C1

Electrophysiological demonstration of an osmotically sensitive Na^+-Ca^{2+} exchanger in bovine articular chondrocytes

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Articular cartilage, which protects long bones at articulating joints, comprises an extracellular matrix (ECM) which is maintained by chondrocytes, the sole cell type resident in the tissue. The absence of articular cartilage results in osteoarthritis and there is, therefore, clinical significance in understanding the mechanical mechanisms which determine cartilage turnover.

Physicochemical factors associated with joint loading, for example changes to extracellular osmolarity, determine ECM turnover by chondrocytes (Urban et al., 1993). In this way, healthy cartilage is remodelled such that it can withstand prevailing stresses. In previous work we have shown that chondrocyte membrane transport mechanisms, notably those determining intracellular Ca^{2+} ($[Ca^{2+}]_i$), can operate as mechanotransduction pathways through their effects on intracellular composition.

Chondrocyte $[Ca^{2+}]_i$ is regulated in part by sodium-calcium exchange (NCE; Ponte and Hall, 1994). In the present study, the activity of NCE in bovine articular chondrocytes has been measured using standard electrophysiological techniques and its sensitivity to extracellular osmolarity determined. Data are presented as mean ± SEM, n ≧ 3.

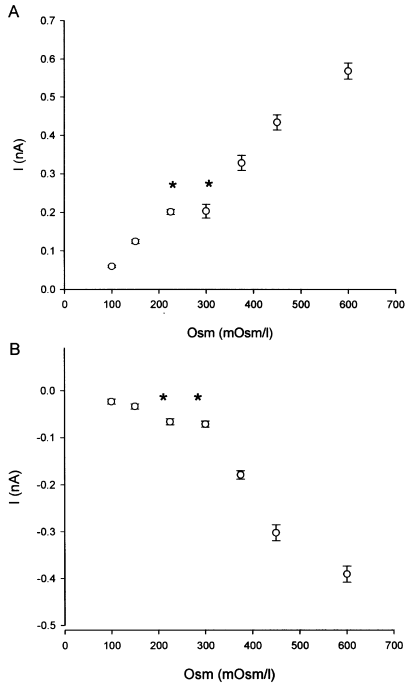

Figure 1. The effect of extracellular osmolarity on NCE current. Osmolarity was changed by the addition of mannitol. n ≥ 4 in each case. NCE current was measured at +80 mV (A) and -120 mV (B).

Articular cartilage was obtained from the metacarpophalangeal joints of steers slaughtered at abbatoir and chondrocytes were isolated by collagenase digestion. NCE activity was assessed at room temperature by measuring the NCE mediated current (I_{NCE}) recorded in whole-cell configuration (Convery and Hancox, 1999). Measurements obtained using a descending ramp protocol (from +80mV to -120mV, holding potential -40mV) demonstrated the existence of I_{NCE} which could be completely inhibited by Ni^{2+} (5mM). Furthermore, I_{NCE} could be partially inhibited by benzamil (500 μM) and KBR4973 (50 mM).

I_{NCE} was recorded in cells bathed in solutions of differing osmolarity (Figure 1). Increases in external osmolarity resulted in a rise of both outward and inward net currents in comparison with control (300 mOsm l^{-1}) whereas reductions in osmolarity of greater than 50% evoked a fall in the magnitude of both outward and inward currents. In both cases, the changes in net current were not apparent in cells treated with Ni^{2+} (5mM; data not shown).

This study is the first direct demonstration of osmotically sensitive I_{NCE} in articular chondrocytes and implicates this transporter in the mechanotransduction processes occurring in these cells.

Ponte MR & Hall AC (1994). *J Physiol* **475.P**, 105P

Convery MK & Hancox JC (1999). *Pflugers Arch* **437**, 944-954

Urban JP *et al.* (1993). *J Cell Physiol* **154**, 262-270

Arthritis Research Campaign, EPA Cephalosporin Fund, Universidad Tecnologica de Pereira.

