Comparison of highly endurance trained and untrained human muscles using cDNA arrays

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The expression of a set of 588 widely expressed genes coding for proteins involved in a wide variety of cellular functions was studied on biopsies from m. vastus lateralis of seven highly endurance trained males (professional cyclists) and seven untrained males. Their average $\dot{V}_{O_2,max}$ was 78 ml O_2 min^{-1} kg^{-1} (S.D. = 8.8) and 35 ml O_2 min^{-1} kg^{-1} (S.D. = 6.6), respectively. Biopsies were obtained from m. vastus lateralis using the Bergström technique (Bergström, 1962) with permission from the Ethical Committee of the University of Bern. Total RNA (0.7–2 μg) was obtained from cryostat sections (10–25 mg) of the biopsies and used to probe Clontech Atlas Human 588 arrays with ^{32}P labelling (Wittwer *et al.* 2002).

A comparison of the array scans with the ones from rat m. soleus (Wittwer *et al.* 2002) showed considerably greater inter-individual variability in the human samples (R^2 for trained *vs.* untrained human samples 0.41–0.81, compared with 0.72–0.92 for atrophied *vs.* normal rat m. solei). Statistical analysis was done with the non-parametric Mann-Whitney *U* test. Fifteen of 408 detected transcripts were significantly different ($P < 0.05$) between the cyclists and the untrained samples, differing between 25 % and 4-fold. They included indicators of enhanced cell destruction and replacement (e.g. the mRNAs of cyclin B1 or inhibitor of apoptosis protein 1). This is compatible with enhanced degeneration/regeneration due to greater wear and tear in the cyclists. The increased prohibitin mRNA corresponds to their enhanced mitochondrial volume. Other changed mRNAs coded for immune status indicators (e.g. CD 33, leukaemia inhibitory factor and interleukin-3 receptor) and signal transduction proteins (protein kinase C α and tie-1). Despite the low number of differing transcripts (well within the range of false positives), their nature and direction of change fits structural and biochemical adaptations of highly endurance trained muscles, where known.

In conclusion, the expression of these widely expressed mRNAs was remarkably similar between the muscles of highly trained athletes and untrained males. The statistical parameters (correlation coefficients and median absolute deviations, which were similar for cyclists and untrained) suggest a considerable individual component in a human muscle's adaptation to a given level of activity.

Bergström, J. (1962). *Scand. J. Clin. Lab Invest.* **14**, suppl. 68.

Wittwer, M. *et al.* (2002). *FASEB J.* **16**, 884–886.

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

The role of hypocapnia in the development of syncope during orthostatic stress

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Orthostatic stress induced by prolonged motionless standing, results in blood 'pooling' in dependent regions and may lead to hypotension and syncope. In addition to its hydrostatic effects prolonged orthostasis is often accompanied by hyperventilation, with resulting hypocapnia and it is possible that this may also contribute to the development of syncope. The purpose of this study was to try to evaluate the likely role of hypocapnia in the development of syncope both by its effects on the cerebral circulation and on peripheral vascular resistance.

We studied patients who had been referred for orthostatic stress testing due to suspected attacks of posturally related syncope. Subjects were studied on a combined tilt/lower body suction device to determine orthostatic tolerance as time to presyncope. Patients were categorised as having normal or low orthostatic tolerance by comparison with previous data. We recorded the following: ECG (chest wall leads), finger and brachial arterial blood pressures (Finapres photoplethysmography and oscillometric sphygmomanometer), end-tidal CO_2 (infrared analyser), brachial arterial blood velocity (Doppler) and middle cerebral artery velocity (transcranial Doppler). From changes in brachial and middle cerebral pressures and velocities we calculated changes in vascular resistances. The effects were then determined of changes in end-tidal CO_2 achieved by hypo-ventilation (aided by added dead-space) and hyperventilation. All data were then compared using Student's unpaired *t* tests; values are expressed as means ± S.E.M.

