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Skeletal muscle regeneration following catecholamine-induced injury

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Many chronic heart failure (CHF) patients experience skeletal muscle wasting which has been shown to be associated in part with the elevated catecholamines (Anker *et al.* 1997). We have previously observed both myocyte necrosis and apoptosis in the heart and soleus muscle of the rat following a single injection of the synthetic catecholamine, isoprenaline (Ellison *et al.* 2002). This raises the possibility of catecholamine involvement in the progressive functional deterioration of the skeletal and cardiac musculature in CHF. Here we describe the regeneration of the soleus muscle, following damage inflicted by a single injection of isoprenaline.

Seven independent groups of male Wistar rats (*Rattus norvegicus*) (250 ± 7 g) (mean \pm S.E.M.) received 5 mg of isoprenaline kg^{-1} (s.c.). All experimental procedures conformed to the UK Animals (Scientific procedures) Act 1986. Animals were killed by cervical dislocation at seven time points over a 28-day period and the soleus muscles were quickly isolated and snap frozen. Regenerating fibres were detected using an anti-embryonic/neonatal myosin heavy chain 1°Ab (Novacastra) on 5 mm cryo-sections. The 1°Ab was detected and visualised using a HRP-conjugated rabbit anti-mouse 2°Ab (Dako) and DAB. Image analysis was used to quantify the number of normal and regenerating fibres in the whole cross section of each soleus muscle. Three \times one-way ANOVA were employed to analyse the data for total fibre number, normal and regenerating fibres, and Tukeys *post hoc* analysis to locate the differences.

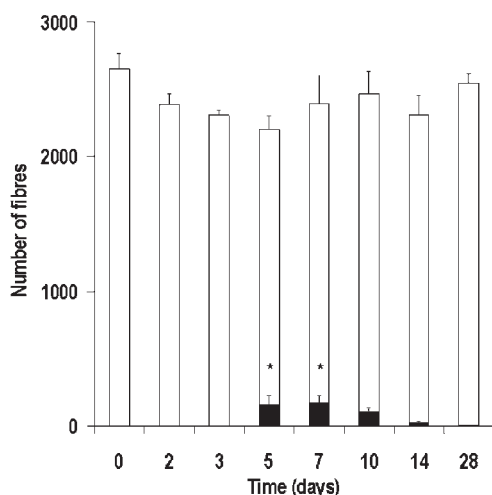


Figure 1. Changes in total fibre number in whole soleus. Number of normal (open column) and regenerating (filled column) fibres over a 28 day period, following a single 5 mg injection of isoprenaline per kilogram ($n = 3-5$). All data are means \pm S.E.M. *Significant differences ($P < 0.05$) between the number of regenerating fibres at 5 and 7 days *versus* 28 days.

No changes in total fibre number were observed in control animals over the 28-day experimental period. In contrast, 5 days after isoprenaline administration fibre number was reduced to 2196 ± 145 (17% loss) (Fig. 1). By 28 days this had returned to 2544 ± 69 . A significant ($P < 0.05$) increase in the number of

regenerating fibres was observed at 5 days ($6 \pm 2.6\%$) and 7 days ($8 \pm 2.6\%$).

These data show that severely injured muscle fibres have the ability to regenerate, with complete restoration of fibres by 28 days following a single injection of isoprenaline. Whether, this process of regeneration is impaired by sustained high levels of catecholamines, as seen in CHF, remains to be ascertained.

Anker SD *et al.* (1997). *Circulation* **96**, 526–534.Ellison G *et al.* (2002). *Journal of Physiology* **543.P**, 100P.

This research was supported by the British Heart Foundation.

All procedures accord with current UK legislation.

O92

Apoptotic and necrotic myocyte death in the heart and soleus muscle of the rat *in vivo*: evidence of secondary necrosis

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We have previously shown that the administration of the β_2 -adrenergic receptor agonist, clenbuterol, induces both apoptotic (Burniston *et al.* 2002b) and necrotic (Burniston *et al.* 2002a) cell death in the heart and soleus muscle of the rat. Here we demonstrate that these two death pathways occur sequentially with a substantial amount of co-localisation.

Male Wistar rats (*Rattus norvegicus*) (287 ± 20 g) were administered (s.c.) 5 mg kg^{-1} of clenbuterol (experimental groups) or the saline vehicle only (controls). Before being humanely killed at specific time points (2–24 h), hearts and soleus muscles were rapidly excised, snap frozen and serial cryosections (5 μm) cut. Apoptosis was detected using an anti-caspase 3 antibody (Ab; R&D systems). For the detection of necrotic myocytes, all animals received an injection (i.p.) of an anti-myosin Ab 1 h prior to the clenbuterol challenge. This Ab is too large to be admitted through the membrane of viable myocytes, but can enter and bind to the sarcomeric myosin of necrotic myocytes *in vivo*. Primary Ab binding was then amplified using secondary immunoperoxidase techniques and visualised with Nova Red (Vector Laboratories). Cell death was quantified in the subendocardial region of the heart and mid-belly of each soleus using image analysis. Results are expressed as percentage area, or percentage number of damaged fibres for the heart and soleus, respectively.