C2

TSC2 phosphorylation at Thr1462 increases in response to resistance and decreases in response to endurance training-like stimulation in rat skeletal muscle

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We have previously reported a switching behaviour to either AMP kinase (AMPK) or protein kinase B (PKB) signalling in response to endurance and resistance training-like muscle stimulation. Others have shown that tuberin (TSC2) could be phosphorylated by AMPK (Inoki et al., 2003) and PKB (Manning et al., 2002). The aim was to investigate whether the observed AMPK-PKB effects were reflected by variations in TSC2 Thr1462 phosphorylation in response to endurance and resistance training-like stimulation.

Male wistar rats were humanely killed and their rat extensor digitorum longus (EDL) or soleus muscles were incubated in oxygenated Krebs-Henseleit buffer and electrically stimulated either continuously at 10 Hz for 3 h (endurance training-like low frequency stimulation; LFS) or for 10 sets of 6 repetitions of 3 s-100 Hz bursts (resistance training-like high frequency stimulation; HFS). Muscles were frozen, protein extracted and Western blotting was carried out using primary antibodies against phospho-TSC2 (Thr1462) and total TSC2.

TSC2 phosphorylation at Thr1462 (normalized to total TSC2) increased significantly to 3.13±0.23 directly after HFS but was not significantly different 3 h thereafter (Figure 1). In contrast, TSC2 Thr1462 phosphorylation decreased significantly to 0.24±0.02 directly and 0.55±0.06 3 h after LFS. Total TSC2 decreased significantly to 0.82±0.02 directly after HFS and increased to 1.27±0.02 after LFS but was not significantly different 3 h after stimulation.

TSC2 Thr1462 phosphorylation is both sensitive to LFS and HFS. The previously reported PKB activation by HFS can explain the acutely increased TSC2 Thr1462 phosphorylation. The LFS effect on TSC2 Thr1462 phosphorylation is not PKB-induced because PKB phosphorylation was not affected by LFS. However, AMPK which is activated by LFS, phosphorylates TSC2 at Thr1227 and Ser1345 (Inoki et al., 2003). The AMPK-phosphorylated sites are close to the Thr1462 site and their phosphorylation by AMPK could potentially inhibit Thr1462 phosphorylation. The total TSC2 data support the hypothesis that Thr1462 phosphorylation controls TSC2 degradation (Inoki et al., 2002). To conclude, the inhibitory effect of LFS and activating effect of HFS on downstream regulators of protein synthesis is likely to be mediated via TSC2 Thr1462 phosphorylation and TSC2 content. TSC2 is thus the downstream executer of a signalling switch between AMPK and PKB-dependent signalling in response to LFS and HFS, respectively.

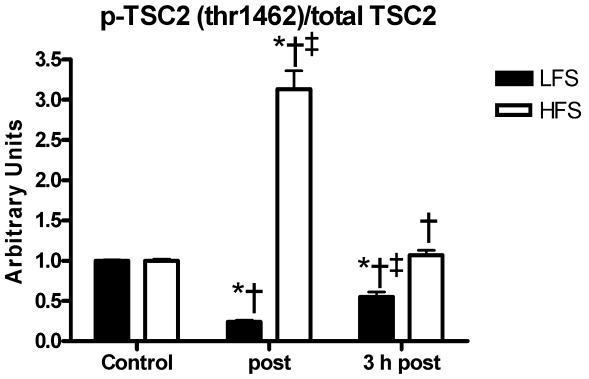

Figure 1 TSC2 phosphorylation at Thr1462/TSC2. (mean ± SEM; n=8) ($p<0.05$).*Significantly different from control; significant difference between LFS and HFS; Significantly higher in EDL.