Twenty patients had lower than predicted times to presyncope (16.5 ± 2.0 min) and twelve were normal (32.8 ± 0.8 min). There was no difference in the values of end-tidal CO_2 at the end of the test in the two groups (3.9 ± 0.2 % in early fainters, and 3.7 ± 0.19 % in normals). Changes in end-tidal CO_2 induced opposite changes in vascular resistance in the two regions: a decrease in CO_2 caused cerebral vasoconstriction and forearm vasodilatation. The sensitivities of the two vascular beds to CO_2 were calculated as percentage changes in resistance divided by changes in end-tidal CO_2. In the cerebral circulation, the sensitivities of the two groups of patients were not significantly different (−26.3 ± 2.4 and −22.7 ± 1.8 units, in early fainters and normals, respectively). In the forearm circulation the CO_2 sensitivity was significantly greater in the early fainters (17.8 ± 2.0 and 10.6 ± 2.3 units, $P < 0.003$).

The results are compatible with the view that hyperventilation and hypocapnia contribute to orthostatic intolerance and that the reason that some patients have a poor tolerance to orthostatic stress may be partly explained by the greater sensitivity of their peripheral circulation, and hence vasodilatation, to a lowering of blood levels of CO_2.

All procedures accord with current local guidelines.

Baroreflex responses to asphyxia and inspiratory resistance

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Obstructive sleep apnoea (OSA) has been found to predispose to hypertension, independently of common risk factors. The mechanisms that link these two conditions, however, are not clear. We propose that either the asphyxia and/or changes in inspiratory resistance that occur in OSA may change the gain and/or setting of arterial baroreceptors, leading to blood pressure being maintained at a higher level.

We studied eight healthy subjects (aged 21–62 years, 4 males, 4 females). The stimulus to carotid baroreceptors was changed using a neck chamber and graded pressures of −40 to +60 mmHg. We assessed the forearm vascular resistance responses from changes in blood pressure (Finapres) divided by brachial flow velocity (Doppler ultrasound). Stimulus response curves were defined during: (i) sham (no additional stimulus), (ii) breathing an asphyxic gas (12 % O_2, 5 % CO_2), (iii) inspiratory resistance ~10 mmHg, (iv) asphyxia and resistance. Sigmoid functions were applied to the curves and the maximum differentials (equivalent to peak gain) and the corresponding carotid pressures (equivalent to 'set point') were determined. All data are presented as means ± S.E.M. Statistical analyses were performed using paired *t* tests.

The sham test had no effect on blood pressure, gain or 'set point'. Asphyxia alone increased blood pressure (+7.0 ± 1.1 mmHg, $P < 0.0005$) and displaced the curve to higher pressures by +16.8 ± 2.1 mmHg ($P < 0.0005$), but had no effect on gain. Inspiratory resistance alone had no effect on blood pressure or 'set point'. However, it reduced gain from −3.0 ± 0.6 to −2.1 ± 0.4 units ($P < 0.05$). The combination of both asphyxia and inspiratory resistance increased blood pressure (+7.5 ± 2.5 mmHg, $P < 0.02$) and 'set point' (+16.8 ± 4.9 mmHg, $P < 0.02$) and reduced gain (−1.8 ± 0.6, $P < 0.02$).

The results of the present study show that inspiratory resistance reduces the gain of the baroreflex, and in combination with asphyxia also shifts the curve to hypertensive levels. If these changes are sustained they would provide a mechanism linking hypertension and OSA.

All procedures accord with current local guidelines.

Effects of water drinking on cardiovascular responses and cerebral autoregulation during orthostatic stress

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Orthostatic symptoms and syncope are common, even in apparently healthy subjects. Drinking water improves orthostatic hypotension in patients with autonomic failure. The aim of this study was to examine the effects of water in healthy volunteers. In addition to measuring its effects upon orthostatic tolerance (OT), we assessed its effects upon the cardiovascular system and cerebral autoregulation.

A randomised, controlled, cross-over study was performed on thirteen healthy volunteers (aged 31 ± 2 years) who drank 500 ml (test) or 50 ml (control) of water on different days after an overnight fast. The Leeds Teaching Hospitals Research Ethics Committee approved the study. We recorded finger arterial pressure (Finapres, calibrated against a sphygmomanometer), heart rate (ECG), stroke volume (impedance plethysmography), middle cerebral artery blood velocity (MCV, transcranial Doppler ultrasonography) and end-tidal CO_2 (nasal catheter). From these data we determined cardiac output, peripheral and cerebral vascular resistance. Fifteen minutes after drinking OT was assessed as time to presyncope during head-upright tilt and combined lower body suction (El-Bedawi & Hainsworth, 1994). The efficiency of autoregulation was quantified as the correlation coefficient (R) of the relationship between MCV and cerebral arterial pressure (CBP), determined as pressure changed during the orthostatic stress. A low value of R indicates good autoregulation. Data are presented as means ± S.E.M. Statistical significance was assessed using Student's paired *t* test. Correlations were assessed using the Spearman ranked correlation coefficient.

