 increased the I_{sc} by 10.3 ± 6.5 μA cm^{-2} ($P < 0.05$, $n = 4$). These results suggest that TXA_2 receptor and prostaglandin EP receptors are present not on the serosal side but on the mucosal side in human gall bladder epithelium.

Heintze K *et al.* (1975). *Prostaglandins* **9**, 309–322.

LaMorte WW *et al.* (1986). *Am J Physiol* **251**, G701–709.

Moser AJ *et al.* (2000). *J Lab Clin Med* **135**, 82–88.

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

PC12

Effect of acute hypocapnic hypoxia on circulating leucocyte subsets, neutrophil activity and plasma anti-oxidant status during exercise at 50 % normoxic $\dot{V}_{O_2,peak}$

C. Douglas Thake and Rubina Mian

Sport Science and Physiology Group, School of Science and the Environment, Coventry University, Coventry CV1 5FB, UK

Symptoms of infection are common in trekkers at high altitude and more prevalent among those with acute mountain sickness (AMS) (Murdoch, 1995). Since hypoxia results in a given level of work being more strenuous than when performed at sea level (Lawler *et al.* 1988) these increased infection rates may well be influenced by changes in immune cell distribution and function that are known to be modulated by exercise intensity, duration and fitness (McCarthy & Dale, 1988). Furthermore, recent evidence indicates that anti-oxidant defence mechanisms are overwhelmed on ascent to high altitude and may also be implicated in the aetiology of AMS (Bailey *et al.* 2001).

With local ethics committee approval, using a randomised single blind cross-over design, eight healthy male subjects (age 21.6 ± 2.3 years, $\dot{V}_{O_2,peak}$ 3.84 ± 0.59 l min^{-1}; means ± S.D.) breathed a hypoxic (H; F_{I,O_2} = 14 %) gas mixture or normoxic (N; F_{I,O_2} = 20.9 %) room air via a mouthpiece for 150 min. Between 60 and 90 min subjects exercised on a cycle ergometer at a predetermined workload to elicit 50 % normoxic $\dot{V}_{O_2,peak}$ (70 r.p.m.). Venous blood was taken at baseline (−30 and 0 min), 60, 90 and 150 min. Total leucocyte and subset counts, neutrophil oxidative activity (qualitative nitroblue tetrazolium (NBT) reduction test) and plasma anti-oxidant status (peroxynitrite ABEL® Pholasin® assay system; Knight Scientific Ltd, Plymouth, UK) were measured. Leucocyte data were corrected for changes in plasma volume (Dill & Costill, 1974). Data were compared between conditions at each time point using ANOVA.

The magnitude of leucocytosis induced by 30 min exercise was greater in H (7.0 ± 1.7 to 10.1 ± 2.8 × 10^9 l^{-1}) compared with N (6.9 ± 1.4 to 8.6 ± 2.1 × 10^9 l^{-1}; $P < 0.005$). This was achieved by a relative neutrophilia (H, 4.5 ± 1.4 to 6.0 ± 2.3 × 10^9 l^{-1}; N, 4.2 ± 1.3 to 5.0 ± 1.7 × 10^9 l^{-1}; $P < 0.05$) and lymphophilia (H, 1.9 ± 0.4 to 3.3 ± 0.9 × 10^9 l^{-1}; N, 2.0 ± 0.5 to 2.7 ± 0.7 × 10^9 l^{-1}; $P < 0.05$) in H. The relative neutrophilia remained evident after 60 min of recovery ($P < 0.05$). NBT-positive neutrophils were greater in H at 60 min ($P = 0.06$) and peaked immediately post-exercise (H, 2.8 ± 1.3 × 10^9 l^{-1}; N, 2.4 ± 1.0 × 10^9 l^{-1}; $P = 0.08$). This difference became significant at 150 min ($P < 0.05$). Post-exercise anti-oxidant levels were greater than baseline (H, 398 ± 55 to 452 ± 73; N, 394 ± 45 to 415 ± 37 Vitamin E analogue equivalent units; VEA eq units μmol l^{-1}) and remained elevated in H at 150 min (H, 421 ± 49; N, 395 ± 43 VEA eq units μmol l^{-1}). However, these differences were not significant despite $n = 6$ and $n = 7$ subjects respectively having greater anti-oxidant values in H.