Inoki K et al (2002). Nat.Cell Biol. 4, 648-657.
Inoki K et al. (2003). Cell 115, 577-590.
Manning et al. (2002). Mol.Cell 10, 151-162.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C3

Comparison of the effects of an acute bout of resistance and endurance exercise on the blood glucose response during an OGTT in young healthy humans

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Endurance and resistance training are known to improve insulin sensitivity in healthy, sedentary and insulin resistant individuals. A single bout of endurance exercise has been suggested to improve insulin sensitivity for up to 48 hours (Perseghin et al. 1996). There is evidence to show that increased glucose uptake and glycogen synthase activity may be directly related to decreases in muscle glycogen post-exercise (Nielsen et al. 2001). On the other hand, very little is known about the acute effects of resistance exercise in humans. The aim of this study therefore was to investigate the blood glucose response to a 75 g oral glucose tolerance test (OGTT) 6 h after a high intensity bout of either resistance (RE) or endurance exercise (EE). In an attempt to replete muscle glycogen stores and thus pinpoint potential effects to the insulin signalling cascade, a carbohydrate/protein beverage was consumed within the first h post-exercise

18 subjects were investigated both after exercise and in a control trial without exercise. 9 subjects (Age = 21 ± 1 years, BMI = 22.8 ± 0.6 kg/m^2, ♂/♀ = 6/3) completed 1 h of EE (cycle ergometry at 75% Wmax) and 9 (Age = 23 ± 0 years, BMI = 23.9 ± 0.3 kg/m^2, ♂/♀ = 6/3) completed a RE bout (3 sets of 10 repetitions to failure) covering all major muscle groups. All subjects ingested 1.5 L of a carbohydrate (200g) protein beverage (50g) both within 1 h after exercise and in the control trial. An OGTT was performed 6 h post-exercise. Only water was consumed in the period between ingestion of the beverage and the start of the OGTT.

Blood glucose concentrations following the OGTT shown by area under the curve decreased 16% in the RE group (822 ± 68 v. 694 ± 23 mmol l^{-1} 120 min) but were unchanged in the EE group (784 ± 40 v. 834 ± 59 mmol l^{-1} 120 min) ($P < 0.05$). Blood insulin concentrations remained unchanged in both the RE and EE groups (4978.74 ± 706.46 v. 4458.07 ± 597.79 μU ml^{-1} 120 min and 4117.61 ± 590.28 v. 3918.04 ± 351.65 μU ml^{-1} 120 min respectively) ($P > 0.05$).

The results suggest that improved insulin sensitivity following a single bout of RE or EE occurs through different mechanisms. It is probable that the improvement of EE reported in previous studies is dependent on the depletion of glycogen stores and, therefore, is not seen in this study. On the other hand the results show that the lowered blood glucose response following RE is still present after re-feeding with carbohydrate and protein immediately post-exercise. Therefore, in line with recent animal studies (Hernandez et al. 2000), these results suggest that the reduction in blood glucose following RE may involve other mechanisms such as increased activation of the insulin signalling cascade.

Hernandez JM et.al. (2000). J Appl Phys 88(3):1142-1149
Nielsen J N et. al. (2001). J Physiol 531.3:757-769
Perseghin G et. al. (1996). N Engl J Med 335:1357-62

The authors would like to thank Zoe Jessop, Paula Hendricks, Laura Wright and Oliver Morley for their assistance and all subjects for their time and effort.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C4

Two weeks of GH administration does not increase the expression of insulin-like growth factor-I mRNA splice variants in the skeletal muscles of young men

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Elevated growth hormone (GH) in blood leads to increased IGF-I derived from the liver. IGF-I is also expressed locally, particularly in skeletal muscle. However, the relationship between circulating GH and localised IGF-I mRNA expression in muscle is unclear. Recently, (Hameed et al. 2004) the mRNA levels of two splice variants of the IGI-I gene (IGF-IEa and MGF) were studied in muscle obtained from elderly males who were administering daily rhGH (0.5IU/m^2/week for 5 weeks). IGF-IEa which is indistinct from mature liver-derived IGF-I, was significantly elevated, MGF remained unchanged. GH and IGF-I levels fall with increasing age but the effects of rhGH administration on young muscle are unknown. This is important as many athletes and bodybuilders use rhGH in an attempt to improve performance. The aim of this study was to examine the effects of exogenous rhGH on the expression of all three IGF-I mRNA splice variants (IGF-IEa, Eb and Ec) in adult males.

