*Proceedings of the Nutrition Society* (2013), **72 (OCE1)**, E9 doi:10.1017/S0029665113000116

*6th International Workshop on Immunonutrition, 15th–17th October 2012, Palma de Mallorca*

# Ascorbic acid (Redoxon®) supplementation modulates gene expression in peripheral blood mononuclear cells specifically upon an inflammatory stimulus. A pilot study

R. Canali[1], G. Leoni[1], L. Natarelli[1], E. Azzini[1], L. Barella[2], O. Sancak[2] and F. Virgili[1]

[1]*National Research Institute for Food and Nutrition, Rome and* [2]*Bayer Consumer Care Ltd, Basel*

A significant amount of evidence suggests a positive association between ascorbic acid (vitamin C) status and health. The metabolic and biochemical properties of ascorbic acid have been extensively documented, most of which are associated with the chemical nature of the molecule. While the role of other vitamins, such as A, D or E as signaling molecules has been demonstrated, the role of ascorbic acid in gene expression modulation needs further investigation. In order to study the effects of vitamin C supplementation on gene expression and compare its effects between physiological and inflammatory conditions, a pilot study was set up utilizing microarray and qPCR technologies. Five healthy, non-smoker volunteers were supplemented with 1g vitamin C, Redoxon®, per day for five consecutive days. Before and at the end of the supplementation, blood samples from the subjects were collected under fasting conditions to isolate plasma and peripheral blood mononuclear cells (PBMNC). PBMNC were isolated from each subject and were split in three parts. One aliquot was used to isolate RNA for the Affymetrix gene 1.0 ST array analysis which allows examining the expression of 28,869 genes. The other two aliquots were resuspended in RPMI 1640 containing 10% of autologous plasma, one of which was incubated with an inflammatory stimulus (10 μg/ml LPS) for 5 hours at 37°C, while the second aliquot was used as a baseline control. To assess the effect of vitamin C supplementation on cellular response to inflammation, we have used a StellArray® system that allows the simultaneous evaluation of the expression of about one hundred genes by qPCR. This system measures the expression of genes that regulate the NFκB signaling pathway.

Vitamin C supplementation raised the mean plasma ascorbic acid concentration to 1.69 mg/dl from the mean baseline concentration of 0.87 mg/dl. Microarray experiments have shown that ascorbic acid supplementation resulted in 40 genes differentially expressed with a FC (Fold Change) difference of $\leq \geq 0.5$ and an ANOVA $p$ value $\leq 0.05$. Only one gene (CANX) was detected with a FC >1. Usually, in gene expression studies, the FC value threshold commonly considered indicative of a biological difference due to gene expression is $\leq \geq 1$. On the other hand, it is reasonable to assume that nutritional molecules widely consumed with the diet induce physiological/moderate changes on gene expression. Analysis of overrepresented biological pathways identified 16 differentially expressed genes related to the ribonucleoprotein complex biosynthesis, translation, RNA processing and chromatin organization. Moreover, 10 out of the remaining 39 genes are annotated in genomic databases as pseudogenes, characterized by the loss of their protein-coding ability. qPCR was used as a validation tool to confirm gene expression results obtained from microarray analysis. However, the small differences in FC observed suggest that the modulation in basal gene expression has little biological relevance. On the contrary to the moderate changes observed under physiological conditions; upon inflammatory activation, vitamin C supplementation resulted in a markedly different modulation of gene expression (FC $\leq \geq 1$). Specifically MyD88 dependent pathway which eventually leads to the activation of the NF-κB and AP1 transcription factors was differentially activated. This study demonstrates that ascorbic acid has, moderate influence on gene expression under physiological conditions, while it is associated with a significantly different gene expression profile upon inflammatory stimulation.