

# **Investigation of antioxidant and triglyceride status of rats consuming a diet enriched by a complex phytonutrient supplement, NanoGreens<sup>10 tm</sup>.**

**Alexander S. Krylov<sup>1</sup>, Marina V. Ivanova<sup>2</sup>, Constantine B. Shumaev<sup>2</sup>, Galina G. Konovalova<sup>2</sup>**

<sup>1</sup> Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Baltijskaya, Moscow, Russia

<sup>2</sup> Cardiology Research Center, Ministry of Health, Cherepkovskaya 15A, Moscow, Russia

## **The aim of the study:**

This investigation examined the influence of a complex phytonutrient supplement (NanoGreens<sup>10tm</sup>) on total antioxidant capacity (TAC), endogenous antioxidant enzyme activity (SOD), and triglyceride concentration in laboratory animals.

## **Introduction: Oxidative stress, antioxidant protection, and blood lipids.**

Oxidative stress occurs in any organism as a result of free radical processes. Oxidative stress is ameliorated by endogenous antioxidant enzyme activity and exogenous dietary antioxidants. During oxidative stress the formation free radicals, also known as reactive oxygen species (ROS), induce oxidation of non-saturated fatty acids in biological membranes and lipoproteins, and oxidation of cell proteins, including DNA. Such oxidative stress is a major factor in all the common diseases of aging. Special phytonutrient formulations may lessen oxidative stress by both contributing exogenous antioxidants and promoting endogenous antioxidant enzyme production.<sup>1</sup>

It is well known that an increase of blood lipids above normal ranges is strongly correlated with cardio vascular disease and metabolic syndrome. Some phytonutrients, particularly polysaccharide soluble fibers, and nutrients such as in lecithin, have demonstrated salubrious effects of blood lipids.<sup>2,3</sup>

## **Material and Methods:**

NanoGreens<sup>10</sup> is a complex phytonutrient supplement (CPS) containing whole foods and food extracts and concentrates derived from fruits, vegetables, grass, algae, herbs, spice, bran, and lecithin. It is designed for human consumption. One serving (12 gm) provides about 2 gm of protein, 8 gm carbs, and 1.5 gm fats. One serving of NanoGreens<sup>10</sup> provides over 50% of the RDV for Vit. C and Vit. A (as beta-carotene). It provides less then 5% the RDV for the other vitamins and minerals. It is extremely rich in phytonutrients, providing approximately 7,000 Oxygen Radical Absorbance Capacity units (ORAC) per serving.<sup>4,5</sup>

Total number of rats was 74. Half of them were controls and ate a usual feed. The second group was given complex phytonutrient supplement (CPS). The everyday portion of CPS was 15 gram per kg of weight (3 grams per one rat). This dosage was designed to be equivalent, metabolically, to 24 gm daily dosage in humans. The total antioxidant capacity (TAC) was measured by test kit (Randox Ltd.) and expressed in Trolox units (TEAC). The enzymatic activity of Superoxide dismutase (SOD) was determined by the method of concurrent inhibition. The concentration of triglycerides in blood serum was determined by test kit (Lachema, from the Czech Republic).

## **Results:**

The results observed with intake of CPS included statistically significant influence on levels of total antioxidant capacity (TAC), enzymatic activity of liver SOD, and triglycerides.

The fastest and sharpest influence of CPS intake was on the total antioxidant capacity (TAC) in plasma. Figure 1 demonstrates the dependence of TAC on duration of CPS feeding. One can see that as the rats started CPS, the level of TAC is increased sharply. The amplitude of this increase is very high (42%). During the period of days 3 to 6 this effect disappears to a level close to control. Subsequently, during the second week, the level of TAC demonstrates a second peak (28%), and then maintains an increased level (15%) over control. Such oscillations during the first days of intake are very normal for many pharmacokinetic processes.