With ethical approval, 8 healthy but untrained males (mean age 24.3) were recruited into a randomised double blind-with crossover trial. Subjects were randomly assigned to administering daily injections of rhGH (via subcutaneous injection 0.5IU/kg/week) or placebo for a 2-week period. Following 2 weeks washout, the groups were reversed. Following local anaesthesia (1% lignocaine), muscle biopsies were obtained at the end of each dosing phase from the vastus lateralis muscle. IGF-IEa, Eb and Ec (MGF) mRNA transcripts were analysed using real-time RT-PCR (Hameed et al 2004). Blood taken from the anticubital vein was analysed by ELISA for GH and mature IGF-1.

The daily administration of rhGH resulted in a significant increase in circulating IGF-I and GH levels. However, no significant change in the mRNA of the three IGF-I transcripts was observed (Table 1). In apparent contrast to the muscles of older men who have lower GH levels, the muscles of young men remain unresponsive to rhGH administration in terms of IGF-I gene transcription.

mRNA isoform	Placebo	Growth Hormone
Muscle IGF-IEa	$2.14 \times 10^{-5} \pm 5.62 \times 10^{-6}$	$1.49 \times 10^{-5} \pm 3.06 \times 10^{-6}$
Muscle IGF-IEb	$8.46 \times 10^{-9} \pm 4.17 \times 10^{-9}$	$1.83 \times 10^{-8} \pm 1.51 \times 10^{-8}$
Muscle IGF-IEc (MGF)	$3.60 \times 10^{-7} \pm 2.94 \times 10^{-7}$	$1.14 \times 10^{-7} \pm 2.95 \times 10^{-8}$
Serum [IGF-I] (nmol/L)	32.5 ± 2.95	106.2 ± 4.87 *

Data are means ± S.E.M. mRNA is expressed as ng mRNA / mg total RNA. *Significant ($P<0.05$) rise in [IGF-I] detected as a result of GH administration compared to placebo at equivalent time points (Paired t-test)

Hameed M *et. al.* (2004). *J.Physiol.* **555,**231-240

This work was funded by the World Anti-Doping Agency

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C5

Cultured muscle cells as a system for the analysis of IGF-I splicing regulation by factors present in the circulation.

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The IGF-I gene is complex and in human muscle it can be spliced into three alternative isoforms at the 3' end: IGF-IEa, IGF-IEb, and IGF-IEc (also called MGF; Hameed et al. 2003, 2004). In rodent muscle, overexpression of the IGF-IEa or MGF results in hypertrophy (Musaro et al. 2001; Yang and Goldspink, unpublished observations). The mechanisms by which IGF-I gene expression is regulated at the muscle level may be mechanical (McCoy et al., 1999), hormonal or a combination of both. For example, in elderly subjects, recombinant human Growth Hormone (rhGH) treatment increases the expression of IGF-IEa, but when combined with exercise markedly increases the MGF isoform (Hameed et al, 2004).

It has recently been shown that muscle cells grown in 3-D collagen matrixes upregulate IGF-I transcript expression in response to stretching of the matrix (Cheema et al., 2004). In the present work we have studied muscle cells in culture with the aim of determining if either or both of the two splice variants of IGF-I would be upregulated when treated with GH and/or IGF-I, in the absence of mechanical signals C2C12 myoblasts were grown to 50% confluency in medium containing 10% foetal calf serum (FCS). The cells were transferred to medium containing i) 1%FCS only, ii) 100 ng/ml rhGH iii) 100 pg/ml IGF or iv) both. In the untreated cells (i) both isoforms (IGF-IEa and MGF) were present. Treatment with rhGH alone lead to an increase in IGF-IEa and MGF of about 3 fold over control (Table 1). However, treatment with IGF-I abolished expression of both isoforms. When used in combination, the inhibitory effect of IGF-I overode the GH stimulation of IGFIEa and MGF transcription.