These data indicate an augmented cellular immune response and a probable paradoxical increase in plasma anti-oxidants after exercise in moderate hypoxia. The significance of these phenomena and the relative contributions of hypoxia, modulating relative work intensity, and hypoxia *per se* remain to be elucidated.

Bailey DM *et al.* (2001). *Aviat Space Environ Med* **72**, 513–521.

Dill DB & Costill DL (1974). *J Appl Physiol* **37**, 247–224.

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McCarthy DA & Dale MM (1988). *Sports Med* **6**, 333–363.

Murdoch DR (1995). *Aviat Space Environ Med* **66**, 148–151.

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

PC13

Microvascular filtration capacity and venous distension induced vasoconstriction in the calves of women during the menstrual cycle

C. Jackson, J. Gamble and M.D. Brown

School of Sport and Exercise Sciences, University of Birmingham, Birmingham B15 2TT, UK

In explanation of premenstrual fluid retention, previous studies reported higher capillary filtration coefficients in women in the luteal phase of the menstrual cycle than at other times (e.g. Tollan *et al.* 1993). In contrast, Gooding *et al.* (2002) found no cycle-related differences. The discrepancy may relate to use of a single large venous congestion pressure step to measure filtration in the first studies as opposed to small incremental pressure steps in the second since only the former triggers a local veni-arteriolar vasoconstrictor reflex (Gamble *et al.* 1993). This has been shown to be attenuated in the luteal phase in skin when activated by venous distension on limb dependency (Hassan *et al.* 1990), and reduced ability to regulate precapillary resistance could contribute to increased capillary pressure and higher filtration values. This study aimed to examine changes in filtration capacity during the cycle and investigate whether local vasoconstriction in the whole limb is altered between phases.

With local University of Birmingham ethical approval and informed consent, calf microvascular filtration capacity (K_f) was assessed in thirteen healthy women (18–22 years) with normal menstrual cycles and not taking oral contraceptives, by applying small cumulative increases in pressure to a thigh cuff and measuring calf swelling by mercury-in-silastic strain gauges. K_f was significantly lower in the early luteal ($4.65 \pm 0.50 \times 10^{-3}$ ml min^{-1} 100 ml^{-1} $mmHg^{-1}$, mean ± S.E.M.) than in the menstrual, early follicular or late luteal phases (5.99 ± 0.76, 6.56 ± 0.78, $6.67 \pm 1.29 \times 10^{-3}$ ml min^{-1} 100 ml^{-1} $mmHg^{-1}$, respectively, $P < 0.05$, repeated measures ANOVA). Mean arterial pressure and resting calf blood flow were unchanged through the cycle. Calf blood flow was also measured by further brief venous congestion when the thigh cuff had been inflated to 50 mmHg for 7–10 min, as in the single step filtration method. Venous distension led to a reduction in flow of 49 ± 9 % in the menstrual phase but changes in flow (−11 ± 17 %) were non-significant in the early luteal phase.

The limb vasoconstrictor response to venous distension is clearly attenuated in the early luteal phase of the cycle, which could underlie the higher limb filtration capacity values measured with a large single step venous congestion method. The reason for lower early luteal K_f values with small venous congestion steps remains to be determined.

Gamble J *et al.* (1993). *J Physiol* **464**, 407–422.

Gooding KM *et al.* (2002). *J Physiol* **539.P**, 85*P*.

Hassan A *et al.* (1990). *Clin Sci* **78**, 39–47.

Tollan A *et al.* (1993). *Acta Obstet Gynecol Scand* **72**, 238–242.

