158 HEME OXYGENASE PROTEIN EXPRESSION IN SKELETAL MUSCLE.

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Chronic endurance exercise increases the mitochondrial protein content of skeletal muscle. Included in this increase are the heme-containing cytochromes. An evaluation of heme synthesis and degradation can provide insight into mechanisms of mitochondrial assembly in muscle. The rate-limiting step in the degradation of heme is catalyzed by heme oxygenase (HO). The level of this enzyme and its expression in skeletal muscle has never been investigated. Thus, we evaluated the protein levels of HO using an anti-HO polyclonal antibody. Immunoblot analyses of liver, spleen, soleus and heart of saline- and $CoCl_2$-treated (250 μmol/kg body weight) male rats (250-300g; n = 6-7/group) were performed, and signals quantified by laser densitometry. $CoCl_2$ was used to induce HO activity. Liver contained the highest level of HO and two isoforms were present (HO-1, HO-2). In spleen and soleus, HO was expressed at lower ($p < 0.05$) levels, corresponding to 13.2 ± 3.7 % and 34.2 ± 14.9% of liver, respectively. However, only one of the liver HO isoforms (HO-1) was detected in soleus muscle. Despite the high mitochondrial content in heart, HO levels were not detectable. After 1-6 hours of $CoCl_2$ treatment, HO levels were induced 2.6-fold in spleen ($p < 0.05$), but were not significantly increased (0.90 ± 0.16 and 0.98 ± 0.10 fold) above the saline-treated animals in soleus and liver ($p > 0.05$), respectively. Thus, HO is expressed in skeletal muscle, but examination of selected tissues (eg. heart) indicates that its expression is not strictly coupled to mitochondrial content. Thus, in heart the degradation of heme may occur via an intramitochondrial pathway. While the level of HO is not induced in muscle by $CoCl_2$, further work will determine the role of chronic exercise in HO expression.

159 EFFECT OF VITAMIN E OR IBUPROFEN ON INDICATORS OF MUSCLE DAMAGE AND EVIDENCE FOR OXIDATIVE STRESS

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This study assessed the impact of Vitamin E (VE) or Ibuprofen (IB) upon creatine kinase (CK) and thiobarbituric acid reactive substances (TBARS) appearance following novel eccentric muscle contractions in 23 college-aged females. The VE group (n=10) ingested 800 IU/d alpha-tocopherol for 14 d prior to and for 4 d after the exercise bout while the IB group (n=5) took 1200 mg/d ibuprofen for 3 d prior to and 4 d after the exercise bout. A placebo (P, n=8) group followed a supplementation schedule similar to VE. Samples were taken before supplementation, preexercise, immediately, 24, 48, and 72 h postexercise, to allow immunochemical determination of circulating CK, and, fluorimetric measurement of urinary TBARS which was expressed as a ratio of TBARS:creatinine (T:C). Two-way ANOVA demonstrated no significant group x time interaction for T:C, however, a significant group main effect was detected ($p < .01$). Subsequent inspection proved the group effect was due to a lower T:C production in IB ($p < .05$). CK results were predictable in that it was elevated at all times postexercise and demonstrated great intersubject variability. At this time the mechanism underlying the IB effect upon T:C is unknown. If our assumption that the T:C ratio is an accurate marker of oxidative stress is correct, then chronic IB administration seems to have a beneficial, albeit limited, effect upon oxygen free radical activity.