We conclude that muscle tissue can upregulate IGF-I isoform expression as a direct result of hormonal stimulation or stretch stimuli. The isoforms of IGF-I seem equally sensitive to GH stimulation in vitro.In vivo, a negative feedback mechanism may modulate the action of GH on IGF-I transcription in muscle tissue in by circulating or local IGF-I expression.

	IGF-IEa	MGF
control	9.02 ± 0.01	1.46 ± 0.18
GH	28.30 ± 1.84	4.50 ± 0.71
IGF-I	0.22 ± 0.05	0.03 ± 0.01
GH+IGF-I	0.15 ± 0.03	0.02 ± 0.01

Data are Means ± S.D. of three separate experiments. * Significant difference ($P<0.5$) relative to control (unpaired t-test).

Cheema U. et al (2004) J Cell Physiol, in press
Hameed, M. et al. (2003) J Physiol, 547: 247-254.
Hameed M. et al, (2004) J Physiol,555:231-40.
McKoy, G. et al. (1999) J Physiol, 516: 583-92.
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This work is funded by the World Anti-Doping Agency.

C6

Transcript profiling identifies genes that define lack of responsiveness to endurance training in humans

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Adaptation to aerobic training is highly variable. We determined whether there was a molecular basis for training responsiveness. 24 male subjects undertook supervised aerobic training; cycling at 75% of peak VO2 (4 times per week, 6 weeks). The study was approved by the Institute ethics committee. Peak aerobic capacity, submaximal heart rate response (@75% of peak VO2) and 15min exercise performance (KJ) were quantified. Subjects were ranked on the basis of the sum of their % improvements across these 3 variables. Muscle gene expression was studied in the top 8 (HRG, 24±1yr, 183±3cm, 77±6kg, Baseline VO2peak =3.5±0.3 l/min) and compared with the 8 lowest ranked subjects (LRG, 23±1yr, 180±3cm, 77±3kg, Baseline VO2peak =3.7±0.1 l/min). Cumulative increase in aerobic fitness index was 10±2% in the LRG and 55±4% in the HRG (P<0.0001). There was no relationship between baseline physiological variables and the magnitude of improvement observed (n=24).Genes associated with extracellular matrix remodelling were influenced by training. Transforming growth factor β (TGFβ) signalling influences a variety of extracellular processes by precipitating the association of receptor kinases on the cell surface. Typically associated with muscle growth inhibition, TGFβ-2 is over-expressed in damaged skeletal muscle (Murakami et al 1999). Higher A2M (a negative regulator of TGF-β2) combined with reduced TGFβ2 expression and a typical (for this family) compensatory increase in receptor expression (TGFβR2) suggests that withdrawal of TGF-β2 signalling is important for muscle adaptation to exercise. In addition, THBS4, a member of a gene family that regulate TGF-β signalling appears related to increased aerobic fitness. A loss of function polymorphism in THBS4 is associated with coronary events in humans (Stenina et al 2004) and our data indicates that aerobic fitness may be a potential explanatory link. In the present study, suppression of the TGFβ2 system appeared essential for gains in aerobic fitness.

	HRG		LRG		
Gene	**Fold Δ**	**P-value**	**Fold Δ**	**P-value**	**Ratio**
COL3A1	9.2±1.2	0.0002	0.9±0.3	0.8	**P=0.00004**
A2M	3.4±0.6	0.004	1.6±0.4	0.2	**P=0.02**
THBS4	9.2±1.2	0.0002	4.3±1.5	0.1	**P=0.02**
TGFÎ²2	0.4±0.2	0.009	1.6±0.4	0.2	**P=0.02**
TGFÎ²R2	3.9±0.9	0.02	1.3±0.2	0.2	**P=0.02**

Murakami, N. I.S. McLeenan, I Nonaka et al. (1999) Muscle & Nerve 22, 889-898.

Stenina OI, TV Byzova, JC Adams et al. (2004) Int J Biochem Cell Biol. 36:1013-30.