No cell death was found in the hearts or solei of animals receiving only the saline vehicle (zero time point). In contrast, administration of clenbuterol induced both apoptotic and necrotic cell death in both striated muscles (Fig. 1). Apoptosis was first observed after 2 h, and necrotic myocyte death at 4 h following clenbuterol administration. The clearance rate of apoptotic cell death also occurred earlier than that of necrosis with no apoptosis evident in either tissue after 24 h.

The sequential nature of the myocyte apoptosis and necrosis suggests that some of the necrotic cells may have been apoptotic cells which have undergone secondary necrosis. This was confirmed by double immunofluorescence labelling, which showed co-localisation of apoptotic and necrotic cells in both the cardiac and soleus muscles.

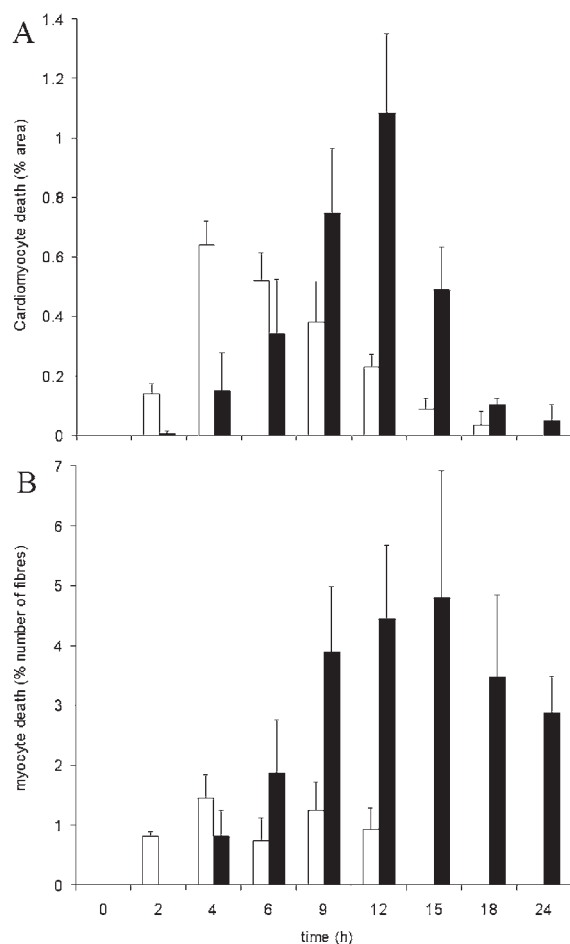


Figure 1. Time course of apoptosis (open columns) and necrosis (filled columns) in the heart (A) and soleus muscle (B) in response to a single injection (s.c.) of 5 mg kg⁻¹ clenbuterol. Data are presented as means \pm S.E.M., $n = 3$ in each independent group.

Burniston JG *et al.* (2002a). *J Appl Physiol* **93**, 1824–1832.

Burniston JG *et al.* (2002b). *J Physiol* **543.P**, 39P.

This work was supported by the British Heart Foundation

All procedures accord with current UK legislation.

O93

Stretch-induced contractile activation in resting intact neonatal rat muscle fibres requires a functionally excitable membrane

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In resting intact muscle fibres isolated from neonatal rats, a large triangular length change (amplitude $\sim 20\%$ of initial muscle length, L_0) has three specific effects. Firstly, it leads to a transitory increase in resting muscle tension (Mutungi & Ranatunga, 2001) and secondly to an increase in intracellular calcium levels $[Ca^{2+}]_i$ (Mutungi & Edman, 2002) both of which occur during the

stretching phase. The stretch–release cycle is then followed by a twitch-like tension response that is $\sim 30\text{--}50\%$ of the isometric twitch tension and is affected by 2,3-butanedione monoxime (BDM) and sarcomere length in the same manner as active twitch tension (Mutungi & Ranatunga, 2001) suggesting that it probably arises from actively cycling cross-bridges. The aim of the present experiments was to investigate the possible mechanisms underlying this stretch-induced increase in $[Ca^{2+}]_i$.

The experiments were performed at 20°C using small muscle fibre bundles ($\sim 10\text{--}15$ muscle fibres) isolated from neonatal rats (5–12 days old) killed using either carbon dioxide or sodium pentobarbitone (given intraperitoneally). Both slow (soleus) and fast (extensor digitorum longus, EDL) twitch muscles were used. The preparations were mounted horizontally between two stainless steel hooks, one attached to a servomotor and the other to a tension transducer in a muscle chamber with a glass bottom. After setting the initial length for optimum twitch tension, the preparations were subjected to rapid stretch–release cycles (amplitude $\sim 25\%$ L_0) in the presence and absence of 1–2 mM lidocaine and 25 mM potassium. The changes in muscle tension and $[Ca^{2+}]_i$ were then monitored. The levels of intracellular calcium were monitored using Fluo-3 AM following the procedure described by Caputo *et al.* (1994).

As the results in Fig. 1 show, the addition of lidocaine completely abolished both active twitch tension and the stretch-induced twitch-like tension response. Similar results were obtained with 25 mM potassium. Furthermore, both compounds also abolished the stretch-induced increase in $[Ca^{2+}]_i$ and their effects were completely reversible. These results suggest that a large stretch, in resting neonatal rat muscle fibres, induces a depolarisation wave that leads to $[Ca^{2+}]_i$ release by perturbing Na^+ channels.

