6.2±1.4CU respectively). L-NAME induced similar hindlimb vasoconstriction in all groups (~5CU) and attenuated the hypoxia-induced increase in IntFVC though this was only significant in N rats (9.7±1.5 vs 4.0±1.0CU) – suggesting a reduced role in CHU rats for NO in the hypoxic dilatation of skeletal muscle. SNP restored baseline IntFVC and allowed for a larger NO-dependent increase in IntFVC during hypoxia in both N and LCHU (9.7±1.8 & 7.8±1.1CU respectively) but not in ECHU (5.7±1.4CU). Protein analysis showed similar levels of eNOS in all groups in TA, but in SOL muscle eNOS was increased in ECHU and LCHU compared to N rats.

These results suggest that NO acts in a similar manner in both N and LCHU albeit to a lesser extent in the latter to produce an NO-dependent dilatation in hypoxia, further, it suggests this mechanism is not functional in ECHU. The fact that eNOS protein was unchanged (TA) or increased (SOL) suggests that the functional changes are not due to the lack of total eNOS protein, but more likely a reduction in NO bioavailability or eNOS function and this may have implications for the control of muscle blood flow under differing conditions.

(1) Barker D.J.P. (1998). Clin. Sci. 95:115-128

(2) Williams S.J. et al (2005). J. Physiol. 565:125-135

(3) Bryan P.T. & Marshall J.M. (1999). J. Physiol. 514:163-175

(4) Coney A.M. & Marshall J.M. (2006). Early Human Dev. 82:518

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

C86

Hypoxia-induced impairment in rat respiratory muscles during development

J. Carberry[1], A. Bradford[2] and K.D. O'Halloran[1]

[1]School of Medicine and Medical Science, University College Dublin, Dublin 4, Ireland and [2]Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin 2, Ireland

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%O_2/5%CO_2) or hypoxic (95%N_2/5%CO_2) conditions. Force-frequency relationship and fatigue index (*ie* 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/cm^2, 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, hyperoxia 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*.

Supported by the Health Research Board,Ireland and University College Dublin.

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

C87

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

M.L. Lucas, P.J. Tainsh, S. McColl, H.L. Kenward, P.C. Watson, L.C. Gilligan, V. Graham and Y.B. Nelson

IBLS, N&BS, Glasgow, UK

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

The authors would like to thank the Chancellor's Fund of the University of Glasgow for financial support.

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

C88

Mouse duodenal iron transport is decreased following chronic exposure to hepcidin

T. Chaston[1], J. Marks[2], E. Debnam[2], S.K. Srai[3] and P. Sharp[1]

[1]Nutritional Sciences, King's College London, London, UK, [2]Department of Physiology, University College London, London, UK and [3]Department of Structural & Molecular Biology, University College London, London, UK

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

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

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

Nemeth E & Ganz T (2006) *Annu Rev Nutr* **26**, 323-342.

Chaston T *et al*. (2008) *Gut* **57**, 374-382.

This work was funded by a project grant from the Biotechnology and Biological Sciences Research Council.

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

C89

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

R.M. Erskine[1], H. Degens[1] and D.A. Jones[1,2]

[1]Institute for Biomedical Research into Human Movement and Health, Manchester Metropolitan University, Alsager, UK and [2]School of Sport and Exercise Sciences, University of Birmingham, Birmingham, UK

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

histological feature X?' where 'X' could be a cell type, or histological region; alternatively, the format can be 'the characteristics of histological feature X are...' where students must choose from a list of options. This latter format makes it easy to incorporate questions on function, and it has been helpful to design quizzes in conjunction with lecturers on relevant topics. This helps ensure the integration of histology with physiology and anatomy.
In the 2nd Year BVSc exam, a three-slide quiz was adapted by replacing a drop-down form with optical marking sheets. This is a conservative option, as the scoring/analysis features of the software are still under development. The ease of transition to optical marking demonstrates the flexibility of the quizzes as an assessment vehicle. Student feedback on the quizzes was extremely positive. For example, >95% of students rated the quizzes 'excellent' or 'good' in terms of usefulness both as a learning tool and as a revision aid.
In summary, students regard on-line quizzes, facilitated by Digital Slidebox, as a highly useful learning tool and revision aid.
Guest access to a demonstration on-line quiz is available at: http://137.222.11.10
Please login as a New User using the activation key 'psdemo'.

We gratefully acknowledge the technical support of Debbie Martin, Debi Ford, Graeme Cappi, Mally McLane, Peter Dickens and Paul Buttner.

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

PC57

Dietary regulation of bovine ruminal UT-B urea transporter expression

G. Stewart[1], E. Scriven[1], C. Smith[1] and N. Simmons[2]

[1]Faculty of Life Sciences, University of Manchester, Manchester, UK and [2]School of Cellular and Molecular Biosciences, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne, UK

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 an ordinary forage diet (RO) and 3 which had been fed a concentrate diet (RC). Using a ^{32}P-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.

Stewart GS and Smith, CP (2005) Nutr. Res. Rev. 18, 49-62.

Stewart GS, Graham C, Cattell S, Smith TPL, Smith CP and Simmons NL (2005) AJP Regul Integr Comp Physiol 289: R605-R612

This work was funded by The Royal Society (GS), Kidney Research UK (GS) and AstraZeneca (NLS).

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

PC58

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

I. Rudenko, V. Kovalyova and L. Ostapchenko

Biological, National Taras Shevchenko University, Kyiv, Ukraine

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

stress) and Ca-Mg ATPase (in 1.4 times under ethanol, the activity of this enzyme under stress is almost undetectable). Under aspirin there were no significant changes in the activity of these enzymes. Exploration of protein kinases activity showed wide variety of changes. Activity of cyclic adenosine monophosphate-, cyclic guanosine monophosphate-, calcium-, and phospholipid-dependent protein kinases and tyrosine kinase activities were evaluated. Under aspirin ulceration activity of all explored protein kinases was decreased in 1.4 times, activity of PK-G showed no changes. Under ethanol was determined increased activities of PK-A, PK-G and decreased activity of tyrosine protein kinase. Increased activity of all explored protein kinases under stress was also found.
This study indicates that stomach ulcer is a complex disease, which leads to different changes in biochemical pathways. Plasma membranes and membrane-associated enzymes and molecules are involved in the ulcer development. Different factors of ulceration lead to changes in different signal transduction pathways.

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

PC59

Hepcidin inhibits iron efflux from human intestinal Caco-2 cells

B. Chung, T. Chaston and P. Sharp

Nutritional Sciences, King's College London, London, UK

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, $^{59}FeCl_3$ (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.

Nemeth E & Ganz T (2006) *Annu Rev Nutr* **26**, 323-342.

Chaston T *et al*. (2008) *Gut* **57**, 374-382.

Yamaji S *et al*. (2004) *Blood* **104**, 2178-2180

This work was funded by a project grant from the Biotechnology and Biological Sciences Research Council. BC is part-funded by the Overseas Research Student (ORS) Award Scheme.

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

PC60

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

G.E. Jones, D. Cotterrell and I. McDowall

Biologcial Sciences, University Of Chester, Chester, UK

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 that 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