Gustafsson T, A. Puntschart, L. Kaijser, et al. (1999) Am J Physiol. 276:679-85, 1999.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C8

The angiopoietin system is transcriptionally regulated by endurance exercise in humans

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The molecular regulation of human skeletal muscle angiogenesis has not been fully described. Despite this lack of knowledge many gene therapy trials have been initiated, many with disappointing results (Rajagopalan et al 2003). Ang1, the endothelial cell Tie2 agonist, is thought to facilitate the maturation of vascular endothelial growth factor (VEGF) stimulated collateral vessel growth through regulation of smooth muscle cell recruitment and by acting as a chemo-attractant factor, directing endothelial cell orientation (Nishishita et al 2004). The physiological regulation of this system, in human muscle tissue has not been previously examined. 24 male subjects undertook supervised aerobic training; cycling at 75% of peak VO2 (4 times per week, 6 weeks). The study was approved by the Institute ethics committee. Peak aerobic capacity, submaximal heart rate response (@75% of peak VO2) and 15min exercise performance (KJ) were quantified. Subjects were ranked on the basis of the sum of their % improvements across these 3 variables. Muscle gene expression was studied in the top 8 (HRG, 24±1yr, 183±3cm, 77±6kg, Baseline VO2peak =3.5±0.3 l/min) and compared with the 8 lowest ranked subjects (LRG, 23±1yr, 180±3cm, 77±3kg, Baseline VO2peak =3.7±0.1 l/min). Cumulative increase in aerobic fitness index was 10±2% in the LRG and 55±4% in the HRG (P<0.0001).

Changes in hypoxia inducible factor 1α (HIF-1α) gene expression, the master regulator of hypoxia responsive genes, did not relate to the increase in aerobic fitness. Ang1 and Tie2 expression were upregulated in the high responder group, indicating that activation of the angiopoietins system, 24 hours following exercise, may be essential for a functional angiogenesis response, following the earlier increase in VEGF expression (Gustafsson et al 1999). The lack of difference in Ang2 between groups, suggests that the vessel stabilizing/maturing role of ANG1 is a more important hallmark of successful adaptation to exercise. In conclusion, pro-angiogenesis processes may determine gains in aerobic fitness in humans following aerobic exercise.

	HRG		LRG		
Gene	Fold Δ	P-value	Fold Δ	P-value	Ratio
HIF-1α	1.7±0.3	0.04	1.7±0.3	0.07	P=0.9
Tie1	3.5±1.6	0.005	1.8±1.0	0.04	P=0.05
Tie2	3.2±1.8	0.01	1.3±0.8	0.3	P=0.02
ANG1	2.7±1.5	0.02	1.0±0.5	0.9	P=0.009
ANG2	2.0±1.0	0.02	2.7±2.5	0.1	P=0.5

Data was generated using TaqMan Real Time PCR (Gustafsson et al 1999).

Values represent mean ± SEM. P value generated using un-paired t-test.

Ratio P values reflect the comparison between the fold change observed in the HRG versus the LRG using un-paired t-test.

Rajagopalan S, Mohler ER 3rd, Lederman RJ, et al. (2003). Circulation 108:1933-8.

Nishishita, T and PC Lin. (2004). J Cellular Biochemistry 91:584-593.

Gustafsson T, A. Puntschart, L. Kaijser, E. Jansson and CJ Sundberg. (1999). Am J Physiol. 276:H679-85, 1999.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C9

No effect of treadmill running on human plasma ghrelin concentrations

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Ghrelin is a recently discovered hormone that is secreted by the stomach (Kojima *et al.* 1999). Ghrelin concentrations rise just before meals and decrease rapidly after meals suggesting that ghrelin is involved in the regulation of appetite (Cummings *et al.* 2002). This is supported by the fact that infusion of ghrelin leads to a short-term increase in hunger in humans (Wren *et al.* 2001). Moreover, ghrelin concentrations are elevated throughout the day following diet-induced weight loss indicating an attempt by the body to regain lost weight (Cummings *et al.* 2002). Little is known regarding the effects of exercise on plasma ghrelin. We hypothesised that an intense bout of exercise would lead to a short-term suppression of appetite, as has been shown previously (Katch *et al.* 1979), and that this effect would be mediated by suppressed concentrations of plasma ghrelin.

