687 CYTOPLASM-TO-MYONUCLEUS RATIOS IN ATROPHIC SKELETAL MUSCLE.

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The purpose of this study was to determine if 28 days of hindlimb unloading (HU) would produce significant changes in the relationship between myonuclear number, cellular volume, and myosin type in single fibers from atrophic soleus and plantaris muscle. Adult female white Wistar rats were randomly assigned to either control or experimental groups. HU for 28 days was accomplished using the method of Morey-Holton (1981). Single fibers from soleus and plantaris muscle were stained for DNA and double immunolabeled with fast or slow MHC monoclonal antibodies. After HU of 28 days, muscle atrophy occurred by cross-sectional area losses of 30% in fast plantaris and 44% and 55% in fast and slow soleus, respectively. Mean ± SEM cytoplasmic volume / myonucleus ratios significantly decreased in atrophic fast plantaris, fast soleus, and slow soleus fibers (39 ± 3; 16 ± 2; 12 ± 1 $\mu m^3 \times 10^3$) as compared to controls (65 ± 7; 29 ± 5; 33 ± 2 $\mu m^3 \times 10^3$), respectively. Myonuclei / mm significantly increased in slow soleus (185 ± 2) as compared to control (154 ± 11). There was no change in the myonuclei / mm in the other HU groups. These data suggest that a constant myonuclear number does not decrease proportionately to maintain a constant cytoplasmic volume to myonuclear number during atrophy. Myonuclear number is preserved in spite on losses of cell volume.

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688 MYOCARDIAL RESPONSE TO GLUTATHIONE & GLUTATHIONE ESTER SUPPLEMENTATION DURING PROLONGED EXERCISE

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Myocardial glutathione (GSH) status was investigated at rest and after prolonged exercise (E) with or without free GSH (GSH-F) and GSH ethyl ester (GSH-E) supplementation. Male Swiss-Webster mice (2 mo, N=56) were randomly divided into four groups: control (C), fed a standard chow diet and water ad libitum; fasted for 24 h and injected i.p. with saline (F); and fasted for 24 h and injected with either GSH or GSH-E (6 mmol/kg body wt) 1 h before E. Half of each group of mice were subjected to an exhaustive bout of swimming and killed immediately after E. The other half was time-matched and killed at rest (R). Endurance time for C, F, GSH, and GSH-E groups was 326±19, 237±17 (P<0.05), 351±22, and 348±27 min, respectively.

(μmol/g w w)		GSH	GSSG	GSH:GSSG	TGSH
Control	R	0.93±0.03	0.11±0.02	8.8±0.4	1.14±0.05
	E	0.75±0.04* (-20%)	0.09±0.01 (-18%)	8.0±0.7	0.94±0.03*(-18%)
Fasted	R	0.99±0.05	0.12±0.01	8.8±0.5	1.22±0.07
	E	0.84±0.04 (-15%)	0.11±0.01 (-8%)	8.0±0.5	1.05±0.04* (-14%)
GSH-F	R	0.82±0.06†	0.14±0.01‡	6.7±1.0	1.11±0.05
	E	0.75±0.06 (-9%)	0.11±0.01* (-21%)	7.2±0.8	0.97±0.03 (-13%)
GSH-E	R	0.80±0.01†	0.14±0.01‡	6.0±0.8	1.07±0.09
	E	0.70±0.01 (-13%)	0.12±0.01 (-14%)	6.4±1.2	0.94±0.08 (-12%)

These data show that (1) GSH-F or GSH-E decreased GSH content (†P<0.05) and increased glutathione disulfide (GSSG) levels (‡P<0.05) in the myocardium; and (2) GSH and total GSH concentrations were decreased with E (*P<0.05), whereas the GSH:GSSG ratio was maintained constant due to the decline of GSSG. It is concluded that there is an increased oxidation of GSH in the heart during prolonged exercise and that GSH supplementation cannot reverse the decrease of GSH. (AHA Grant-in-aid).

689 EFFECT OF GLUTATHIONE SUPPLEMENTATION ON POST EXERCISE NEUTROPHIL FUNCTIONS.

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We tested the effects of Glutathione (GSH) and N-Acetylcysteine (NAC) supplementation on exercise induced leukocyte margination and on neutrophil oxidative burst activity to evaluate the importance of antioxidant protection in neutrophil-mediated inflammatory events. Rats (8 week old Han-Wistar; n≥7 per group) received GSH or NAC supplementation or saline for control as one single intraperitoneal (i.p.) injection (1g/Kg). Exercising animals were subjected to treadmill running until exhaustion. Leukocyte margination was measured indirectly by changes in leukocyte counts in peripheral blood. The oxidative burst activity of peripheral blood phagocytes was determined by the chemiluminescence (CL) method. Exhaustive treadmill exercise significantly decreased the leukocyte count in peripheral blood in the control group (8300 SD 1400/μL vs 5470 SD 1150/μL, p<0.001). Such leukocyte margination was not seen in GSH and NAC supplemented animals. Also, neutrophil counts were significantly higher in the resting GSH and NAC supplemented groups than the control group (p<0.05). Post exercise CL activity of total blood was significantly higher in NAC and GSH supplementation groups compared to corresponding controls (p<0.01). Our findings suggest that GSH and NAC can induce leukocyte and neutrophil mobilization and decrease leukocyte margination, and may be useful in preventing excess leukocyte infiltration into damaged tissues.

