P18-30
Elevated lipid peroxidation biomarkers and low antioxidant status in atherosclerotic patients with increased carotid and iliofemoral intima-media thickness
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Purpose: The aim of the study was to explore the correlation between intima-media thickness (IMT) of the major arteries as a clinical marker of atherosclerosis with biomarkers of oxidative stress.

Material and methods: Plasma levels of the major antioxidant micronutrients of the organism (HPLC) as well as malondialdehyde (MDA) (HPLC) and 8,12-isoprostanes $F2_{\alpha}$-VI (8,12-iPF2_{α}-VI) (GC/MS) were measured in 30 patients with atherosclerosis of carotid and iliofemoral arteries. Patients were compared to a matched group of 62 controls. Patients were grouped according to IMT: A < 0.5 mm, B 0.6 -1.0 mm, and C > 1.1 mm.

Results: In patients, independent of fruit and vegetable intake, lower levels of antioxidant micronutrients as well as 8,12-IPF2_{α}-VI levels almost doubled ($p < 0.001$) and MDA levels increased by one third ($p < 0.01$) were observed compared to controls. Antioxidant micronutrients with the exception of β-cryptoxanthin and γ-tocopherol were higher and IPF2_{α}-VI and MDA levels were lower in the IMT A group compared to the B and C groups.

Conclusion: We conclude that the use of isoprostane measurement as biomarker of oxidative stress and of IMT evaluation in cardiovascular pathologic conditions should be encouraged in the performance of clinical studies on the role of antioxidants and oxidative stress in atherosclerosis.

P20-1
Endothelial Stress, in NOS Activity and Oxidative Damage.
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Purpose: In endothelial cells,the expression of the inducible nitric oxide synthase (iNOS) and the resulting high-output nitric oxide synthesis have often been assumed as detrimental to endothelial function,but recent publications have demonstrated a protective role resulting from iNOS espression and activity.It will be a question for research.

Material and methods: To adress this question,we used antisense-mediated iNOS knockdown during proinflammatory cytokine challenge in primary endothelial cell cultures and studied endothelial function by monitoring the expression of stress defense genes.

Results: Under these conditions,cytokine addition results in full iNOS protein expression with minimal nitric oxide formation,concomitant with a significant reduction in stress response gene expression and susceptibility to cell death induced by reactive oxygen species. taken together,our data suggest that cytokine-induced endogenous iNOS expression and activity have key functions in increasing endothelial survival and maintaining function.

Conclusion: Thus suppression of iNOS expression or limited substrate supply,as has been reported to occur in atherosclerosis patients,appears to significantly contribute to endothelial dysfunction and death during oxidative stress.

P20-2
Iron-Sulfur Cluster is a Preferential Target of Peroxynitrite in Proteins
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Purpose: Iron-sulfur clusters are involved in diverse physiological processes including energy metabolism, intracellular iron homeostasis, heme and biotin biosynthesis, and DNA repair. Modification of iron-sulfur clusters will not only inactivate the proteins that contain iron-sulfur clusters, the iron released from iron-sulfur clusters will further augment cellular oxidative damages via the Fenton reaction. The goal of the research is to determine the relative sensitivity of iron-sulfur clusters and tyrosine residues in proteins to peroxynitrite.

Material and methods: An E. coli endonuclease III was purified and treated with peroxynitrite. The enzyme activity of the endonuclease III was measured using a fluoresent-labeled DNA substrate, and the nitrotyrosine in endonuclease III was quantified using the Western blotting.

Results: When endonuclease III was incubated with increasing amounts of peroxynitrite, almost all iron-sulfur clusters were disrupted before any nitrotyrosine was formed by peroxynitrite. Re-assembly of iron-sulfur clusters in the peroxynitrite-treated endonuclease III restored the enzyme activity. Nevertheless, formation of nitrotyrosine appeared to block restoration of the endonuclease III activity even after re-assembly of iron-sulfur clusters.

Conclusion: We conclude that iron-sulfur clusters are the preferential targets of peroxynitrite, and that disruption of iron-sulfur clusters in endonuclease III leads to inactivation of the enzyme activity which can be restored by re-assembly of iron-sulfur clusters.

P20-3
Thioproline induces an anorexic effect, associated with improved survival and neurological function in mice
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Purpose: Thioproline (l-4-thioproline, l-thiazolidine-4-carboxylic acid) is produced in the catabolism of 5-hydroxytryptamine.

Material and methods: Male mice were supplemented with thioproline at 2.0 g/kg of food from 28 weeks of age and for the rest of mice lives. Mice were weekly weighed. Mice were utilized to construct the survival curves and were subjected every 2 weeks to two behavioral assays. Brain and liver mitochondria were isolated, and we measured: 1) biochemical markers of oxidative stress; 2) mitochondrial electron transfer activities; 3) mitochondrial nitric oxide synthase (mtNOS). The antioxidant capacity of thioproline in vitro and the effect over mitochondrial respiration were measured.

Results: Thioproline-supplemented aged mice had, compared with control mice: a) 20% lower spontaneous food intake and a 10% lower body weight; b) 23% increased maximal life span, associated with improved neurological functions; c) a decrease of the age-dependent oxidative damage in brain and liver mitochondria; and d) the age-associated decrease in brain NADH-dehydrogenase, cytochrome oxidase and mtNOS activities was markedly prevented by thioproline intake. Thioproline in vitro neither exhibited direct antioxidant activity nor had any effect on electron transfer or mtNOS functional activities of brain and liver mitochondria.

Conclusion: Thioproline dietary supplementation decreases spontaneous food intake and increases survival and brain function in mice.