Eighteen volunteers (9 men and 9 women) participated in this study which was approved by Loughborough University's Ethical Advisory Committee. The age, body mass index and maximal oxygen uptake (VO_2max) of the participants (mean ± S.E.) were: 24.8 ± 0.9 y, 22.9 ± 0.6 kg m^{-2} and 57.7 ± 2.2 ml kg^{-1} min^{-1}. Participants completed two, 3-h trials (exercise and control) on separate days in a randomised balanced design. The exercise trial involved a 1-h treadmill run at 75% of VO_2max followed by 2 h of rest. The control trial involved 3 h of rest. Both trials were completed in the morning following an overnight fast. No food was consumed during the trials. Venous blood samples were drawn via a cannula at 0, 0.5, 1, 1.5, 2 and 3 h. Plasma from these samples was frozen at -80°C. Total ghrelin concentration was determined from plasma samples using an enzyme immunoassay (Phoenix Pharmaceuticals). Appetite was assessed following each blood sample using a 15-point 'hunger scale'. Data were analysed via two-factor (time × trial) repeated measures ANOVAs using SPSS version 11.0 for Windows. Significance was set at $P<0.05$.

Hunger scores were significantly lower in the exercise trial compared to the control trial (mean of six values: 11 ± 1 versus 14 ± 1 respectively). However, venous plasma ghrelin concentrations did not differ significantly between trials or over time (see Figure 1). These findings indicate that an acute bout of intense treadmill running suppresses appetite but this effect does not appear to be mediated by plasma ghrelin concentrations.

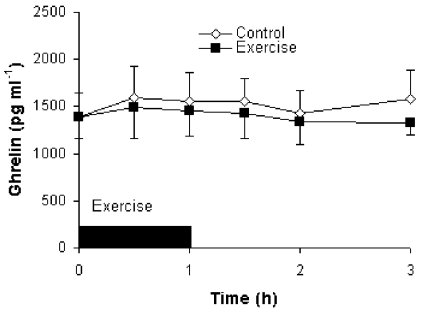

Figure 1

Cummings, D.E. *et al.* (2002) *N Eng J Med* **346**, 1623-1630.
Katch, V.L. *et al.* (1979) *Am J Clin Nutr* **32**, 1401-1407.
Kojima, M. *et al.* (1999) *Nature* **402**, 656-660.
Wren, A.M. *et al.* (2001) *J Clin Endocrinol Metab* **86**, 5992-5995.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C10

Dose-response relationship during hyperaminoacidaemia between insulin and leg protein turnover in healthy young men studied by tracer amino acid exchange

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Insulin is anabolic in human skeletal muscle but our knowledge of the dose-response relationships between its availability and protein turnover is poor. We set out to remedy this in studies of 8 healthy men (20.4 ±1.2 y, BMI 23.8 ± 2.6 kg/m2) in whom we measured leg amino acid balance, and rates of appearance and disappearance of D5-phenylalanine (Phe) from arterio-venous concentrations and enrichments of Phe, as indices of leg protein breakdown and synthesis. We made measurements before and during a hyperinsulinaemic-euglycaemic clamp (octreotide (30 ng/kg/min), glucagon (15 ng /kg/h) and 20 % glucose) during mixed amino acid infusion (18 g/h, Glamin?). Leg protein metabolism was measured in the post-absorptive (PA) state and during 3 h of infusion of insulin aimed to achieve plasma concentrations of ~5, ~30, ~80 and ~180 mU/l.

