Hypoxia-induced impairment in rat respiratory muscles during development

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We studied the effects of hypoxia on contractile and endurance properties of respiratory muscles in the developing rat. Wistar rats aged postnatal day (P)19 and P29 were killed humanely and the diaphragm and sternohyoid (a representative pharyngeal dilator) muscles were surgically removed. Isometric contractile properties of isolated muscle strips were measured in tissue baths containing physiological salt solution at 30°C under hyperoxic (95%O₂/5%CO₂) or hypoxic (95%N₂/5%CO₂) conditions. Force-frequency relationship and fatigue index (i.e. ratio of force at 5min of fatigue to initial force) were examined. Fatigue was assessed in response to repeated tetanic contractions (40Hz, 300msec train duration) every 2 sec for 5 minutes. Hypoxia decreased specific force in the sternohyoid muscle but had no effect on diaphragm muscle force (peak force in sternohyoid muscle at P19 was 5.5±0.9 vs. 2.8±0.5*, P29 was 9.2±1.2 vs. 3.6±0.8*, mean±SEM N/cm², hyperoxia vs. hypoxia, P<0.05 ANOVA). We found that in vitro hypoxia significantly reduced muscle endurance in both the sternohyoid and diaphragm muscle. We also observed an age-dependent decrease in endurance for both muscles in hyperoxic and hypoxic groups. Thus, sternohyoid fatigue index for P19 was (65.7±4.5% vs. 21.7±2.7%), P29 was (37.4±2.7% vs. 2.7±1.7%); mean±SEM hyperoxia vs. hypoxia, P<0.05 ANOVA). Diaphragm fatigue index for P19 was (80.7±2.7% vs. 27.9±2.7%), P29 was (62±4.5% vs. 19.7±1.8%, mean±SEM, hypoxia vs. hypoxia, P<0.05 ANOVA). We conclude that hypoxia impairs respiratory muscle function. Our results suggest that the sternohyoid muscle is more vulnerable to hypoxic insult than the diaphragm muscle. We speculate that this is due to their different fibre type characteristics. The mechanism for hypoxia-induced muscle impairment remains unknown. However, the effects of hypoxia may have implications for the control of airway patency in vivo.

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

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The interaction of stretch, intraluminal pressure and E. coli heat stable (Sta) enterotoxin on jejunal fluid absorption in the anaesthetised rat


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A perfused intestinal loop preparation was used to measure luminal uptake of fluid in vivo by means of fluid volume recovery from the jejunum of the anaesthetised rat (70 mg/Kg i.p. sagatal). All procedures were carried out in conformity with current UK legislation. Data is given as the mean plus the standard error with the number of experiments in brackets. Significance was tested by ‘t’-test. Distension of the jejunum with a polythene loop in the lumen reduced fluid absorption (p<0.001) from 116±18(7)ul/cm/hr to 40±5(10)ul/cm/hr. Heat-stable (Sta) toxin from E.coli reduced fluid absorption further to 14±13(6)ul/cm/hr in the stretched intestine, not significantly different from zero fluid absorption. Distension by 30 cm hydrostatic pressure reduced fluid absorption (p<0.01) to 52±10(6)ul/cm/hr. Combination with Stas reduced fluid absorption to 29±10(5). Lack of net secretion implies that distension does not initiate a secretory event but prevents absorption. The lack of super-imposition of Stais and distension effects implies a common absorption mechanism inhibited by both.

Low rates of fluid absorption by coil distension were not restored by serosal application of lidocaine, i.e. hexamethonium or luminal perfusion of atropine. In contrast, luminal atropine did restore fluid absorption in jejunum distended by hydrostatic pressure, from 52±10(6)ul/cm/hr to 103±15(5)ul/cm/hr, not significantly different from the undistended jejunal value. The neural component to the inhibition of absorption is likely to be mediated through an axon reflex within a cholinergic neuron. In contrast, neither i.v. hexamethonium, serosal lidocaine nor luminal atropine restored fluid absorption after exposure
to STa, making unlikely a local neural component to the action of STa.
Luminal carbachol (1 mM) reduced net fluid absorption to 31.9 ± 7.6 (6) μl/cm/hr that was significantly lower than control values and comparable to absorption rates after exposure to STa. Isotonic choline chloride perfused in combination with STa suppressed sodium independent fluid absorption. Additionally perfusing with 1 mM carbachol gave no further decrease in fluid absorption, indicating that no secretion process was detected that could worsen inhibited absorption. This indicated that a cholinergically mediated secretory process was unlikely to be present in the proximal jejunum. The reduction in fluid absorption after STa exposure and after pressure distension is likely to be the result of a final convergence of both pathways on sodium: hydrogen ion exchange with the pressure distension mediated by the internal release of acetylcholine through an initiation of the intestino-intestinal stretch reflex.