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

PC14

Collagen synthesis in human tendon, ligament and muscle

J. Babraj*, H. Langberg†, M. Kjaer†, M. Krogsgaard†, K. Smith*, T. Waddell*, P. Rickhuss*, D.J.R. Cuthbertson*, W. Meier-Augenstein* and M.J. Rennie*

**Division of Molecular Physiology, University of Dundee, Dundee DD1 4HN, UK and †Sports Medicine Research Unit, Bispebjerg Hospital, DK-2400, Copenhagen NV, Denmark*

We wished to study adaptations in human musculoskeletal connective tissue in relation to physical activity. We previously developed a method to measure human bone collagen synthesis (Babraj *et al.* 2002) and have now applied it to the study of collagen turnover in human tendon, ligament and muscle. These studies had the subjects' informed consent and approval from ethics committees in Tayside and Copenhagen. We studied eight overnight fasted subjects (24 ± 8 years; means ± S.D. throughout); four were undergoing surgical repair of the anterior cruciate ligament. In all subjects, a flooding dose of [1-^{13}C]proline (3.75 g of 20 atoms percent) was administered I.V. and blood samples taken for up to 120 min. Four other subjects also received a primed constant infusion of [1-^{13}C]leucine (1 mg kg^{-1} h^{-1}), started 2 h before the proline bolus. Surgical biopsies were taken from tendon and ligament immediately after induction of general anaesthesia. Muscle biopsies were taken by the conchotome technique under local anaesthesia (lignocaine 1 %) in the non-surgical subjects. Collagen was isolated from connective tissue and muscle, and myofibrillar and sarcoplasmic proteins were isolated from muscle using standard methods; fractional synthesis rates were calculated from the incorporation of proline into collagen hydroxyproline and incorporation of both leucine and proline into muscle proteins, determined by gas-chromatography-combustion-isotope ratio mass spectrometry. Using leucine or proline, there were no differences between the myofibrillar and sarcoplasmic fractional synthetic rates (grand means: 0.043 ± 0.013 and 0.077 ± 0.008 % h^{-1}, respectively) obtained, confirming that the flooding dose of proline does not stimulate protein synthesis, an assumption underlying the method. Human muscle collagen had a markedly lower synthesis rate (0.016 ± 0.002 % h^{-1}) than myofibrillar or sarcoplasmic protein. We also demonstrate for the first time the feasibility of direct measurement of collagen synthesis in human tendon (0.052 ± 0.014 % h^{-1}) and ligament (0.042 ± 0.004 % h^{-1}).

The synthetic rates were higher than in muscle collagen but similar to those in mature bone collagen (0.04–0.06 % h^{-1}), indicating substantial collagen metabolic activity. These data suggest that the technique will be applicable to the study of human musculoskeletal connective tissue adaptation.

Babraj JA *et al.* (2002). *Biochem Soc Trans* **30**, 61–65.

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

PC15

Prior heavy exercise increases the time to exhaustion during subsequent peri-maximal exercise in humans

Daryl P. Wilkerson*, Mark Burnley†, Katrien Koppo‡ and Andrew M. Jones*

**Department of Exercise and Sport Science, Manchester Metropolitan University, Hassall Road, Alsager ST7 2HL, †Department of Sport Science, University of Wales, Aberystwyth SW23 2AX, UK and ‡Department of Movement and Sports Sciences, Ghent University, Belgium*

Exercise which elicits a metabolic acidaemia has been shown to reduce substrate-level phosphorylation (Rossiter *et al.* 2001) and speed the overall $\dot{V}_{O_2}$ kinetics (Gerbino *et al.* 1996; Burnley *et al.* 2000) during subsequent high-intensity exercise. It has been proposed that these effects may predispose to increase exercise tolerance (Gerbino *et al.* 1996; Rossiter *et al.* 2001). The purpose of this study was therefore to test the hypothesis that prior heavy exercise increases time to exhaustion during subsequent peri-maximal exercise.