160 MYONUCLEAR RESPONSE OF REGULATORY PROTEINS AND TWO MYOSIN ISOFORMS SHORTLY AFTER STIMULATION.

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The study has two aims (1) to determine the earliest molecular changes in response to chronic stimulation and (2) to determine whether single myonuclei can co-transcribe both fast IIX and slow myosin heavy chain isoforms. (1) Tibialis anterior, TA, muscles from Sprague Dawley rats were stimulated in plantar-flexed position at 5 Hz for 15 to 120 minutes. Extracted RNA was quantitatively assayed on filters with labelled cDNA probes specific for *creb, c-fos,* myogenin and MyoD1. *creb* increases five-fold above control by 1 hour but no changes in the muscle regulatory factors were detected. (2) TA muscles from female New Zealand White rabbits stimulated continuously at 10 Hz for 4-10 days were quantitatively analyzed with myogenin and MyoD1 probes by filter hybridization, and for fast IIX and slow myosin mRNA by *in situ* hybridization in serial sections. Myogenin and MyoD1 were unchanged. Nuclear domain hybridization data from over 100 myonuclei show steady increases in slow myosin from 15.6% in the control to 62.4% nuclei at 7 days; IIX expression rises from 24% in control, peaks at 43% at 7 days then falls back to control levels by 14 days. Coexpression of both IIX and slow transcripts was 28.2 ± 4.5% from 4 to 10 days. We conclude that an early event in fiber type transformation is up-regulation of *creb,* a cAMP regulatory element binding protein. Later individual nuclei transcribe two myosins concurrently.

161 EFFECT OF EXERCISE AND TWO POLYUNSATURATED: SATURATED FAT DIETS ON ADIPOCYTE SENSITIVITY TO ADENOSINE IN SWINE.

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Twenty-four female mininture Yucatan swine were used in this study to determine the effects of exercise and two polyunsaturated:saturated (p:s) fat diets on isolated adipocyte sensitivity to the anti-lipolytic agent, adenosine. Swine were fed ad libitum a diet containing 37% of kcal as fat with a p:s of 0.3 or 1.0, and were treadmill-exercised or remained sedentary. Littermate pairs were assigned to the 4 experimental groups in a fractional factorial design. After 3 mos., over-the-shoulder biopsies were taken and adipocytes isolated. Cells were incubated for 90 mins with no addition, epinephrine (epi, 10^{-4} to 10^{-9}M), isoproterenol (iso,10^{-6}M), or 10^{-6}M epi plus 1 of 8 levels of phenylisopropyladenosine (PIA,10^{-10} to 10^{-8}M), and glycerol release was measured. Backfat thickness was also measured by ultrasonography at the site of the biopsy. Our findings revealed that 1) adipocytes from swine fed a diet with a p:s ratio of 1.0 had a greater release of glycerol/cm^2 surface area (SA) compared to controls (CO) when stimulated by iso (34.8±11.9 vs 26.6±11.3 nmol, $p < .05$) 2) when stimulated by 10^{-6}M epi adipocytes from exercised swine (ES) released greater amounts of glycerol/cm^2 SA compared to CO (104.3±31.9 vs 74.9±19.0 nmol, $p < .05$) and per 10^4 cells compared to CO (32.2±10.5 vs 22.2±5.6 nmol, $p < .05$), 3) backfat thickness was lower in ES compared to CO (2.40±.29 vs 2.95±.49 cm, $p < .005$) and ES had a lower backfat:body weight compared to CO (.033±.005 vs .039±.004 cm/lb, $p < .005$) 4) neither exercise nor diet had a significant effect on adipocyte sensitivity to PIA inhibition of lipolysis. We conclude that ad libitum feeding of a high fat diet, regardless of the p:s ratio, overrides the exercised-induced decrease in adipocyte sensitivity to adenosine previously reported by this lab.