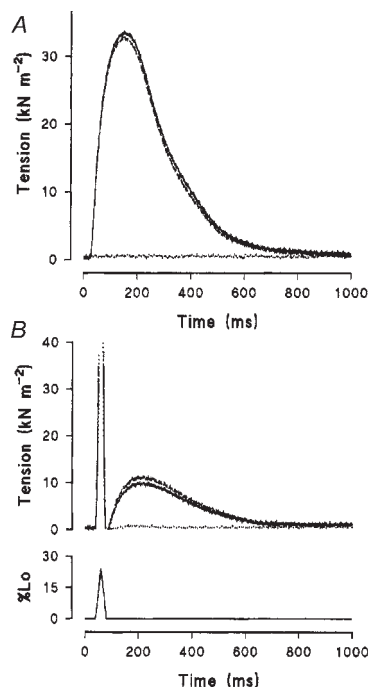


Figure 1. Effects of lidocaine on active twitch tension (A) and the stretch-induced twitch-like response (B) in an EDL (a fast) muscle fibre bundle isolated from a 6-day old rat. Note that the addition of 1.5 mM lidocaine reversibly abolishes both active twitch tension and the stretch-induced tension response. Similar results were obtained in 7 fast and 7 slow muscle fibre bundles.

Caputo C *et al.* (1994). *J Physiol* **478**, 137–148.

Mutungi G & Edman KAP (2002). *J Physiol* **543.P**, 103P.

Mutungi G & Ranatunga KW (2001). *J Physiol* 536.P, 88P.

This work was supported by grants from The Wellcome Trust (G.M.) and the Swedish Medical Research Council (K.A.P.E.).

All procedures accord with current UK legislation.

O95

Creatine supplementation for 5 days has no further effect in stimulating human muscle protein synthesis after resistance exercise

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Creatine dietary supplementation by training athletes is associated with increases in muscle mass but the mechanism is unknown. There is no effect of creatine supplementation on muscle protein turnover at rest in the post-absorptive or fed states (Louis *et al.* 2001). However it is possible that any anabolic effect of creatine is only seen after muscle contractile activity. To test this we studied the effects of creatine supplementation on muscle protein synthesis (MPS) in six healthy men (72.7 ± 2.3 kg, 177 ± 5.1 cm, 21.1 ± 1.6 years, means \pm s.d.) after strenuous exercise. The local ethics committees (Université Libre and Hôpital Erasme, Bruxelles) provided approval. We used a protocol which allowed measurements of MPS (as incorporation of $[1-^{13}\text{C}]$ leucine administered by a primed constant infusion at $1 \text{ mg kg}^{-1} \text{ h}^{-1}$) at rest then again 3 h after strenuous exercise. With the left leg, subjects performed 20 sets of 10 repetitions of leg extension-flexion on a Cybex at 80 % of 1-repetition maximum. Quadriceps muscle biopsies were taken using lignocaine (1 %) local anaesthesia in both the rest and exercise legs at the end of the exercise, and again 3 h later. From the end of exercise, subjects were fed orally, every 20 min (21 g maltodextrin + 6 g milk protein h^{-1}) for 3 h. The study was carried out twice, 2 weeks apart; the second study was immediately the subjects had ingested creatine at 20 g day^{-1} for 5 days. The incorporation of $[1-^{13}\text{C}]$ leucine into myofibrillar and sarcoplasmic protein was measured by standard methods using gas chromatography-combustion-mass spectrometry after protein separation and hydrolysis.

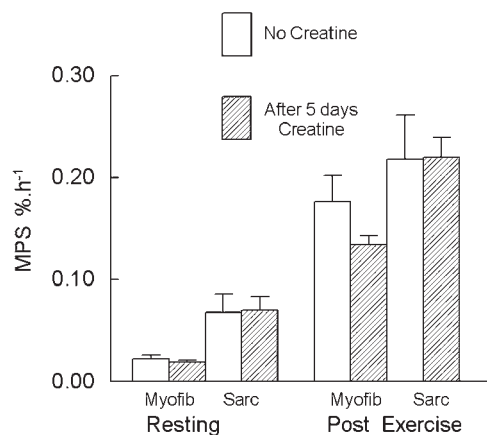


Figure 1. Effect of strenuous exercise on muscle protein synthesis before and after 5 days of creatine supplementation of the diet (values are means \pm s.d.).

The results (Fig. 1) confirm the lack of an effect of creatine on resting myofibrillar and sarcoplasmic protein synthesis. Strenuous exercise stimulated MPS (myofibrillar ~ 8 -fold and sarcoplasmic ~ 3 -fold, $P < 0.01$, ANOVA) but the stimulatory effects were no greater after the subjects had ingested creatine. Thus, any anabolic effect of creatine on muscle in training athletes is unlikely to be due to contraction-dependent effects.

Louis M *et al.* (2001). *J Physiol* 539.P, 92P.

This work was supported by MRC, The Wellcome Trust and The University of Dundee. M.L. is funded by the Fonds du patrimoine pour la recherche médicale, Université catholique de Louvain.