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### C88

**Mouse duodenal iron transport is decreased following chronic exposure to hepcidin**

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Hepcidin, the main circulating iron regulatory hormone exerts its actions by binding to the iron efflux protein ferroportin, inducing transporter degradation and thereby inhibiting iron release from cells [1]. We have shown previously that injection of hepcidin into mice results in a rapid (within 4h) decrease in serum iron and a concomitant decrease in ferroportin expression in splenic macrophages [2]. Interestingly, duodenal ferroportin expression in the same animals was not altered within this time frame [2]. The aim of this study was to examine the effects of longer-term exposure to hepcidin on duodenal iron transport.

Male C57BL/6 mice (aged 4 weeks) were given injections of hepcidin (10μg/mouse, i.p.) or an equivalent volume of saline at 24h intervals. A final injection of hepcidin was administered 4h prior to experimentation. In anaesthetised animals (sodium pentobarbitone, 60 mg/kg, i.p.), tied-off duodenal segments were washed with saline, followed by air, filled with Hepes-buffered saline (pH6.5) containing 0.2mM ⁵⁹Fe, complexed with 4mM ascorbate and incubated for 10 min. At the end of the exposure period, the amount of ⁵⁹Fe in the duodenal mucosa and the animal carcass were determined by gamma counting. Data are presented as mean ± SEM, and were analysed using one-way ANOVA and Tukey’s post hoc test with differences considered significant at P<0.05.

Iron transfer from the duodenal mucosa to the animal was significantly decreased in both 24h and 72h hepcidin treated mice compared with the control group (Table 1). Interestingly, iron retention within the mucosal tissue was significantly elevated in 24h hepcidin treated mice compared with the other two experimental groups, suggesting that enterocytes were still able to take up iron despite the inhibition of the efflux pathway. Taken together with our previous data [2], we propose that duodenal enterocytes are less sensitive than splenic macrophages to a hepcidin challenge, and that this is consistent with the relative importance of these two cell types in maintaining body iron homeostasis.

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mucosal retention (μg/mucosa)</th>
<th>Mucosal transfer (μg/mucosa)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>158.4 ± 11.8</td>
<td>433.2 ± 115.9</td>
<td></td>
</tr>
<tr>
<td>24h hepcidin</td>
<td>347.0 ± 79.8*</td>
<td>8.8 ± 3.3*</td>
<td>*P&lt;0.05</td>
</tr>
<tr>
<td>72h hepcidin</td>
<td>124.1 ± 14.3</td>
<td>19.3 ± 2.5*</td>
<td>*P&lt;0.05</td>
</tr>
</tbody>
</table>


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### C89

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

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**BACKGROUND:** The maximal force a muscle can generate depends on the number of sarcomeres in parallel and thus its physiological cross sectional area (PCSA). However, the increase in muscle strength with training is widely reported to be greater than expected from the increase in size (1). The aim of the present investigation was to systematically address potential problems that may be caused by changes in voluntary activation and coactivation and changes in muscle architecture during maximum voluntary contraction (MVC). To our knowledge, this is the first study to investigate the effect of resistance training on specific tension taking into account the structural differences between the four quadriceps muscles.

**METHODS:** Fourteen healthy male volunteers aged 21 ± 3 yrs performed unilateral leg-extension (4 sets of 10 repetitions at 80% 1RM), 3 times/wk for 9 weeks. Quadriceps tendon force (Fₜ) was calculated by correcting maximum isometric torque obtained at the optimum knee angle for antagonist coactivation (estimated from electromyographic activity), voluntary activation (using the interpolated twitch technique), patella tendon moment arm length and the ratio of quadriceps tendon force to patella tendon force (2). The PCSA of each quadriceps muscle was calculated by dividing the volume measured...
Dietary regulation of bovine ruminal UT-B urea transporter expression

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Ruminants, such as cattle, need to recycle nitrogen through the process of urea nitrogen salvaging (UNS) in order to maintain nitrogen balance (1). The process of UNS requires large amounts of urea to pass into the gastrointestinal tract and previous studies have suggested that this occurs through ruminal facilitative UT-B urea transporters (2). In this study we have investigated the effect of dietary intake on bovine ruminal UT-B urea transporter expression.

Ruminal tissue samples were obtained from 6 adult cows, 3 which had been fed a concentrate diet (RC) and 3 which had been fed an ordinary forage diet (RO) and 3 which had been fed a concentrate diet (RC). Using a 32P-labelled full-length bUT-B cDNA probe, northern analysis detected no difference in the level of the 3.7kb bUT-B transcript between ruminal RNA samples from the two diets (NS, Unpaired T-Test). In contrast, western analysis of ruminal protein samples using a recently characterized bUT-B antibody detected significant differences between the two groups. For example, a 36 kDa bUT-B signal representing unglycosylated bUT-B2 was significantly greater in RC compared to RO ruminal protein (P<0.05, Unpaired T-test). Finally, using 10µM sections of methanol-fixed ruminal tissue, immunolocalization studies showed that while the bUT-B signal was found predominantly in the stratum basale in RO samples, it was found mainly within cells of the stratum granulosum in RC samples.

Our results therefore provide strong evidence that ruminal UT-B urea transporter protein expression is altered by dietary intake. Since ruminal microflora, short-chain fatty acids and pH are altered by concentrate feeding, further work on these factors are required to understand the cellular basis of UT-B expression.