162 EFFECTS OF LACTACIDOSIS ON ION REGULATION IN RESTING CAT HINDLIMB

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This study determined the effects of lactic acidosis on ion regulation in resting perfused cat hindlimb. Hindlimbs (n=7, 45.3±4.8 g; mean±SE) were perfused by popliteal artery at 36°C with a 4:1 solution of artificial plasma to cat blood (final hematocrit of 7.1±0.8%) at a flow of 31±2.7 $ml.min^{-1}$. Hindlimbs were perfused at low [lactate] (C, 1-2 mM, pH=7.42±0.01) for up to 60 min, and later at high lactate (HL) (25.5±2.9 mM, pH=6.68±0.03) for 6 min. Gasses and other strong ions were nearly identical in the 2 perfusates. Venous perfusate was sampled every 6s for 4 (C) or 6 (HL) min. Plasma was analyzed for ions (Nova Statprofile 5), lactate and Evans Blue (spectrophotometer). Net fluxes were calculated as a-v plasma [ion] times plasma flow (units of $umol.100g^{-1}.min^{-1}$) with consideration for net water flux (J_v). Higher HL osmolality (by 8 mosmol/L) resulted in a J_v of -0.04±0.14 $ml.min^{-1}.100g^{-1}$ (net efflux from tissue) at 20s which decreased exponentially to -0.07±0.04 at 360s. Rapid lactate uptake was mainly due to flux into extracellular tissue compartments. Lactate influx decreased from 158±34 at 60s to 66±38 at 360s. Na^+ influx was 23±9 in C, with no net flux in HL except for a rapid transient of -51±24 between 120-150s. No net flux of Cl^- occurred in C, but net efflux (-30 to -40 $umol.100g^{-1}.min^{-1}$) occurred throughout HL with a rapid transient of -100±24 from 125-145s. HL prompted an immediate efflux of K^+ of -6.2±1.7 at 21s, followed by reduced flux of -1.5±0.9 at 90s, a rapid transient efflux of -3.7±0.9 from 120-150s, and reduced efflux of about 1.8±0.8 until 360s. It is concluded that pronounced lactacidosis transiently disrupts skeletal muscle ion homeostasis. It is speculated that the net efflux of Cl^- may be associated with lactate influx (maintenance of electrical neutrality) and counteract the intracellular acidifying effect of increasing intracellular lactate.

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163 MITOCHONDRIAL SUPEROXIDE DISMUTASE (SOD) CONCENTRATION FOLLOWING PHYSICAL TRAINING AND EXHAUSTIVE EXERCISE

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Manganese-SOD (Mn-SOD) is a mitochondrial antioxidant protein. The effect of 8 wk treadmill training (2.1 Km/h, 2 h/day, 5 days/wk) and exhaustive exercise (EE) on immunoreactive Mn-SOD concentration was studied in rats. Mn-SOD concentrations were assayed by an ELISA using a polyclonal antibody raised against purified rat liver Mn-SOD. Control mean values (μg Mn-SOD/mg protein for tissues and μg/l for plasma) and the corresponding 95% CI are presented in italics. Treadmill training (T) enhanced the specific concentration (SC) of Mn-SOD in the resting skeletal muscles (SM) [red gastrocnemius (RG; *1.44, 1.17-1.71*) and mixed vastus lateralis (*1.57*)], liver (*5.56, 4.26-6.86*) and heart (*9.24, 8.33-10.15*) by 30%, 50% and 14%, respectively. The SC of Mn-SOD in the lung was <200 ng/mg protein. Resting plasma Mn-SOD content was significantly (*) higher in the trained group (*T 3.11 vs. C 2.62*). During EE, control rats ran at a lower intensity (ca. 1.4 Km/h) and a shorter duration compared to T. Following EE, a decrease in the SC of Mn-SOD was seen in the heart of T (13%) and C (9%), liver of T (40%), and RG of C (35%). Parallely, a 40%*, 96%* and 19%* increase in the plasma content of Mn-SOD was observed in T, C (*2.62, 2.33-2.91*), and GSH administered (0.5 g/Kg body wt,i.p, 10' pre-EE) T, respectively. EE dependent loss of Mn-SOD from tissues and appearance in the plasma may be interpreted as a leakage from disintegrated mitochondria. Loss of Mn-SOD from the liver seemed to be dependent upon EE-intensity. GSH administration diminished the EE-dependent increase of plasma Mn-SOD content. A protective effect of T upon RG, perhaps related to an elevated antioxidant status, was seen.