# The Antioxidant Effects of PLGA-based Nanoparticles Loaded with Vitamin E in Rats Treated with Hypercaloric Diet

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*PLGA (Poli-lactic-co-glycolic acid) nanoparticles (NPs) are curently used as drug delivery systems for many types of drugs including antioxidants such as vitamin E. The main aim of our study was to test the antioxidant effects of PLGA-vitamin E on Wistar male rats. Two groups of Wistar rats received a hypercaloric diet for 21 days: the first group received besides the hypercaloric diet a daily dose of PLGA loaded with vitamin E and the control group received only the hypercaloric diet. Spleen cellular lysate has been used to detect biomarkers of oxidative stress such as malondialdehyde, glutathione, advanced human oxidative protein and vitamin E. After 3 weeks of treatment, statistical significant changes have been detected between the two groups.*

*Keywords: PLGA nanoparticles, spleen, hypercaloric diet*

Adopting a hypercaloric and hyperlipidic diet will finally lead to obesity. Obesity is a global health problem which presents many systemic complications such as type 2 diabetes, metabolic syndrome, cardiovascular disease, cancer, non-alcoholic fatty liver disease and even aging [1, 2]. The effects of obesity are greatly studied throughout the world, in humans and especially using laboratory animal studies (mice and rats) [3-6]. The antioxidant properties of vitamin E have been studied so far on different batches of laboratory animals that were obese, hypertensive or presented a metabolic syndrome. Carotenes are hydrophobic molecules in aqueous medium, therefore their absorption from the diet and antioxidant capacity are limited [7-11]. At the moment, many types of antioxidants can be loaded in the structure of PLGA, however vitamin E and lutein are of major interest [12]. PLGA-nanoparticles (NPs) are approved by FDA and currently used as drug delivery systems for cancer, cardiovascular, neuro-degenerative disorders, microbial, parasitic and viral infections, osteoporosis and even oral diseases. Bioavailability, biocompatibility, minimal toxic effects, sustained release and flexibility are the most important benefits of using PLGA [12-16]. An important role in nanoparticles' cellular uptake is played by size, electrical charge, solubility and even the way they are administered. In order to test PLGA properties, previous studies were performed on mice and rats that received NPs with different sizes at different period of times. Using intravenous administration, the NPs were present in liver, spleen, kidney, lung, heart and brain, but in different concentration depending on time and size. Between 0-5 to 24 h, PLGA NPs of 140-200 nm were accumulated mostly in the kidneys and lungs of the rats. Using mice to testing the bio-distribution of PLGA NPs having 120 nm, the results were very different from rats. They were present after 0.5-1 h in higher concentration in liver, then in the heart and the spleen. PLGA with a size of 96 nm after 1.5 to 3 h were present mostly in mice spleen [17]. The main finding of these studies is that an increased size of PLGA NPs conducts to a lower cellular uptake. Until now, many studies have been carried out on laboratory animals, with the liver being the most studied organ [4-6, 18]. The aim of our study was to test the antioxidant properties of PLGA-vitamin E administered to Wistar rats treated with a hypercaloric diet, but this time in the spleen. To the best of our knowledge, this is the first study with this approach made until the present time.

## Experimental part

PLGA nanoparticles synthesis and characterization has already been described in one previously article [18].

### Animal model

Group 1 included 5 Wistar male rats that had been treated during 3 weeks with a hypercaloric diet (butter and sugar) and a daily dose of PLGA-vitamin E (50mg/body weight). The control group was formed by 5 Wistar male rats that received only a hypercaloric diet during 3 weeks. Vitamin E loaded PLGA NPS have been administered by gavage. The rats were obtained from the Animal Facility of Carol Davila University of Medicine and Pharmacy, Bucharest, Romania and the experimental procedures were carried out under Convention 86/609/E.E.C. from November 24, 1986, for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes. After three weeks the rats were sacrificed and tissue samples (spleen) have been collected. We homogenated the spleen using KCl 25%.

### Oxidative stress biomarkers detection

Glutathione (GSH) was detected following the colorimetric reaction, based on the production of a yellow color when 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) was added to compounds with sulfhydryl groups. The absorbance was read at 412 nm [15].

The method used for malondialdehyde (MDA) was based on the reaction of MDA with thiobarbituric acid (TBA) by heating to produce a complex that can be determined spectrophotometrically [16]. Briefly 250$\mu$L of homogenate (spleen) were mixed thoroughly with 2.25 mL working

reactive (10 mL Tricholoroacetic acid (20%) and 30 mL TBA dissolved in $HClO_4$). For the controls, 250μL of KCl were methodically mixed with 2.25 mL working reactive. The samples and the controls were placed in boiling water for 20 min , cooled to room temperature, centrifuged at 5000 rpm for 10 min and the absorbance was measured at 532 nm.

Advanced human oxidative protein (AOPP) levels were determined using the ELISA method (kits were provided by RND-Germany). The microtiter plate is pre-coated with an antibody specific to AOPP. Standards and samples are added to the microtiter plate wells with a biotin-conjugated polyclonal antibody specific for AOPP. Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then, the TMB substrate solution is added to each well and incubated. The enzyme-substrate reaction was stopped by the addition of sulfuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm.