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

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Effect of acute exhaustive exercise and a 6-day period of intensified training on immune function in cyclists

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An increased incidence of upper respiratory tract infections is often reported by individuals undertaking prolonged strenuous exercise and by athletes during periods of heavy training (Gleeson, 2000; Mackinnon, 2000). Therefore, we examined the effects of acute exhaustive exercise performed during normal, intensified and recovery training periods on several aspects of immune function including neutrophil and monocyte oxidative burst, lymphocyte proliferation and T-cell cytokine production.

Following approval by the South Birmingham Local Research Ethics Committee, seven healthy endurance-trained men (age 30 ± 2 years, body mass 75 ± 3 kg, maximal oxygen uptake ($\dot{V}_{O_{2max}}$) $4.55 \pm 0.11 \text{ l min}^{-1}$; means \pm s.e.m.) completed three trials consisting of cycling exercise at a work rate equivalent to 74 % $\dot{V}_{O_{2max}}$ until volitional fatigue. The trials took place before and after a 6-day period of intensified training (IT) and after 2 weeks of light recovery training (RT). Normal training (NT) consisted of ~ 10 h of cycling per week; during IT the training load was increased on average by 73 %. During RT, exercise was limited to no more than 3 h per week for 2 weeks. Training intensity and duration were confirmed by the use of heart rate monitors. Venous blood samples were collected at rest, during and after the exercise trials. Following stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin, samples were stained for T-lymphocyte surface antigens (CD3), and intracellular interferon (IFN)- γ and analysed using flow cytometry. Neutrophil and monocyte oxidative burst activity (reactive oxygen species production) were assessed using *E. coli* (as stimulant) and flow cytometry. Lymphocyte proliferation was determined by $[^3\text{H}]$ thymidine incorporation using phytohemagglutinin as the mitogen and expressed as the stimulation index (stimulated to unstimulated ratio). A two-way (trial \times time) repeated measure ANOVA was used to compare means with *post hoc* Tukey or paired *t* tests as appropriate.

Time to exhaustion in the exercise trials was 107 ± 7 , 85 ± 5 and 100 ± 7 min during NT, IT and RT periods, respectively ($P < 0.01$, NT vs IT). Following acute exhaustive exercise on the NT trial the circulating number of IFN- γ + T-cells ($P < 0.05$),

and the amount of IFN- γ produced per T-cell ($P < 0.05$) was decreased. The circulating number of T-cells producing IFN- γ was lower at rest following the IT period compared with normal training ($P < 0.05$), though the amount of IFN- γ produced per T-cell was unchanged (Table 1). Neutrophil oxidative burst activity and lymphocyte proliferation both fell after acute exercise by about 30% ($P < 0.05$) and both neutrophil and monocyte oxidative burst were lower ($P < 0.05$) at rest after the IT period compared with NT (Table 1). All measured immune functions were back to normal after 2 weeks of RT.

Table 1. Resting immune function variables during normal, intensified and recovery training periods

	Normal training	Intensified training	Recovery training
Neutrophil oxidative burst†	767 \pm 87	612 \pm 115*	789 \pm 116
Monocyte oxidative burst†	178 \pm 18	136 \pm 24*	196 \pm 21
Lymphocyte proliferation	161 \pm 27	117 \pm 40*	172 \pm 23
IFN- γ + T-cells (10^9 cells l^{-1})	0.19 \pm 0.02	0.12 \pm 0.03*	0.20 \pm 0.03
IFN- γ production by T-cells†	197 \pm 36	239 \pm 62	184 \pm 26

Values are means \pm S.E.M. ($n = 7$). * $P < 0.05$ vs. normal training.
†Geometric mean fluorescence intensity in arbitrary units.

These results indicate that resting immune function is decreased after only 6 days of intensified training and these effects are reversible with two weeks of relative rest. In general the immune response to an acute bout of exhaustive exercise was not affected by the weekly training load.

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

Mackinnon LT (2000). *Immunol Cell Biol* 78, 502–509.

This work was supported by GlaxoSmithKline.

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

O97

Effect of exhaustive exercise and intensified training on human T-lymphocyte CD45RO expression

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A previous study suggested that the expression of an activation marker (CD45RO) on T-lymphocytes was significantly higher in athletes diagnosed as suffering from overtraining syndrome (Gabriel *et al.* 1998). The aim of the present study was to determine the effect of acute exhaustive exercise and an intensified training period on human lymphocyte CD45RO expression.

Following approval by the South Birmingham Local Research Ethics Committee, seven healthy endurance-trained men (age 30 ± 2 years, body mass 75 ± 3 kg, maximal oxygen uptake ($\dot{V}_{O_{2max}}$) 4.55 ± 0.11 $l \text{ min}^{-1}$; means \pm S.E.M.) completed three trials consisting of cycling exercise at a work rate equivalent to 74% $\dot{V}_{O_{2max}}$ until volitional fatigue. The trials took place before and after a 6-day period of intensified training (IT) and after 2 weeks of light recovery training (RT). Normal training (NT)

consisted of ~10 h of cycling per week; during IT the training load was increased on average by 73%. During RT, exercise was limited to no more than 3 h per week for 2 weeks. Training intensity and duration were confirmed by the use of heart rate monitors. Venous blood samples were collected at rest, during and after the exercise trials. Samples were stained for T-lymphocyte surface antigens (CD3, CD4, CD8, CD45RO and CD45RA) and analysed using flow cytometry. A two-way (trial \times time) repeated measures ANOVA was used to compare means with *post hoc* Tukey or paired *t* tests as appropriate.