690 EFFECT OF EXERCISE AND ETHANOL ON ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION IN BRAIN REGIONS OF RAT.

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We have reported that exercise training alters the antioxidant enzymes and glutathione levels in certain brain regions of rat. Since individuals performing exercise may also consume alcohol which is quickly absorbed and may affect the brain antioxidant system. Therefore, the aim of this study is to investigate the effect of ethanol ingestion after acute exercise on antioxidant enzymes and lipid peroxidation in brain regions of rat. Male Fisher-344 rats were sacrificed after exercise, ethanol, and exercise + ethanol treatment and brain regions were isolated and superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR) and lipid peroxidation (MDA) were determined. The results indicate that acute exercise (at 100% VO_2 max) increased CAT and GSH-Px activities (155% and 247% of SC) and MDA level (157% of SC) in cerebral cortex. Ethanol 20% (8 ml/kg po) increased MDA in cerebral cortex, medulla and hypothalamus (173%, 136%, and 143% of SC) respectively; whereas, striatal CAT, GSH-Px and GR increased (192%, 238%, and 152% of SC); CAT and GR activities enhanced (214% and 144% of SC) in medulla and GSH-Px activity (289% of SC) in hypothalamus. In summary, acute exercise as well as ethanol exerted oxidative stress to many of the brain regions which control respiration, cognitive function, heat production, emotional expression and motor function. The combined effect of acute exercise and ethanol seems to augment antioxidant enzymes and also an increase in MDA level in various areas of the brain.

691 SKELETAL MUSCLE GLUTATHIONE STATUS IN EXERCISE: EFFECT OF GSH AND GSH ETHYL ESTER SUPPLEMENTATION

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The effects of free glutathione (GSH-F) and GSH ethyl ester (GSH-E) supplementations on GSH status in the quadriceps (Q) and the costal diaphragm (D) muscles were investigated at rest and after prolonged exercise (E). Male Swiss-Webster mice (2 mo, N=56) were randomly divided into 4 groups: controls (C), fed a chow diet and water ad libitum; fasted for 24 h and injected saline, i.p. (F); and fasted for 24 h and injected either GSH-F or GSH-E (6 mmol/kg body wt, i.p.) 1 h before E. Half of each group of mice was subjected to an exhaustive bout of swimming prior to kill. The other half was time-matched and killed at rest (R). Endurance time for F was shorter (237±17 min, P<0.05) vs. that in C (326±19), GSH-F (351±22), and GSH-E (348±27). GSH content in Q did not alter with GSH-F, but decreased 22% (P<0.01) in GSH-E compared to F. Glutathione disulfide (GSSG) content in Q was increased with GSH-E, resulting in a decreased GSH:GSSG ratio (P<0.05). Exercise decreased GSH content in Q of F, GSH-F, and GSH-E mice by 20, 14, and 18%, respectively (P<0.05), but not in C. GSH-F or GSH-E supplementation did not change GSH or GSSG content in D. However, a greater decline of GSH was found after E in the D of C (13%), F (35%), GSH-F (30%), and GSH-E (17%), with no effect on GSSG or GSH:GSSG ratio. We conclude that GSH supplementation does not improve GSH status in skeletal muscle during prolonged exercise, and therefore, the enhanced endurance performance with GSH-F and GSH-E may be caused by mechanisms independent of muscle GSH antioxidant reserve. (Supported by AHA Grant-in-Aid and AHA-IL).

692 EFFECT OF CAFFEINE AND OTHER XANTHINES ON CYTOCHROME *c* OXIDASE ACTIVITY.

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Evidence exists that caffeine (1,3,7 trimethylxanthine) administration may result in an increase in oxygen consumption during exercise beyond that which would be expected due to a shift in substrate utilization. Possible mechanisms of action for caffeine include: inhibition of phosphodiesterase, increases in norepinephrine, and adenosine receptor blockade. An additional untested mechanism may involve direct regulation of the respiratory chain in the mitochondria. Cytochrome *c* oxidase (CcO) is a potential target for regulation of oxygen metabolism since this enzyme is responsible for >90% of the oxygen utilized in humans. To probe the effects of various xanthines on CcO we have examined steady-state turnover activity of CcO with it's physiological substrate, cytochrome *c*, in the presence and absence of caffeine, theophylline (1,3 dimethylxanthine) and xanthine. Absorption spectra were obtained using a Milton Roy Spectronic 3000 diode array spectrophotometer. Our results indicate that caffeine and theophylline have no effect on CcO activity, while xanthine increased the relative rate of CcO by 26%(k_1 = 0.036 s^{-1}, in the absence of xanthine and k_1 = 0.05 s^{-1} in the presence of 5mM xanthine). These results suggest the possibility of an active role for caffeine metabolites in regulating respiratory activity.