Vitamin E detection was done using the competitive ELISA method (kits provided by Biocompare -USA). This technique utilizes a monoclonal antibody for the target antigen and a target antigen for HRP conjugate. The samples and buffer were incubated together with the target antigen HRP conjugate in a pre-coated plate during one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme and a blue colored complex is formed. The stop solution is added and the final color became yellow. The target antigen concentration in each samples are interpolated from the standard curve.

*Statistical analysis*

Statistical analysis was performed using *Student's t-test* to compare and correlate clinical parameters with biochemical biomarkers. Statistical significance was set at a p-value $< 0.05$.

## Results and discussions

Reactive oxygen species (ROS) are harmful to the body when are present in a higher concentration and will interact with essential biomolecules such as DNA, proteins and carbohydrates [20]. Oxidative stress (OS) is installed into the human body in many systemic pathologies including obesity caused by a hypercaloric diet [3, 4, 6, 20].

To test the presence of ROS, many biomarkers can be used such as MDA, GSH, AOPP, enzymatic and non-enzymatic antioxidants. Obesity was, is and will be studied on humans and model animals. Over the past years, many cross-sectional studies have been completed in order to observe the direct relationship between obesity and OS [21-23].

MDA is the end product of the lipid peroxidation process, widely used as an indicator of lipid damage. Plasma levels of MDA were found to be increased in obese diabetic patients (men and women) *versus* the normal group, and the MDA / SOD ratio decreased in obese patients [24, 25]. Olusi and co-workers detected in obese patients (children and adults) increased plasma levels of MDA *versus* non-obese patients. They also observed decreased levels for superoxide dismutase (SOD) and gluthation peroxidase (GPX) in erythrocytes at morbidly obese patients [26]. Increased plasma levels for MDA and vitamin E had been detected by Myara and co-workers at obese patients [27]. Besides lipids, free radicals will also attack proteins, so an important biomarker of protein damage is AOPP. This biomarker that is not the end product of protein damage, represents an enhancer of tissue damage and has not been detected until now in animal studies. Piwowar A and co-workers detected higher levels of AOPP biomarkers at patients with type 2 diabetes [28]. Glutathione (GSH), one of the most important intracellular antioxidants displayed decreased levels in obesity in human and animal studies [3-5].
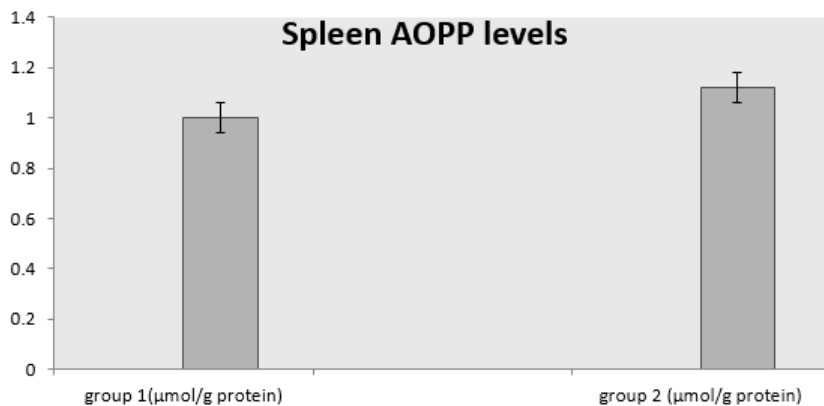


Fig. 1. AOPP levels obtained in spleen homogenates in the two groups of rats included in the study
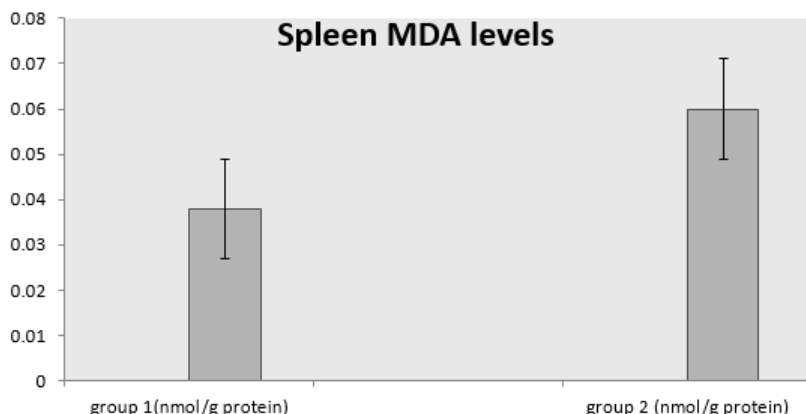