Table 1. CD45RO expression on T-cells in response to acute exercise during normal training

	Rest	Post-exercise	1 h post-exercise
CD4+CD45RO+ (GMFI)	335 \pm 50	278 \pm 44*	314 \pm 57*
CD8+CD45RO+ (GMFI)	201 \pm 27	171 \pm 29	134 \pm 19

Values are means \pm S.E.M. ($n = 7$). * $P < 0.05$ vs. Rest. GMFI: geometric mean fluorescence intensity in arbitrary units.

Table 2. CD45RO expression on T-cells at rest during normal, intensified and recovery training periods.

	Normal training	Intensified training	Recovery training
CD4+CD45RO+ (GMFI)	335 \pm 50	254 \pm 46	305 \pm 41
CD8+CD45RO+ (GMFI)	201 \pm 27	164 \pm 47	176 \pm 26

Values are means \pm S.E.M. ($n = 7$). * $P < 0.05$ vs. normal training. GMFI: geometric mean fluorescence intensity in arbitrary units.

Time to exhaustion in the exercise trials was 107 ± 7 , 85 ± 5 and 100 ± 7 min during NT, IT and RT periods, respectively ($P < 0.01$ NT vs. IT). Following the acute exercise trial during NT the CD45RO expression fell significantly ($P < 0.05$) on CD4+ cells (Table 1). However, the circulating number of T-cells expressing CD45RO at rest (about 400 and 180 cells mm^{-3} for CD4+CD45RO+ and CD8+CD45RO+ cells, respectively) was not different in the NT, IT and RT periods and the CD45RO expression at rest tended to be lower during the IT period (Table 2).

These results indicate that T-cell CD45RO expression is not increased by acute exercise or short-term increases in the training load.

Gabriel HHW *et al.* (1998). *Med Sci Sports Exerc* 30, 1151–1157.

This work was supported by GlaxoSmithKline.

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

O98

Effect of feeding different amounts of carbohydrate during prolonged exercise on human T-lymphocyte intracellular cytokine production

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Prolonged strenuous exercise is immunosuppressive and this may account for the increased incidence of upper respiratory

tract infection following endurance events (Nieman, 1997). Regular consumption of a 6% (w/v) carbohydrate (CHO) beverage during prolonged exercise has been shown to attenuate the rise in stress hormones and the fall in lymphocyte proliferation (Nieman & Pedersen, 2000) compared with ingestion of the same volume of a placebo drink. Exercise may induce temporary immunosuppression via an inhibition of T-lymphocyte intracellular cytokine production (Starkie *et al.* 2001). However, it is not clear if CHO ingestion can prevent the fall in T-cell interferon (IFN)- γ and interleukin (IL)-2 production during prolonged exercise. Therefore, we examined the effects of feeding beverages containing different amounts of CHO (glucose and glucose polymers) during prolonged cycling on T-cell intracellular cytokine production.

Following approval by the South Birmingham Local Research Ethics Committee, seven healthy endurance-trained men (age 25 ± 1 years, body mass 77 ± 1 kg, maximal oxygen uptake ($\dot{V}_{O_{2max}}$) 4.56 ± 0.06 l min⁻¹; means \pm S.E.M.) completed three trials consisting of cycling exercise at a work rate equivalent to 65% $\dot{V}_{O_{2max}}$ for 2.5 h in temperate ambient conditions ($\sim 25^\circ\text{C}$, $\sim 50\%$ relative humidity). In random order, subjects consumed beverages containing 0, 6.4 or 12.8% w/v CHO. The volume of drinks consumed was 500 ml just before exercise and 200 ml every 20 min during exercise. The trials took place in the morning after an overnight fast and were spaced 1–2 weeks apart. Venous blood samples were collected at pre-exercise, immediately post-exercise and at 2 h post-exercise. Following stimulation with phorbol 12-myristate 13-acetate and ionomycin, samples were stained for T-lymphocyte surface antigens (CD3, CD4 and CD8), and intracellular IFN- γ , IL-2 and IL-4 and analysed using flow cytometry. A two-way (trial \times time) repeated measures ANOVA was used to compare means with *post hoc* Tukey or paired *t* tests as appropriate.

At 2 h post-exercise on the 0% CHO (placebo) trial the number of circulating IFN- γ +T-(CD3+) cells was significantly lower than at pre-exercise (0.15 ± 0.03 vs. $0.29 \pm 0.04 \times 10^9$ cells l⁻¹, respectively; $P < 0.05$) and the amounts of IFN- γ and IL-2 (but not IL-4) produced per T-cell (both CD4+ and CD8+ cells) were decreased ($P < 0.05$) compared with rest (Table 1). With CHO supplementation (both 6.4 and 12.8% CHO drinks) the number of circulating IFN- γ +T-cells, and the amount of IFN- γ , IL-2 and IL-4 produced per T-cell were unchanged from rest (Table 1).

