With prolonged exposure to a microgravity environment, astronauts experience muscle unloading and significant loss in skeletal muscle mass. Subsequently, during readaptation to Earth’s gravity astronauts (as well as animals) can experience varying degrees of muscle damage while reloading atrophied muscles. Additionally, the astronauts can suffer from post-flight symptoms of severe muscle pain, marked muscle edema, and high blood levels of muscle specific markers creatine kinase (CKmm) and myoglobin [Sayers and Clarkson, 2003]. Subsequently, excess protein loads arising from injured muscle could ultimately lead to increasing levels of oxidative damage resulting from the release of iron from heme (e.g., myoglobin and hemoglobin) and non-heme proteins [Menshikova et al, 1999; Fang et al., 2002]. However, sources of oxidative stress and subsequent damage have remained elusive. Furthermore, regions of ischemia and anoxia can cause lysis of the vascular endothelium and disruption of the sarcosome. In damaged muscle fibers, extracellular edema, extravasated erythrocytes, macrophages and neutrophils have been observed. Studies aboard COSMOS flights revealed microhemorrhages, thrombi and segmental necrosis [Krippendorf and Riley, 1994]. In addition, exposure to radiation while in space as well as bouts of ischemia/reperfusion are undoubtedly contributing factors for increases in oxidative stress and damage occurring during readaptation. The purpose of this study was to determine whether the release of free radical generators such as xanthine oxidase (XO) could account for both the high levels of iron and oxidative stress observed during the post-suspension (PS) period.

Methods: Studies were conducted using the rat hindlimb suspension and paired-feeding paradigms. Mature male Sprague Dawley rats were suspended (S) for a period of 2 weeks. Non-suspended (NS) control rats were handled similarly to the suspended rat groups [tail casts in place and line attached], but all 4 feet remained in contact with the cage bottom. Following suspension, post-suspended (PS) rats were released from the 30° head-down tilt position and allowed to move about the cage (same as NS controls) for periods of 0.5 or 24 h. Sample Collection: Following sacrifice with beuthanasia, blood was collected via cardiac puncture into heparinized tubes, centrifuged at 1,000-x g (15 min @ 4°C), and the plasma transferred to 250 µL cryotubes and stored at -80°C. Slow and fast-twitch muscles (soleus and plantaris) were harvested, weighed and immediately frozen and stored at -80°C. Analysis: Plasma myoglobin content was determined by ELISA. XO activity was determined colorimetrically using the Molecular Probes kit (A-22182). Absorbance was read at 560 nm in a 96-well plate. Iron was also determined colorimetrically using the FerroZine method. Total glutathione was determined by reverse phase fluorescence HPLC.

Results: After 30 minutes post-suspension, there was no significant difference in plasma XO activity when compared to non-suspended (NS) controls (Figure 1). However, plasma XO activity (182.4± 32.0%, p< 0.001) was significantly greater (p<0.001) than in the NS group after 24 hrs of post suspension. With increasing XO activity, there is a concomitant rise in the level of oxidative stress. However, in the presence of oxidative stress modifiers such as iron or other metals, free radical production is greatly increased. Cell culture experiments using human retinal cells treated with ferrous iron in concentrations ranging from 0.5 to 2.5 µg/mL showed a dose-dependent increase in cell death when exposed to 30 minutes of UV-C radiation [von Deutsch et al., 2005]. Similarly, plasma iron content increased over that 24-hour post-suspension period (Figure 2). Although the levels were not statistically significant at the set time points, the mean iron content (121.2± 6.2%, p= 0.038) was significantly greater than NS control rats. Similarly, the total iron content for unloaded soleus and plantaris muscles (% total Fe increase: 118.4 ± 8.4% for soleus and 121.5 ± 9.3% for plantaris) were significantly
increased over that of the non-suspended controls (Figure 3).

Since the release of myoglobin into the blood stream is an indication of muscle trauma upon reloading, plasma myoglobin levels were measured (Figure 4). In suspended (S) rats, the concentration of plasma myoglobin was significantly less (35.0± 6.6%, p= 0.013) than that observed in the non-suspended (NS) controls (100.0± 21.2%), while at 24 hrs levels were significantly greater (283.1± 74.4%, p= 0.033). Times 0.5 and 1.0 hours were not significantly different from the NS control.

During recovery, the increases in plasma iron content and XO activity were offset by decreases in total glutathione (GSH) levels over the observed 24-hr post-suspension period. In both suspended and 0.5-hour post-suspension recovery rats, GSH levels were not significantly different from NS controls (Figure 5). However, plasma GSH levels were significantly less in the 1-hour (74.8 ±7.0% of NS control) and 24-hour (64.1± 5.3% of the NS control) post-suspension recovery groups.

**Discussion**: Results from these studies demonstrated that plasma XO activity, myoglobin and iron content all increased during the post-suspension period. Unloading causes significant increases in muscle total iron content, more so in the soleus than the plantaris. The greater amount of total iron in the soleus is reasonable because of the higher content of myoglobin and heme oxygenase-1.

In contrast, total glutathione levels decreased during the initial 24-hour post-suspension period. The observed changes were presumably due to oxidative damage arising from a multitude of sources including the release of XO as part of the damage cascade. However, the underlying mechanism critical for triggering musculoskeletal and vascular damage upon reloading is unclear. Factors contributing to increases in oxidative stress levels during recovery can include: space radiation-induced inflammation resulting from exposure to space radiation, reductions in endogenous antioxidant protection, local ischemia/reperfusion and eccentric movements during recovery. The resulting injury to the vascular endothelium and skeletal muscle can potentially result in the release of enzymes known as free radical generators (e.g., XO) and free radical modifiers. Modifiers to free radical damage include iron and myoglobin (as a source for iron release). Modifiers such as iron can enter into the Fenton and Haber-Weiss reactions and help catalyze the conversion of peroxides and superoxides into hydroxyl radicals. Subsequently, hydroxyl radicals can participate in lipid peroxidation reactions, causing direct damage to membranes and cellular macromolecules (receptors, DNA, etc). Thus, high plasma XO activity (along with other free radicals generators) will ultimately result in high plasma iron content and the initiation of the oxidative damage cascade. In contrast to the observed increased XO activity, plasma total GSH levels decreased during the 24-hour post-suspension period. With increasing XO activity, total glutathione levels decreased. This suggests that the level of oxidative stress increases with rising XO activity.

**Conclusions**: In PS rats, reductions in plasma glutathione levels suggest increased levels of oxidative stress induced, in part, by the muscle reloading. The insights gained from these studies can lead to the development of more efficient countermeasures to aid astronauts during readaptation to Earth’s gravity. This work was supported, in part, by NASA Grant NCC9-112, NIH Grants 1R25RR17694, 5P20RR011104, and MBRS Grant 506GM08248.

**References**