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Exploration of the biochemical processes in rat gastric mucosa under the experimental ulceration

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World statistics says that stomach ulcer is still one of the most common diseases in the many European countries. On the cell level an important role in ulcer development play violations in the system of the biochemical processes. The aim of this study was to explore biochemical processes in the cells of the stomach gastric mucosa in rats under different models of experimental ulceration.

Wistar rats 150 grams weight were used in the experiment. The research were carried out using the aspirin (acetate), stress and ethanol methods of experimental ulceration. To induce stressful ulceration we used social immobilizing stress (Groisman, Carevina). Ethanol ulceration was induced by administration of 1 ml of 80% ethanol per os. To induce acetate ulceration we administered aspirin in doze of 150 microliters per kilogram of body weight 5 times a day during 3 days.

A complex exploration of the state of the plasma membranes of rat gastric mucosal cells under different models of ulceration was carried out. It was proven, that lipid peroxidation (LPO) processes play key role in ulcer development irrespective from the factors of ulceration. Under all explored conditions, the LPO products content in the homogenate of gastric mucosa was increased, while an activity of antioxidant enzymes was decreased. A decreased content of all groups of phospholipids was found in plasma membrane fraction. The most significant changes were found under stress – in 2 times. Cholesterol content was increased under stress and ethanol more than in 2 times. Decreased activity of membrane-associated enzymes was also found under ethanol and stress: Na-K ATPase, 5’ nucleotidase, (in 1.8 times under ethanol, in 1.5 times under...
Hepcidin inhibits iron efflux from human intestinal Caco-2 cells

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Hepcidin, a 25 amino acid produced by hepatocytes in response to iron loading and inflammation, is the main circulating iron regulatory hormone [1]. Hepcidin is predicted to exert its regulatory action by binding to the iron efflux protein ferroportin, inducing transporter degradation and thereby inhibiting iron release from cells [1]. Our work in macrophages supports this proposed mechanism for the action of hepcidin [2]. However, hepcidin does not alter ferroportin expression in intestinal epithelial cells [2,3], suggesting that they may be less responsive to hepcidin. To further test this hypothesis we measured changes in iron transport in Caco-2 cells exposed to hepcidin. Caco-2 cells were grown for 21 days on Transwell inserts. At the start of each experiment, 59FeCl3 (10μM) was added to the apical medium and hepcidin (1μM) was added to the basolateral medium. The cellular accumulation of iron and its release into the basolateral medium were measured over the following 24h by gamma counting. Data are presented as mean ± SEM, and were analysed using Student’s unpaired t-test with differences considered significant at P<0.05.

Despite our previous observation that hepcidin does not alter ferroportin expression [2,3], iron efflux from Caco-2 cells into the basolateral medium was significantly decreased in hepcidin-treated cells (normalised data at 24h time-point; control, 1.00 ± 0.08; + hepcidin, 0.68 ± 0.05, P=0.004, n=11). This was associated with a significant increase in cellular iron accumulation after 24h (normalised data; control, 1.00 ± 0.04; + hepcidin, 1.58 ± 0.21, P<0.01, n=11). Our data support the notion that in intestinal epithelial cells, hepcidin can block iron release without inducing degradation of ferroportin. Together with our macrophage data, this suggests that the actions of hepcidin are cell-type specific.


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Effect of leg massage on heart rate variability (HRV) in healthy subjects

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Cardiac autonomic activity was assessed by heart rate variability (HRV) following a 30min leg massage on resting subjects

Methods
HRV was measured for 5mins in a supine position at baseline in ten healthy males (24.7±3.4yrs) using the Polar Precision Performance software (Polar Electro, Finland). Subjects then received either a manual leg massage (MM) to both legs, or rested (R) for 30mins. HRV was then measured again for 5mins.

Results
Compared to Rest, heart rate for MM was significantly lower (Figure 1). This was accompanied by a significant decrease in an indicator of sympathetic activity (LFnorm) and an increase in parasympathetic activity (HFnorm) for MM. LF:HF Ratio, which is an indication of sympatho-vagal balance also decreased, indicating a parasympathetic effect exerted by manual leg massage. In contrast, during R, LFnorm was higher and HFnorm lower and not significantly different from baseline.

Rate pressure product, which is a surrogate measure of myocardial oxygen consumption and cardiac workload, decreased significantly from 8218±444units at baseline to 7154±502units (p=0.021) for MM, which was significantly lower than R (8126±488units) at the end of the 30mins, indicating a lower workload of the heart.

Baseline leg skin temperature was 31.5±0.5°C. At the end of the 30mins, temperature had increased to 33.4±0.33°C for MM, which was significantly higher than R (31.5±0.4°C).

Baseline perception of feeling measured on a 13 point bipolar scale was 2 (IQR 1.25, 3; ‘Fairly Good’). Feeling improved over the 30min period for MM; and was +6 (IQR 5, 6 ‘Very Good’). This was significant higher than R (2 (IQR 1.25, 3)). The results indicate that MM had the greater effect when compared to R, inducing a relaxation response.

Conclusion
Manual leg massage is effective at decreasing cardiac sympathetic and increasing parasympathetic activity, reducing the