Table 1. Intracellular cytokine production in CD4+ and CD8+ T-cells at 2 h post-exercise expressed as the percentage of the pre-exercise value

	0% CHO (placebo)	6.4% CHO	12.8% CHO
CD4+/IFN- γ	68 ± 8^a	82 ± 4	101 ± 7^b
CD8+/IFN- γ	65 ± 7^a	90 ± 4^b	98 ± 9
CD4+/IL-2	73 ± 7^a	83 ± 5	110 ± 9^b
CD8+/IL-2	82 ± 8	84 ± 2^a	109 ± 7^b
CD4+/IL-4	92 ± 3	100 ± 3	96 ± 2
CD8+/IL-4	94 ± 9	110 ± 8	102 ± 5

Values are means \pm S.E.M. ($n = 7$). $P < 0.05$ vs. ^apre-exercise, ^bplacebo.

These results indicate that the regular ingestion of 6.4% or 12.8% CHO drinks during prolonged exercise prevents the fall in T-cell IFN- γ and IL-2 production and that this may be the mechanism for the previously observed attenuation of exercise-induced immunosuppression by CHO supplementation.

Nieman DC (1997). *J Appl Physiol* **82**, 1385–1394.

Nieman DC & Pedersen BK (2000). *Nutrition and Exercise Immunology*. CRC Press, Boca Raton, FL, USA.

Starkie RL *et al.* (2001). *Am J Physiol Cell Physiol* **281**, C1233–1240.

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

P131

Effects of KB-R7943 on sodium-withdrawal contractures in rat slow-twitch skeletal muscle

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In skeletal muscle the evidence supporting the functional role of Na⁺/Ca²⁺ exchange is based on results obtained when the extracellular medium was switched to a low-Na⁺ solution. Under these conditions the exchanger operates in its reverse mode transporting Ca²⁺ into the cell. Recently KB-R7943 (KBR) has been reported to inhibit in cardiac cells the reverse mode of Na⁺/Ca²⁺ exchange with a lower potency for the forward mode and other ion transport systems (Iwamoto *et al.* 1996). The aim of the present study was to test the effect of this novel agent on sodium-withdrawal contractures generated in slow skeletal muscle.

Experiments were conducted on small bundles (2–4 cells) isolated from soleus muscle and mounted in the experimental chamber already described by Joumaa *et al.* (2002). Adult male Wistar rats were anaesthetised by an ether vapour flow and then killed by cervical dislocation. Experiments were performed in intact fibres in the presence of 2 μM cyclopiazonic acid (CPA), which was shown to improve low-Na⁺ contracture (Même *et al.* 1997).

In intact fibres, exposure to Na⁺-free medium induced repeatable transient contracture with an amplitude of 107.3 ± 4.4 mN, a time-to-peak tension of 7.9 ± 0.4 s and a time constant of relaxation of 13.1 ± 1.5 s (means \pm S.E.M., $n = 10$). KBR induced a dose-dependent decrease in the amplitude of Na⁺-free contracture and up to 50 μM the effects were fully reversible. For each experiment the log dose–response curve for inhibition was fitted according to a sigmoid equation. The apparent K_i and the Hill coefficient were 11.8 ± 1.9 μM and 1.7 ± 0.4 , respectively (means \pm S.E.M., $n = 10$).

In saponin-skinned fibres, the amount of Ca²⁺ taken up at 1 min of loading time in pCa 7.0 solution was estimated by using the amplitude of the contracture due to caffeine application (10 mM). In the presence of KBR there was no significant change in the reduction in amplitude of caffeine contracture by CPA (control: 0.74 ± 0.05 mN; CPA: 0.59 ± 0.03 mN; KBR (20 μM): 0.57 ± 0.04 mN, $n = 12$, $P < 0.05$, Student's paired *t* test).

In Triton X-100 fibres, the Ca²⁺ sensitivity of contractile proteins (pCa₅₀) was not significantly modified in the presence of 2 μM of CPA and/or KBR (5–20 μM) (control: 6.312 ± 0.009 ; CPA: 6.294 ± 0.015 ; KBR (20 μM): 6.304 ± 0.008 , $n = 9$, $P > 0.05$).

It is proposed that KBR could be useful to study under normal physiological conditions the role of the exchanger in the excitation–contraction coupling of mammalian skeletal muscles.

Iwamoto T *et al.* (1996). *J Biol Chem* **273**, 22391–22397.

Joumaa WH *et al.* (2002). *J Pharmacol Exp Ther* **300**, 638–646.

Même W *et al.* (1997). *J Physiol* **504.P**, 232–233P.

All procedures accord with current National and local guidelines.

P132

Force enhancement during lengthening in mammalian (rat) muscle fibres: the effect of temperature

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It is well known that the force produced during lengthening of an activated muscle is greater than the maximal isometric force, but there is less agreement as to the magnitude of this force enhancement (see Woledge *et al.* 1985). In experiments on isolated frog muscle, the force produced during lengthening has been shown to be up to twice the isometric force (Katz, 1939). During *in vivo* experiments on human muscle, however, the lengthening force shows only modest, if any, increase above the isometric force (Pinniger *et al.* 2000). While muscle temperature is known to influence the contractile process (Ranatunga, 1984), there has been no systematic investigation of the influence of temperature on lengthening force enhancement in isolated mammalian muscle. This was the purpose of this study.