Seven healthy males (27 ± 3 years; 78.4 ± 0.7 kg; means ± S.D.) volunteered to participate in this study which was approved by the institutional ethics committee. The subjects first completed a ramp exercise test on an electrically braked cycle ergometer to determine the gas exchange threshold (GET) and $\dot{V}_{O_2,max}$. Subsequently, the subjects performed square wave transitions from unloaded cycling to power outputs equivalent to 100, 110 and 120 % $\dot{V}_{O_2,max}$ following no prior exercise (control, C) and 10 min after a 6 min bout of heavy exercise (HE) at 50 % Δ (half-way between the GET and $\dot{V}_{O_2,max}$). Four subjects also performed the peri-maximal exercise bouts 10 min after a moderate intensity exercise bout (ME; ~12 min at 80 % GET). For all trials, the time to 'exhaustion' was recorded to the nearest second, where 'exhaustion' was defined as a fall in self-selected pedal rate of > 5 rev min^{-1}. A blood sample was taken from the fingertip immediately before and after each trial for the determination of blood [lactate]. Differences between the C and HE conditions were analysed with paired *t* tests and are reported as means ± S.E.M.

Blood [lactate] was higher at the onset of the peri-maximal exercise bouts when they were preceded by HE (C: ~ 1.1 *vs.* HE: ~2.5 mM; $P < 0.01$) but there was no significant difference in blood [lactate] at end-exercise. Time to exhaustion was increased by prior HE at 100 % (C: 386 ± 92 *vs.* HE: 613 ± 161 s), 110 % (C: 218 ± 26 *vs.* HE: 284 ± 47 s), and 120 % (C: 139 ± 18 *vs.* HE: 180 ± 29 s) $\dot{V}_{O_2,max}$ (all $P < 0.01$). Prior ME, which did not increase blood [lactate], did not affect time to exhaustion nor the time course of the $\dot{V}_{O_2}$ response during exercise ($n = 4$). At 100 % $\dot{V}_{O_2,max}$, $\dot{V}_{O_2}$ was not significantly different between C and the prior HE condition either at 1 min into exercise (C: 3.04 ± 0.09 *vs.* HE: 3.14 ± 0.14 l min^{-1}) or at exhaustion (C: 3.89 ± 0.08 *vs.* HE: 3.88 ± 0.13 l min^{-1}). At 110 % $\dot{V}_{O_2,max}$, $\dot{V}_{O_2}$ was significantly higher at 1 min into exercise following prior HE (C: 3.11 ± 0.14 *vs.* HE: 3.42 ± 0.16 l min^{-1}; $P < 0.05$) but there was no significant difference at exhaustion (C: 3.89 ± 0.08 *vs.* HE: 3.90 ± 0.12 l min^{-1}). At 120 % $\dot{V}_{O_2,max}$, $\dot{V}_{O_2}$ was significantly higher following prior HE both at 1 min into exercise (C: 3.25 ± 0.12 *vs.* HE: 3.67 ± 0.15 l min^{-1}; $P < 0.01$) and at exhaustion (C: 3.60 ± 0.08 *vs.* HE: 3.95 ± 0.12 l min^{-1}; $P < 0.01$).

These results indicate that prior heavy exercise which increases blood [lactate] to 2–3 mM results in an increased time to exhaustion during subsequent peri-maximal exercise. Prior heavy (but not moderate) exercise therefore appears to enhance the aerobic contribution to energy turnover in subsequent high-intensity exercise and retard the rate at which fatigue develops. However, the characteristics of the prior work rate, exercise duration and recovery period that optimise this effect remain to be determined.

Burnley M *et al.* (2000). *J Appl Physiol* **89**, 1387–1396.
Gerbino A *et al.* (1996). *J Appl Physiol* **80**, 99–107.
Rossiter HB *et al.* (2001). *J Physiol* **537**, 291–303.