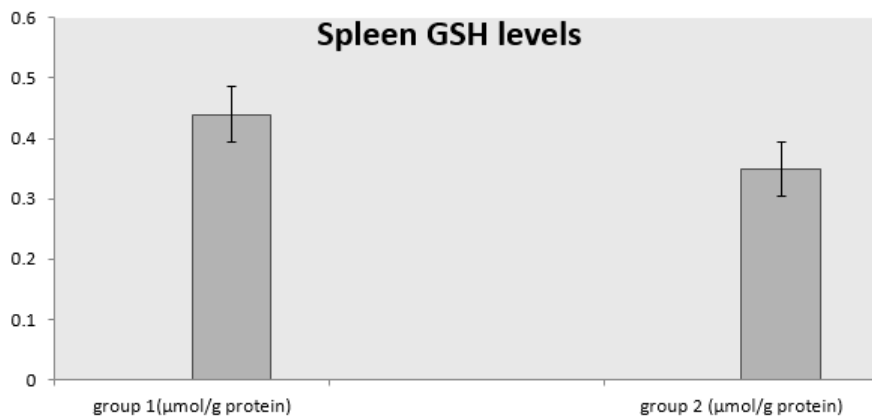Fig. 2. MDA levels obtained in spleen homogenates in the two groups of rats included in the study

Fig. 3. GSH levels obtained in spleen homogenates in the two groups of rats included in the study
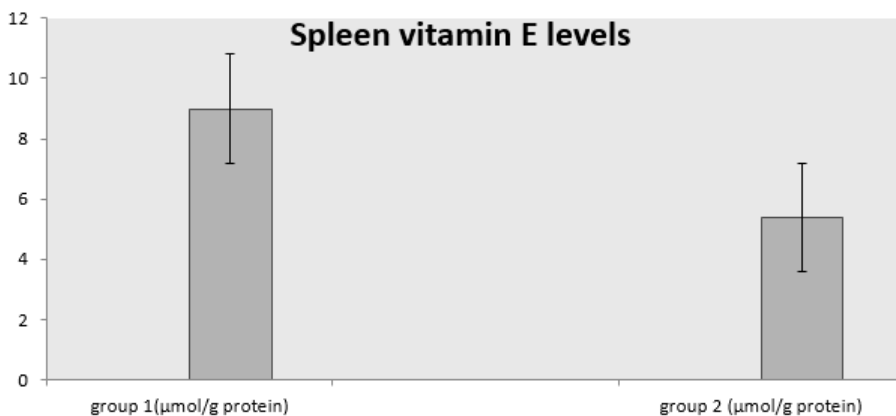


Fig. 4. Vitamin E levels obtained in spleen homogenates in the two groups of rats included in the study

Vitamin E possesses anti-oxidative, anti-inflammatory, anti-obesity, anti-hyperglycemic, anti-hypertensive and anti-hypercholesterolemic properties. These effects have been studied using laboratory animals that received a daily dose of vitamin E per os. Zhao and co-workers treated C57BL/6J male mice with high-fat, who received gamma-tocopherol 10 and 50 mg/kg. After 4 weeks of diet, obesity and hyperglycemia were significantly reduced, but the dyslipidemia did not show any changes [29]. Alcala et al. conducted a study that included obese mice fed on a high-fat diet and that received twice a week α-tocopherol (150 mg/kg) for 28 weeks. After this period, a significantly decrease of hyperglycemia and dyslipidemia and no effects on obesity could be observed [30]. Hasty and co-workers in 2007 did not notice changes in obesity, hyperglycemia and hyperlipidemia in a study using mice treated with a 42% fat diet that received vitamin E (2,000 IU/kg diet) during five weeks [31].

These inconveniences can be overcome by incorporating vitamin E into the PLGA structure. PLGA NPs are approved by USA FDA (Federal Food Administration) because their products of degradation formed usually by hydrolysis (lactic and glycolic acids) are eliminated from the body as carbon dioxide and water. In many studies conducted until now on rats and mice that received a hypercaloric diet, the liver had been the most studied organ [4-6, 18, 23].

In the present study, we also aimed to see the changes occurring in Wistar rats' spleen, treated daily during 3 weeks with a hypercaloric diet, but who have received a daily dose of PLGA NPs loaded with vitamin E. Our results revealed statistically decreased levels of MDA and AOPP at Wistar rats from group 1 *versus* group 2. Increased levels of MDA and AOPP for group 2 reflect the presence of OS illustrated by lipid peroxidation and protein oxidation processes. To test cellular spleen uptake and the release from PLGA NPs, we detected vitamin E at the two groups included in the study. Our results revealed increased levels of vitamin in group 1 *versus* group 2. The higher concentration of vitamin E for group 1 reduced OS, this being reflected also by the statistically increased level of GSH.

## Conclusions

In the 21st century, the population around the world including the Romanian people has a hypercaloric diet. OS is implicated in the pathogenesis of many systemic diseases, including obesity induced by a hypercaloric diet. PLGA NPs are important and promising carriers for many drugs including vitamin E. The spleen antioxidant status was greatly improved in group 1 of Wistar rats that received a daily dose of PLGA loaded with vitamin E *versus* the control group, thus having a protective role against the inflammatory processes involved in obesity and that can promote the development of other systemic disorders.

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