Adult male rats were humanely killed with an intra-peritoneal injection ($> 200 \text{ mg kg}^{-1}$) of an overdose of Sodium pentobarbitone (Euthatal). Small bundles of about five intact muscle fibres, dissected from the flexor hallucis brevis muscle, were mounted horizontally between a force transducer and a servomotor at an initial sarcomere length of $2.4\text{--}2.6 \mu\text{m}$. The fibre bundle was stimulated to maximal tetanic tension and a ramp stretch of $5\% L_0$ (muscle fibre length) was applied on the tension plateau. Ramp stretch velocities between 0.05 and $4.0 L_0 \text{ s}^{-1}$ were used and the force enhancement examined at 5°C increments between 10 to 30°C .

Force responses during constant velocity lengthening were qualitatively similar to those from frog muscle fibres (Lombardi & Piazzesi, 1990). Force responses were analysed for peak force at the end of stretch (P_k), the steady force 500 ms after the stretch (P_s) and the rate of force decay after stretch. To quantify the steady force during lengthening (P_2), P_s was subtracted from P_k and the resultant was normalized to the tetanic force immediately prior to the stretch (P_0).

A small steady force after stretch (P_3) was present in all records and was independent of stretch velocity. When corrected for P_3 , the enhancement of steady force during lengthening (P_2) increased with stretch velocity up to an 'approximate' plateau (above $\sim 0.5 L_0 \text{ s}^{-1}$). In preliminary experiments ($n = 6$), the relative force enhancement decreased with increasing temperature. As a function of isometric force (P_0) the mean ($\pm \text{S.D.}$) lengthening force (at $\sim 4.0 L_0 \text{ s}^{-1}$) was $2.22 (\pm 0.14)$ at 10°C , $1.78 (\pm 0.07)$ at 20°C and $1.59 (\pm 0.02)$ at 30°C . This decrease is probably due to a marked increase in isometric force (approximately 2-fold) in warming from 10 to 30°C . The temperature-dependent modulation of force transients may account, in part, for the differences in the force-lengthening velocity relationship reported for human and frog muscle.

Katz B (1939). *J Physiol* **96**, 45–64.Lombardi V & Piazzesi G (1990). *J Physiol* **431**, 141–171.Pinniger GJ *et al.* (2000). *Eur J Appl Physiol* **81**, 375–383.Ranatunga KW (1984). *J Physiol* **351**, 517–529.Woledge RC *et al.* (1985). *Monographs of the Physiological Society* **41**.

We thank the Wellcome Trust for financial support.

All procedures accord with current UK legislation.

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Twitch tension potentiation induced by rapid laser temperature jumps in intact mammalian (rat) muscle

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A rapid temperature jump (T-jump) induces a biphasic tension rise to a new steady level in maximally Ca^{2+} -activated skinned fibres (Ranatunga, 1996), and in intact fibres during tetanic contraction (Ranatunga & Coupland, 2002). The aim of this study was to examine the effect of rapid T-jumps during twitch contraction in intact fibre bundles from a mammalian muscle.

The foot muscle, flexor hallucis brevis, was isolated from rats killed with an intraperitoneal injection of an overdose of sodium pentobarbitone (Euthatal, $>200 \text{ mg (kg body mass)}^{-1}$). Small bundles of $\sim 5\text{--}10$ fibres ($\sim 2 \text{ mm}$ fibre length) were set up for isometric tension recording in a $50 \mu\text{l}$ trough containing oxygenated Ringer solution. A rapid temperature jump of $\sim 4^\circ\text{C}$ (see Ranatunga, 1996) was induced in the aqueous medium and the fibre bundle by applying an infrared laser pulse ($\lambda = 1.32 \mu\text{m}$, 0.2 ms duration) at different times during twitch contraction.

Preliminary data (means $\pm \text{S.E.M.}$) obtained at a steady temperature of 10°C showed that the effect of a T-jump was dependent on when it was applied relative to the twitch time course. Applied during the rising phase, a T-jump led to a transient increase of force ($31 \pm 2\%$, $n = 6$) compared to the steady temperature control and the rate of tension relaxation was increased. The force increase was less marked ($\sim 4\%$) when a T-jump was placed near the peak of the twitch. Little if any tension change was induced by T-jumps on the relaxation phase. Similar observations were made in three experiments at 25°C , but the force increase induced by the T-jump was less marked. Interestingly, there was a transient increase ($16 \pm 1\%$, $n = 6$) of force induced by a T-jump at 10°C compared to control twitches at the elevated temperature.

Our present observations indicate that an early step in excitation-contraction coupling is perturbed by a T-jump. It is of interest to note that previous studies showed that twitch tension is also potentiated by high pressure (Vawda *et al.* 1997) and by stretch (Mutungi & Ranatunga, 2001).

Mutungi G & Ranatunga KW (2001). *J Muscle Res Cell Motil* **22**, 175–184.Ranatunga KW (1996). *Biophys J* **71**, 1905–1913.Ranatunga KW & Coupland ME, (2002). *Biophys J* **82**, 367a.Vawda F *et al.* (1997). *J Muscle Res Cell Motil* **17**, 631–636.

We thank the Wellcome Trust for financial support.

All procedures accord with current UK legislation.