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

PC16

Stimulation of human quadriceps protein synthesis after strenuous exercise: no effects of varying intensity between 60 and 90 % of one repetition maximum (1RM)

J.L. Bowtell*, D.M. Park*, K. Smith†, D.J.R Cuthbertson†, T. Waddell† and M.J. Rennie†

**Human and Exercise Science Division, South Bank University, London and †Division of Molecular Physiology, Faculty of Life Sciences, University of Dundee, Dundee DD1 4HN, UK*

Strenuous exercise markedly stimulates the rate of human muscle protein synthesis but the relative importance of intensity of contraction and the associated metabolic changes are unknown. In order to answer this question we arranged to measure muscle protein synthesis in nine subjects (7 men, 2 women, aged 29.7 ± 5.5 years, BMI 23.4 ± 1.6). Subjects were randomly assigned to one of three exercise protocols so that the intensity of isometric contraction of the quadriceps could be varied but the total ATP turnover would be constant. The study had the approval of the local ethics committee. The subjects, all postabsoprtive, exercised as follows: high intensity, 3 repetitions, 90 % 1 RM plus 16.66 further sets, with 1 min rest between sets; medium intensity, 8 repetitions at 75 % 1 RM, plus 7 further sets, with 2 min 21 s rests between; low intensity, 15 repetitions at 60 % 1 RM, plus 4.33 further sets, with 3 min 8 s rests between. A primed constant infusion of [1,2-^{13}C]leucine was begun 160 min before exercise and continued for 150 min afterwards. Muscle biopsies were taken under local anaesthesia (lignocaine 1%) immediately after exercise and 90, 120 and 150 min post-exercise. Blood samples were taken intermittently from an antecubital vein. Muscle was separated into myofibrillar and sarcoplasmic components and the fractional protein synthetic rates determined from the incorporation of [^{13}C]leucine by our standard methods.

Basal protein synthetic rates were 0.042 ± 0.014 and 0.057 ± 0.011 % h^{-1} (myofibrillar and sarcoplasmic, respectively, grand means ± S.D.). In all cases the rate of muscle protein synthesis between 90–150 min post-exercise period was stimulated markedly after a lag phase of ~90 min. The increase in myofibrillar protein synthesis was substantially greater than that in sarcoplasmic, as previously observed (Rennie *et al.* 1999). However, the extent of the relative increases (as percentage of the resting value) were not significantly different between the three groups (grand means, 3.4 ± 0.5-fold myofibrillar and 2.6 ± 0.8-fold sarcoplasmic), suggesting that in the first 2.5 h after exercise at least, the extent of the stimulation of muscle protein synthesis is not determined by the intensity of the preceding contractile activity. This does not preclude the possibilities that the duration of any intensity-dose effect extends beyond the period studied or that there may be additional effects in the fed state.

Rennie MJ *et al.* (1999). *J Physiol* **520.P**, 58*P*.

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

PC17

Effects of chronic electrical stimulation on vascular reactivity of the calf assessed by distal circulatory arrest in healthy humans and chronic heart failure patients

S.I. Anderson, M. Rosbergen, M.K. Davies* and M.D. Brown

*School of Sport and Exercise Sciences, University of Birmingham and *Department of Cardiology, University Hospital Trust, Selly Oak, Birmingham, UK*

Patients with chronic heart failure (CHF) exhibit dysfunction of the vascular endothelium. This has been demonstrated as reduced reactive hyperaemic vasodilatation in a limb following release of a proximal occlusion and by impaired flow-mediated ultrasound-detected dilatation of conduit arteries, e.g. brachial, radial, following release of either proximal or distal occlusions. We have shown that distal occlusion (DO) in combination with venous occlusion plethysmography can detect enhanced flow-mediated dilatation in human limbs after a single bout of whole body exercise or local muscle activity induced by electrical stimulation in young healthy subjects (Rosbergen *et al.* 2000). In this study, this method was used to assess the effects of chronic low-frequency electrical stimulation on limb vasodilatation in CHF patients and age-matched control subjects.