Drinking 500 ml water significantly improved OT (36 ± 3 compared with 31 ± 3 min, $P < 0.001$) and increased supine mean blood pressure ($P < 0.01$) due to increased peripheral resistance (106 ± 1 % of baseline compared with 100 ± 1 % after 50 ml water). Water drinking significantly blunted the increase in heart rate after 10 min of tilting (+10 ± 1 compared with +16 ± 2 b.p.m. in control, $P < 0.001$) and the decrease in stroke volume (−38 ± 3 compared with −45 ± 2 % in control, $P < 0.01$). The autoregulation index (R for regression of MCV on CBP) was significantly less after 50 ml water (0.52 ± 0.08 compared with 0.72 ± 0.06, $P < 0.05$). The autoregulation index (R) was significantly inversely correlated with OT ($P < 0.05$). Water had no effect on end-tidal CO_2 levels.

Drinking 500 ml water improved the tolerance to orthostatic stress. This was associated with smaller decreases in heart rate and stroke volume and a larger increase in vascular resistance. Cerebral autoregulation was also significantly improved.

El-Bedawi, K.M. & Hainworth, R. (1994). *Clin. Auton. Res.* **4**, 239–244.

All procedures accord with current local guidelines.

Effects of heat acclimation on plasma volume and orthostatic tolerance in healthy subjects – a preliminary study

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Heat acclimation is usually associated with an increase in plasma volume (Sawka *et al.* 2000), and it is known that expansion of an individual's plasma volume results in an increase in tolerance to orthostatic stress. The aim of this preliminary study was to determine whether orthostatic tolerance was influenced by heat acclimation, induced by repeated bouts of exercise in a hot environment.

The study, which had been approved by the University of Leeds and Leeds Teaching Hospitals ethics committees, was performed on four male and four female volunteers (aged 19–34 years). Heat acclimation was achieved by exercising at approximately 60 % of $V_{O_2,max}$ for two hour-long sessions per week for 4 weeks, at 40°C and 25–35 % humidity. Before, and 2 days after completing the acclimation protocol, the following measurements were made: plasma volume ($n = 5$, Evans' blue dye dilution, El-Sayed *et al.* 1994), orthostatic tolerance ($n = 6$, El-Bedawi & Hainsworth, 1994) and plasma electrolytes. Heart rates and aural temperatures were recorded during each heat exposure. All data are presented as means ± S.E.M. Statistical significance was assessed using paired t tests. Correlations were assessed using the Spearman ranked correlation coefficient.

Following acclimation, there was a reduction in the end-exercise core temperature (38.0 ± 0.1 to 37.6 ± 0.1 °C, $P < 0.005$) and in maximal heart rate (168.6 ± 5.5 to 152.4 ± 4.8 b.p.m., $P < 0.005$). $V_{O_2,max}$ did not change (35.1 ± 3.6 to 32.5 ± 3.1 ml kg^{-1} min^{-1}). There was a significant reduction in plasma Na^+ concentration (143.6 ± 0.9 to 140.9 ± 0.6 mmol l^{-1}, $P < 0.05$), but no change in plasma volume (40.4 ± 3.4 to 41.9 ± 4.2 ml kg^{-1}). Orthostatic tolerance, assessed as time to presyncope, did not change significantly (35 ± 2.4 to 38 ± 2.8 min), but there was a significant negative correlation between orthostatic tolerance and aural temperature during heat exposure, ($r = -0.567$, $P < 0.05$).

All subjects were successfully heat acclimated without affecting aerobic fitness. Mean orthostatic tolerance did not change significantly, but was significantly negatively correlated with the increase in body temperature during heat exposure (a measure of the degree of heat acclimation). The failure of plasma volume and orthostatic tolerance to increase significantly following the heat acclimation may be related to sodium depletion.

El-Bedawi, K.M. & Hainsworth, R. (1994). *Clin. Auton. Res.* **4**, 239–244.

El-Sayed, H. *et al.* (1994). *Clin. Lab. Haem.* **17**, 189–194.

Sawka, M.N. *et al.* (2000). *Med. Sci. Sports Ex.* **32**, 332–348.

All procedures accord with current local guidelines.