P134

Changes to contractile properties, sarcoplasmic reticulum loading and MHC isoform profile in single fibres from mammalian fast- and slow-type skeletal muscle following denervation

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Denervation enables the study of skeletal muscle properties in the absence of neural electrical input or the influence of nerve-derived trophic factors. The pattern of stimulatory electrical activity is of major importance in determining overall muscle properties in fast- and slow-type skeletal muscles, many contractile and biochemical properties of which revert to an intermediate state when neural influence is denied. One problem with studying denervated skeletal muscle is that experiments are usually carried out on bundles of intact fibres or on whole muscle preparations, meaning that events at the single fibre level can only be inferred. Here we have tracked changes in contractile properties, sarcoplasmic reticulum (SR) function and myosin heavy chain (MHC) isoform composition of mechanically skinned single muscle fibres taken from rat soleus and EDL muscles denervated for 0, 7, 21 or 50 days.

All experiments were carried out with approval from the Animal Ethics Committee of La Trobe University. Male Long Evans rats were anaesthetised with gaseous halothane, unilaterally denervated under aseptic conditions and allowed to recover. Untreated age-matched animals were used as controls. Animals were killed humanely by halothane overdose, and EDL and soleus muscles removed and placed under paraffin oil for single fibre isolation and mechanical skinning. Measurement of contractile activation parameters (pCa_{50} , n_H , pSr_{50} , etc.) was performed using previously described solutions and techniques (Fink *et al.* 1986). SR loading was determined as the ratio of Ca^{2+} released after a 2-min load in a solution of pCa 6.2 (0.5 mM EGTA) to that in a solution of pCa 7.10. MHC profiles of single muscle fibres and muscle homogenates were determined as previously reported (Bortolotto *et al.* 1999).

Results showed a significant ($P < 0.05$; Student's unpaired *t* test) decrease compared to control in the SR loading ratio of EDL fibres following denervation, and an increase in that of soleus fibres such that the two became similar in 50-day denervated fibres. Compared to control, pCa_{50} – pSr_{50} values for soleus fibres were more affected by denervation than were EDL fibres, with overall changes in soleus fibres towards fast-type fibre properties clearly apparent. The results can be explained in part by the increased number of hybrid muscle fibres after denervation.

Bortolotto SK *et al.* (1999). *Am J Physiol* **276**, C628–637.

Fink RH *et al.* (1986). *J Physiol* **373**, 513–525.

All procedures accord with current local guidelines.

P135

Effects of Mg^{2+} on halothane induced Ca^{2+} release in skeletal muscle from humans susceptible to malignant hyperthermia

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The inhibitory effect of Mg^{2+} on halothane induced Ca^{2+} release from the SR was examined in mechanically skinned malignant hyperthermia susceptible (MHS) and non-susceptible (MHN) fibres from human vastus medialis.

Muscle was obtained by open biopsy from patients attending for investigation of MH susceptibility at St James's Hospital, Leeds, UK. This was done with institutional Research Ethics Committee approval and informed patient consent. All procedures were carried out according to the Declaration of Helsinki. Approximately 1 g of the muscle used in the *in vitro* contracture test (IVCT), which was performed according to the criteria for malignant hyperthermia (MH) research of the European MH Group (The European Malignant Hyperpyrexia Group, 1984). The IVCT was used as the primary method of categorising MHN and MHS tissue.

Single mechanically skinned fibres were prepared from the remaining 0.2 g of tissue. Preparations were perfused with solutions mimicking the intracellular milieu and changes in $[Ca^{2+}]$ were detected using fura-2 fluorescence. Caffeine (40 mM) was briefly applied at 4 min intervals, at a free $[Mg^{2+}]$ of 1 mM. Each application of caffeine resulted in a transient increase in $[Ca^{2+}]$ within the preparation due to Ca^{2+} release from the sarcoplasmic reticulum (SR). After four to five reproducible caffeine responses were obtained, the preparation was perfused for a further 4 min to allow the SR to re-accumulate Ca^{2+} . The solution within the bath was then exchanged for one containing 1, 0.4, 0.2 or 0.1 mM Mg^{2+} and perfusion stopped to restrict the volume of solution surrounding the preparation to approximately 6 μ l. After a further minute at the selected $[Mg^{2+}]$, 1 mM halothane was rapidly introduced. In MHN muscle, 1 mM halothane never caused a detectable Ca^{2+} release at 1 mM Mg^{2+} . However, halothane-induced Ca^{2+} release did occur when the $[Mg^{2+}]$ was lowered to a threshold level of 0.4 mM in two patients, 0.2 mM in 10 patients and 0.1 mM in one patient. In MHS muscle, halothane-induced Ca^{2+} release was generally apparent following a smaller reduction in Mg^{2+} . Of eight MHS individuals tested, 1 mM halothane induced Ca^{2+} release at 1 mM Mg^{2+} in four patients, 0.4 mM Mg^{2+} in three patients and 0.2 mM Mg^{2+} in one patient.

These results suggest that (i) in MHN fibres, halothane induced Ca^{2+} release is markedly inhibited by physiological levels of cytosolic Mg^{2+} and (ii) the inhibitory effect of cytosolic Mg^{2+} is typically less pronounced in MHS muscle. Reduced inhibition of halothane-induced Ca^{2+} release by cytosolic Mg^{2+} might contribute to the increased susceptibility of MHS muscle to contracture induced by volatile anaesthetics.

This work was supported by the The Wellcome Trust

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