With approval from the South Birmingham Local Research Ethics Committee, eight CHF patients aged 62 ± 11 years (mean ± S.D.), ejection fraction 32 ± 6 %, and 10 age-matched (65 ± 7 years) healthy subjects were recruited. Calf blood flow was measured by venous occlusion plethysmography (Filtrass 2000, DOMED) immediately on release of an ankle cuff inflated to suprasystolic pressure for 10 min, and at 20 s intervals thereafter until return to baseline. Measurements were made before and after a 4 week period during which all participants used electrical muscle stimulators (NT 2000, NeuroTech) to activate calf muscles for 3 × 20 min per day, at a frequency of 8 Hz, pulse duration 250 μs, maximum tolerable intensity.

Prior to stimulation, peak blood flows on release of DO were ~30 % lower in CHF patients than in age-matched controls (3.38 ± 0.32 *vs.* 4.86 ± 0.33 ml min^{-1} 100 ml^{-1}, $P < 0.05$, Mann-Whitney), and both were lower than those reported for young healthy subjects (Rosbergen *et al.* 2000). After chronic stimulation, peak blood flows on release of DO had increased significantly for both CHF and age-matched groups (4.77 ± 0.46 and 6.42 ± 0.59 ml min^{-1} 100 ml^{-1}, respectively, both $P < 0.05$ *vs.* pre-stimulation values, Wilcoxon signed ranks). Thus plethysmography after DO can detect both impairment of flow-mediated dilatation in CHF patients and in ageing, and the positive effects of stimulation therapy. Chronic electrical stimulation offers an alternative means of improving limb vascular function in a targeted fashion that avoids the central cardiovascular limitations of whole body exercise.

Rosbergen M *et al.* (2000). *J Physiol* **523.P**, 235*P*.

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

PC18

Irregular meal pattern and carbohydrate metabolism in healthy young women

H. Farshchi, M.A. Taylor and I.A. Macdonald

School of Biomedical Sciences, University of Nottingham, UK

Irregular meal pattern has become more prevalent during the last decade (Samuelson, 2000). No studies have evaluated the association between irregular meal pattern and carbohydrate metabolism. The purpose of this study was to investigate the impact of irregular meal frequency on blood glucose and serum insulin response.

Nine healthy, lean women aged 18–42 years gave informed vountary consent to participate in a randomised cross-over trial consisting of four phases over a total of 71 days. Subjects were studied after an overnight fast at the start and end of phases 1, 2 and 4. In phase 1, subjects were asked to eat their normal diet for 28 days. In phase 2 (14 days), they were asked to consume similar things as normal but either on six occasions per day (regular meal pattern) or follow a predetermined irregular meal pattern (3–9 meals per day), which had the same total number of meals over the 14 days. In phase 3 (14 days), subjects continued their normal diet as a wash-out period. In phase 4 (14 days), subjects followed the alternative meal pattern to that followed in phase 2.

At each laboratory visit, two baseline blood samples were taken for fasting blood glucose and serum insulin before a high carbohydrate test meal (42 kJ kg^{-1}) was consumed. Blood samples were then taken every 15 min for 3 h and analysed for blood glucose and serum insulin. Total volume of blood sampled at each visit was 70 ml.

There was no difference in fasting blood glucose and serum insulin, or, area under the curve (AUC) for post-prandial blood glucose over the experiment. A significant increase in serum insulin concentration occurred after the test meal in all visits. No significant changes were observed in the peak insulin value or AUC of the insulin response between the pre-diet visits. In contrast, the peak insulin levels at the post-diet visits were significantly different after normal, regular and irregular diet periods (mean ± S.D. (mIU l^{-1}) was 63.4 ± 22.9, 55.4 ± 17.9 and 71.4 ± 25.0, respectively, $P = 0.017$, ANOVA). AUC of insulin response for the post-diet visits were also statistically different ($P = 0.013$, ANOVA).

Irregular meal frequency had no effect on glucose tolerance. However, there was an increased post-prandial insulin response, suggesting a degree of insulin resistance after the irregular meal pattern.