### Total antioxidant status

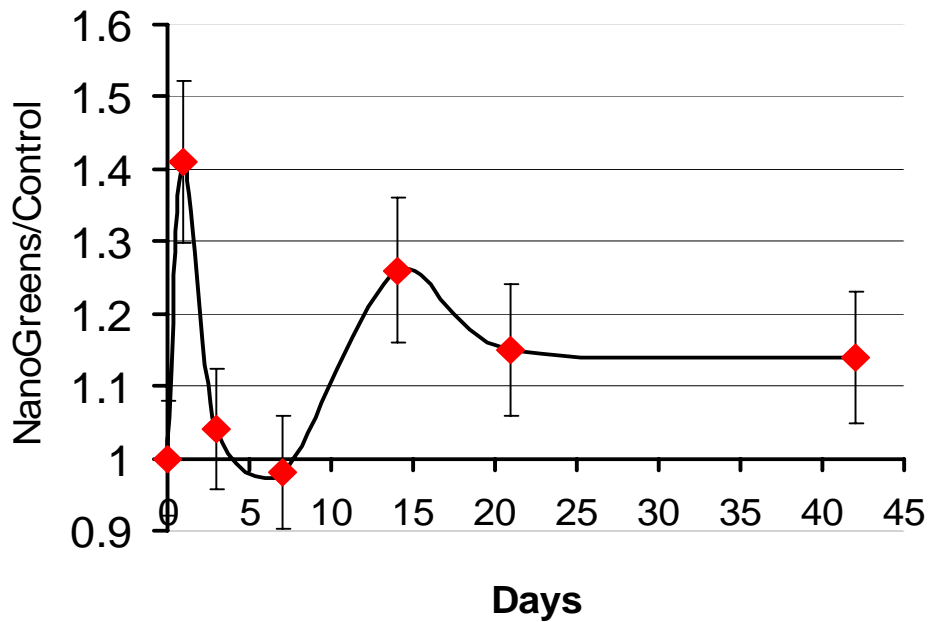


Figure 1. Time course of TAC status during intake of NanoGreens.

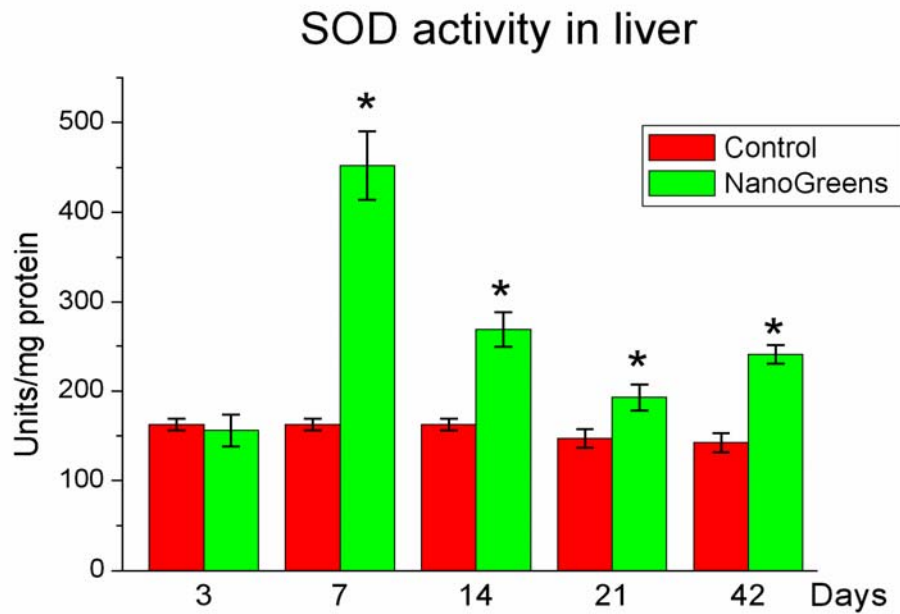


Figure 2. Activity of Superoxide dismutase in liver during CPS intake.

Superoxide dismutase (S.O.D.) consists of several metallo-enzymes that catalyze the formation of hydrogen peroxide and oxygen from superoxide radical and thus protects against superoxide induced free radical damage. Very interesting results are garnered from CPS intake on the activity of enzyme S.O.D. in the liver. SOD is notably increased at day 7. This activity remains increased for all the period of experiment – 42 days. The process is very statistically significant.

