

urine and plasma samples. Hereby, a citrate/acetate buffer at pH 4.75 containing 3.3% methanol is used to separate allantoin from uric acid. The allantoin peak is collected and the compound degraded to glyoxylic acid by subsequent treatment with base and acid at 100 °C. Finally, the hydrazone derivative is formed by adding 2,4-dinitrophenylhydrazine and the final product is rechromatographed ($\lambda=363$ nm). However, at our laboratory, derivatives from various concentrations of allantoin standards yielded irreproducible results after initial chromatography and the amount of hydrazone derivative varied highly with increasing citrate concentrations in the buffer thereby, indicating that degradation of citrate led to the formation of reactive species which possibly intercept the aldehyde intermediate before reaction with the chromophore. Indeed, elimination of the buffer system and chromatography with pure methanol/water yielded excellent results regarding reproducibility and sensitivity of this modified derivatization method. In addition, uric acid and allantoin standards were separated under these conditions. Unfortunately, the application of plasma samples led to inappropriate separation of allantoin and uric acid due to the salt content of the sample. Therefore, we turned to reversed-phase HPLC coupled with tandem mass spectrometry (RP-HPLC-MS/MS-ESI) and, omitting the derivatization step, obtained promising initial results. Further development along these lines should lead to a method which allows the direct determination of allantoin, thereby, avoiding the reported pitfalls concerning derivatization and buffer conditions.

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THE EFFECTS OF RIBOSE INGESTION ON INDICES OF FREE RADICAL PRODUCTION DURING HYPOXIC EXERCISE

John G. Seifert, Andrew Subudhi, Min-Xin Fu, Karen L. Riska, Jeff C. John:
HPL, St. Cloud State University, St. Cloud, MN, 56301
USA; TOSH, Salt Lake City, UT; Oxis Research, Portland, OR

Free radicals are produced through reductive and hypoxic environments, nucleotide metabolism, or ischemia and reperfusion. This study investigated the systemic effects of ribose on indices of free radical production during hypoxic exercise. Seven volunteers cycled twice at lactic acid threshold for 25 minutes while inhaling 16% O₂ and then sat quietly for 60 minutes while breathing room air. One week elapsed between trials in this double blind, cross-over study. Subjects ingested 250 mL of either a control (CT) or 7 g of ribose in 250 mL (RB) immediately before and after exercise. Results: Measured urinary MDA increased significantly during CT as reflected by an increase of 0.2 ± 0.03 nM/mg, but decreased by 0.04 ± 0.03 nM/mg in the RB trial. Plasma reduced glutathione (corrected by hemoglobin) was also significantly less for RB (-0.2 ± 0.29 μ M), but increased by 0.03 ± 0.29 μ M for the CT condition. Plasma uric acid was similar between groups, RB: 4.55 ± 0.06 mg/dL vs CT: 4.67 ± 0.06 mg/dL. Physiologically, heart rate at the end of the 25 minute exercise was significantly lower for RB than CT (175 ± 1.3 bpm vs. 181 ± 1.3 bpm). No differences were observed

between trials for SpO₂ (RB: $87.7 \pm 3\%$ vs. CT: $87.6 \pm 3\%$) or blood glucose (RB: 3.3 ± 1 mM/L vs. CT: 3.5 ± 1 mM/L.).
Conclusion: Pre- and post-exercise ribose ingestion lowered urinary MDA while modifying plasma glutathione status. Pre-exercise ribose ingestion resulted in a reduced heart rate at similar exercise intensities during hypoxic stress.

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SPATIAL ELECTRON SPIN RESONANCE (ESR) IMAGING OF FREE RADICALS IN MOUSE MAXILLOFACIAL REGION

Hirofumi Shoji¹, Hiroyuki Miyazaki¹, Fumihiko Yoshino¹, Yoichi Omori¹, Kazunori Anzai²,

Toshihiko Ozawa² and Masaichi-Chang-il Lee¹

¹ Department of Pharmacology and ESR Laboratory, Kanagawa Dental College, Yokosuka, Japan.

² Department of Bioregulation Research, National Institute of Radiological Sciences, Chiba, Japan.

In recent years, *in vivo* ESR technique, especially ESR imaging, utilizing paramagnetic nitroxyl compound as spin label has been shown to be appropriate applications *ex vivo* and *in vivo* mapping of spatial temporal variations of free radical generation, metabolism, and oxygenation in biological system. Free radicals have been implicated in the pathogenesis of various diseases, including oral diseases. Regarding as inflammatory diseases, oxidative stress-induced tissue damage could be involved in the pathogenic processes of periodontitis and temporomandibular joint disease. We performed that the measurements of the redox state of nitroxyl spin probe as 3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-yloxy (MC-PROXYL) and 3-carbamoyl-2,2,5,5-tetramethyl-pyrrolidine-1-yloxy (carbamoyl-PROXYL) in head region of living mice using *in vivo* ESR imaging system. The obtained ESR images show that the MC-PROXYL, but not carbamoyl-PROXYL, is distributed well in the brain. Interestingly, we found that the ESR image of carbamoyl-PROXYL only distributed well in maxillofacial region compare that of MC-PROXYL. We provide first ESR image that *in vivo* distribution of spin probe as carbamoyl-PROXYL in oral region of non-invasive mice. With further development of ESR instrumentation, this technology holds great promise for non-invasive measurement and spatial imaging of free radical in the animal models of oxidative stress-induced oral disease.

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IDENTIFICATION OF PRODUCTS FORMED BY REACTION OF 2'-DEOXYGUANOSINE WITH HYPOCHLOROUS ACID OR A MYELOPEROXIDASE-H₂O₂-Cl⁻ SYSTEM

Toshinori Suzuki¹, Mitsuharu Masuda¹, Marlin D. Friesen² and Hiroshi Ohshima¹ Unit of Endogenous Cancer Risk Factors, ²Unit of Nutrition and Cancer, International Agency for Research on Cancer, 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France.