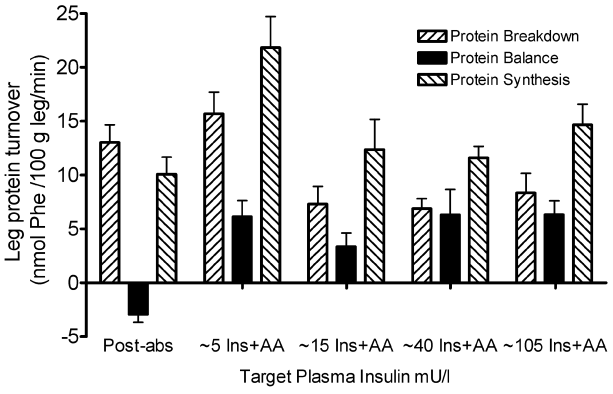

Figure 1. Leg (muscle) protein turnover with increased insulin availability; values are means ± SD. PA = post absorptive.

In the PA state, leg protein balance was negative, with protein breakdown exceeding synthesis. Infusion of amino acids during insulin availability at a fasting level (~5 mU/l) doubled protein synthesis ($P<0.001$) with no effect on breakdown, so protein bal-

ance became positive. However a modest increase in insulin availability (to a target value of ~15 mU/l) suppressed protein breakdown by ~50% ($P<0.001$). Thereafter, further increases oin insulin to concentrations higher than those seen post-prandially (i.e. ~ 105 mU/l) caused no further increase in protein balance, or decreases in breakdown. Leg protein synthesis tended to be lower at all insulin concentrations above the fasting value, possibly due to suppression of protein breakdown limiting intracellular availability of amino acids despite the exogenous amino acid supply. The results suggest (i) that no rise in insulin availability is necessary for amino acids to stimulate leg (probably muscle) protein synthesis, and (ii) that the extent of the insulin suppression of leg (muscle) protein breakdown is almost maximal with modest rises of insulin availability - less than seen during normal feeding. Apparently, switching from a net catabolic to a net anabolic state (as on feeding) may be achieved by amino acid-stimulated increases of protein synthesis alone, without any increase in insulin availability. However small increases in insulin, while not further stimulating protein synthesis, markedly suppress protein breakdown to an extent not increased by further increases in insulin availability.

Supported by BBSRC and Iovate Health Sciences Research Inc.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA1

Differential responses of protein turnover in human muscle fibre types and sex differences in the response to exercise.

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1) Investigations of human protein metabolism rely on the assumption that all muscles in the body are equivalent in terms of protein turnover. However, it is known that in animals the rate of protein synthesis is higher in red, slow-twitch, oxidative muscles than white, fast-twitch, glycolytic muscles. We now have evidence that neither anatomical location nor fibre-type composition are major determinants of the rate of human muscle protein synthesis. Data will be presented that demonstrate that the basal (overnight fasted) and amino acid stimulated rates of myofibrillar and sarcoplasmic protein synthesis are very similar in human muscles of vastly different fibre-type composition (triceps, vastus, soleus); although statistically significant ($P<0.05$), the differences in protein synthesis rates between muscles are not big enough to be considered biologically significant: triceps protein synthesis rate is ~15% and vastus protein synthesis rate ~7% smaller than that of soleus. Therefore, (i) extrapolating the findings from one muscle to whole-body skeletal muscle is valid, at least during fasted and fed conditions, (ii) differences in fibre-type composition will not substantially confound the data obtained from studies in subjects with distinct muscle fibre-type composition (e.g., sedentary persons and athletes; the young and elderly; men and women), and (iii) rodents are poor models for investigations of the effects of interventions and conditions that affect human muscle protein turnover.

2) The investigation of human muscle protein metabolism has been limited largely to measurements made in men; hence, little is known about sexual dimorphism in the regulation of muscle protein kinetics. However, it is well known that men have more muscle mass than women, and there is evidence that resistance exercise training leads to a greater increase in muscle mass in men than in women, probably because of a more pronounced stimulation of muscle protein synthesis to either exercise alone or exercise plus nutrition. The proposed differential responses of muscle protein metabolism could be due to sex per se (i.e., male versus female genotype) or otherwise sex-related differences such as the hormonal milieu and body-composition and their possible interaction with exercise. Evidence for sexual dimorphism in the response to exercise/nutrition available so far and the importance of various factors associated with sexual dimorphism will be presented.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.