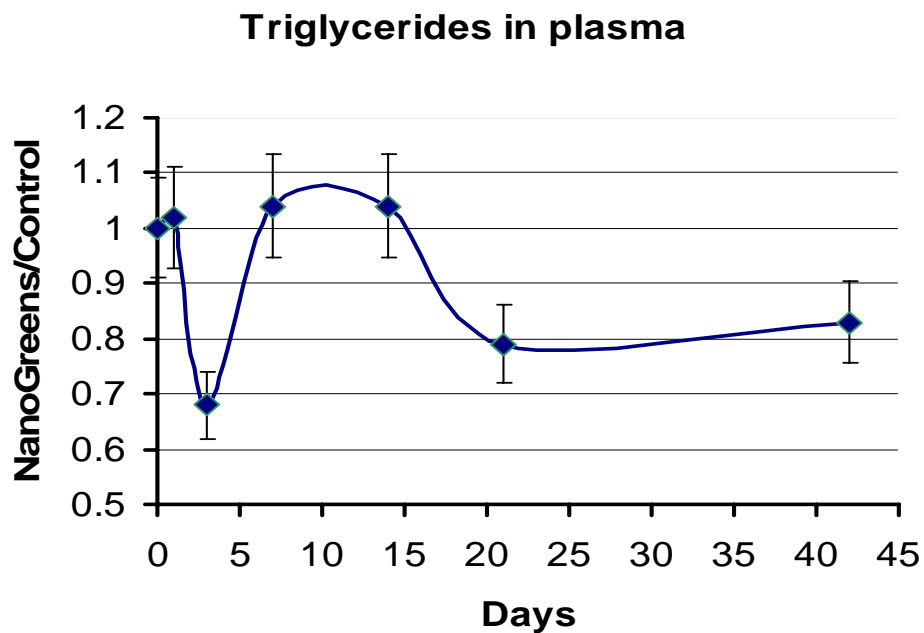


Figure 2. The time course of triglycerides in plasma

At first, the concentration of total triglycerides dropped precipitously (> 30%), then rapidly returned to slightly above control levels for the first two weeks, thereafter dropping and “leveling off” at around a 10% to 25% overall decrease.

## **Discussion**

### *TAC Results*

In human trials assessing the effect on a diet rich in fruits and vegetables on the antioxidant capacity of plasma in humans, it was reported that by “increasing their fruit and vegetable intake to 10 a day (from 5 a day, or twice the previous antioxidant capacity) ...the ORAC value of blood plasma increased between 13 and 15 percent.”<sup>6</sup>

In a study conducted at the Health Research Studies Center in Stanford, CA, a baseline ORAC level of blood serum of subjects was measured prior to ingesting 400 mg of a phytonutrient concentrate with an approximate ORAC of 5000 units. The plasma antioxidant capacity was measured after 30 minutes and 1 hour. Increases of blood ORAC levels averaged 12%.<sup>7</sup>

It should be understood that the main endogenous antioxidants in plasma are *endogenous*, such as urate and albumin. Therefore a 15% average increase in TAC from *exogenous* dietary phytonutrient antioxidants is considered very significant.<sup>8,9,10</sup> The steady, prolonged 15% increase demonstrated in this study appears to be consistent with a phytonutrient rich diet high in fruits and vegetables.

### *S.O.D. Results*

In addition to endogenous albumin, glutathione, urate, and exogenous dietary antioxidants like Vit. A, C and E, *endogenous antioxidant enzymes* are needed to quell destructive free radicals. Superoxide dismutase (SOD) is a vital endogenous antioxidant enzyme known to produce a wide range of health benefits. Its chief function is to convert superoxide anion radicals into hydrogen peroxide and oxygen.

Superoxide is one of the most damaging free radicals. If left unchecked it causes cellular damage leading to the progression of diseases.<sup>11</sup> Unfortunately, a delivery method for SOD has eluded scientists due to the proclivity of the enzyme to denature when exposed to gastric secretions. As a result, exogenous SOD has typically been administered via injection, and its advancement as a supplement has been limited.

Therefore, the induction of SOD activity in the liver may be even more important than above significant increase in TAC vis-à-vis total free radical scavaging activity. In their study on induction of SOD activity in humans via natural phytochemicals (herbs) Nelson, et al. conclude, “*that modest induction of the catalytic antioxidants SOD... may be a much more effective approach than supplementation with antioxidants (such as vitamins C and E) that can, at best, stoichiometrically scavenge a very small fraction of total oxidant production.*”<sup>12</sup>

This favorable increase in S.O.D seen in this study may be from the high concentration of polyphenols which are known to influence Phase I and II detoxication in the liver.<sup>13</sup> The increase of S.O.D. activity may also be related to the increase of metallic ions available for transport to liver (Mg, Zn, Cu).

### *Triglycerides Results*

The CPS formula is rich in oat beta glucan and lecithin (21% phosphatidyl choline). Both have been demonstrated to lower triglycerides separately.<sup>14,15</sup> Lowering of triglycerides by almost 20% is therefore consistent with published research.

As part of the pathogenesis of cardio vascular disease is the oxidation of blood lipids, a product that both lowers blood lipids and enhances antioxidant activity may prove useful in promoting cardio-vascular health.<sup>16</sup>

### **Conclusion:**

Supplementation with the complex phytonutrient supplement, **NanoGreens10**, demonstrated statistically significant and favorable increases on total antioxidant capacity in blood and SOD activity in the liver. It also caused a statistically significant and favorable drop in total triglycerides. The results are hypothesized to be a consequence of the synergistic effects of its complex and dense natural phytonutrient composition along with its naturally occurring micronutrient vitamins and minerals, and lecithin.

The Coordinator of the Project  
Alexander S. Krylov, Ph.D.  
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