Samuelson (2000). *Eur J Clin Nutr* **54**, S21–28.

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

C124/PC18b

The effects of induced alkalosis on sympatho-adrenal responses to high-intensity exercise

Richard Bracken, Denise Linnane and Stephen Brooks

School of Science and Environment, Coventry University, Priory Street, Coventry CV1 5FB, UK

The role of muscle pH as a determinant of sympathetic nervous system (SNS) activity during exercise is not fully understood. Victor *et al.* (1988) demonstrated that the amount of SNS activity during physical exercise was proportional to the muscle cell pH. However, Vissing (2000) found that muscular acidosis was not a prerequisite for SNS activity in exercise. The aim of the present study was to study the effect of induced alkalosis on the sympatho-adrenal response to high-intensity exercise.

With local ethics committee approval, eight male subjects (age 19 ± 1 years (mean ± S.D.), body mass 69.2 ± 9.7 kg, body fat 10.2 ± 2.7 % and $\dot{V}_{O_2,max}$ 43 ± 5 ml kg^{-1} min^{-1}) attended the laboratory on three occasions following preliminary testing. On each occasion subjects were given one of three solutions in a randomised order: placebo (0.3 g (kg body mass)$^{-1}$ of $CaCO_3$ + 1 g NaCl, A) or 0.3 g kg^{-1} (B), or 0.5 g (kg body mass)$^{-1}$ (C) of tri-sodium citrate ($C_6H_5Na_3O_7$,) in 500 ml of water. One hour following ingestion subjects performed a test on an electromagnetically braked cycle ergometer at a workload calculated to elicit 110 % of $\dot{V}_{O_2,max}$ for 2 min. Blood samples (11 ml) taken from an antecubital vein were first used to determine pH and blood base excess (BBE) then, after the addition of EGTA and glutathione, the remaining blood was centrifuged and analysed for plasma catecholamines by HPLC with electrochemical detection (Davies *et al.* 1981). Data were analysed using one-way and repeated measures ANOVA with *post-hoc* testing where appropriate. Significance was established at the $P < 0.05$ level.

Blood pH and BBE were significantly increased following ingestion of C ($P < 0.05$) but not B when compared with A (pH 7.39 ± 0.03 A, 7.40 ± 0.03 B, and 7.42 ± 0.02 C; BBE 2.9 ± 2.9 A, 6.4 ± 1.3 B, and 6.5 ± 0.9 mmol l^{-1} C). All trials demonstrated a significant decrease in pH and BBE immediately, 5, 10 and 15 min post-exercise. However, the blood pH and BBE at all time points post-exercise in conditions B and C were significantly greater than condition A. There were significant increases in plasma noradrenaline (NA) and adrenaline (AD) concentrations immediately (NA 4.6 ± 2.1 A, 4.2 ± 1.8 B, and 4.6 ± 2.2 nmol l^{-1} C; AD 0.6 ± 0.3 A, 0.5 ± 0.2 B, and 0.5 ± 0.3 nmol l^{-1} C) and 5 min post-exercise (NA 2.1 ± 0.6 A, 2.1 ± 0.6 B, and 2.2 ± 0.6 nmol l^{-1} C; AD 0.2 ± 0.1 A, 0.2 ± 0.1 B, and 0.2 ± 0.1 nmol l^{-1} C, $P < 0.05$) but there was no change in the plasma dopamine (DA) concentration. No significant differences in plasma NA, AD and DA concentrations were found between trials at any time.

The results of this investigation demonstrate that although significant alkalosis was induced following sodium citrate ingestion, no effect was observed on antecubital vein plasma catecholamine responses to high-intensity exercise. This suggests that alterations in alkalosis may not be important for sympatho-adrenal activity in exercise.

Davies CL *et al.* (1981). *Ann Chem* **53** , 156–159.

Victor RG *et al.* (1988). *J Clin Invest* **82**, 1301–1305.

Vissing J (2000). *Acta Physiol Scand* **170**, suppl. 647, 5–26.

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