

# Effect of vitamins A, E and a citrus extract on *in vitro* and *in vivo* lipid peroxidation

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**Introduction:** Oxygen, though necessary for life, is a toxic substance. Fortunately, 98% of the oxygen is completely reduced to water in the process of respiration. The other 2% turns into potentially toxic free radical oxidants [1]. These substances can damage proteins, nucleic acids, carbohydrates, and polyunsaturated fatty acids. They can also directly reduce the level of the antioxidant defence molecules in the plasma [2]. Free radical-mediated lipid peroxidation has been implicated in several disease states including cancer, rheumatoid arthritis, postischaemic reoxygenation injury, as well as in the degenerative processes associated with atherosclerosis, diabetes and aging [3].

There has been a paucity of research on the effect of vitamin supplementation on lipid peroxides in humans. The purpose of this research was to examine several antioxidant (AOX) vitamins and their effect on *in vitro* and *in vivo* lipid peroxidation. Vitamins in a natural matrix were studied as they have been shown to have greater animal and human absorption and retention than synthetic vitamins alone [4].

**Materials and methods:** Vitamins and citrus extract were obtained from the Grow Company, Hackensack, New Jersey, USA. Vitamin A was retinyl palmitate in carrot concentrate (250,000 IU g<sup>-1</sup>). Vitamin E was tocopherol acetate in vegetable oils (250 IU g<sup>-1</sup>). Citrus extract was a dried water/alcohol extract of orangette fruit which contained 18.1% bioflavonoids (naringin, naringenin and hesperidin), 50% proteins and 25% carbohydrates.

In order to test for *in vitro* inhibition of lipid peroxidation, we used the procedure of Jain and McVie [5]. Red blood cells (RBC) were isolated from a single healthy individual. 100  $\mu$ L of RBC were incubated with 1 mL of isotonic solutions of normal glucose in Hanks Balanced Salt Medium (5.5 mM) or high glucose (55.5 mM) Hanks solution at pH 7.4 for 24 h at 37°C in a shaking water bath. Water soluble citrus extract was dissolved in the Hanks solution.

Fat-soluble vitamins A and E in methanol were ultrasonified with the Hanks solution. Following the incubation, the procedure of Jain and McVie [5] was used to measure lipid peroxides (LPO). Tetraethoxypropane was used to generate malondialdehyde *in situ* as a standard. The absorbance of the membrane filtered solutions from the RBC incubations was corrected for haemoglobin [6]. Lipid peroxides concentration was expressed as thiobarbituric acid reactive substances (TBARS).

Statistical significance was determined by a two-tailed *t*-test.

All subjects volunteered for the *in vivo* supplementation studies with informed consent. The protocol was approved by the Institutional Human Subjects Committee. Five normal disease-free subjects, four males and one female, aged 19 to 32 years, mean 28  $\pm$  5 years, participated in the single vitamin supplementation studies. Six subjects took part in the

Table 1: Effect of vitamins A, E and a citrus extract on glucose-induced *in vitro* red blood cell lipid peroxidation (means  $\pm$  SD; n in parentheses).

Incubation	Inhibitor (concentration)	TBARS ( $\mu$ M)
Normal glucose control, 5.5 mM (7)	—	5.96 $\pm$ 0.38
High glucose control, 55.5 mM (7)	—	8.27 $\pm$ 0.72*
High glucose (3)	Vitamin A (17.5 $\mu$ M)	2.15 $\pm$ 0.45**
High glucose (3)	Vitamin E (110 $\mu$ M)	6.92 $\pm$ 0.20**
High glucose (3)	Citrus extract (3.60 mg mL <sup>-1</sup> )	1.29 $\pm$ 0.11**

\* As compared with normal glucose control,  $p < 0.01$ .

+ As compared with high glucose control,  $p < 0.05$ .

combination vitamin study. There were five males and one female, aged 20 to 32 years, mean 26  $\pm$  5 years. 5/6 of the subjects were from the previous studies.

Each subject appeared after an overnight fast for a venous blood sampling into an EDTA tube. The plasma was frozen at -20°C until analysed a few days later. A 24 h urine was collected and refrigerated until analysis. TBARS were measured as in the *in vitro* study.

Each subject then consumed single vitamins for a period of 2 weeks and another fasting blood sample and 24 h urine sample was taken. A 2 week wash-out period then ensued followed by another sampling and vitamin supplementation for 2 weeks. Following the four single vitamin supplementations, a combination of A, E and citrus extract was taken for 2 weeks and sampling done as before.

Vitamins were consumed by volume with the following daily doses: Vitamin A 10,000 IU, Vitamin E 800 IU, and citrus extract 4 g. The combined supplement provided the same dose of vitamins A and E and citrus extract as in the single vitamin studies.

Statistics were performed using a two sample *t*-test.

**Results and discussion:** The results of the RBC incubation are shown in Table 1. The high glucose media produced a significantly higher amount of LPO than the normal glucose media as also shown by Jain and McVie [5]. This was hypothesised to be due to the greater concentration of NADPH in the hyperglycaemic media which stimulated the NADPH-dependent cytochrome P-450-like activity of haemoglobin. In turn, this caused an increase in oxygen radicals and a subsequent increase in lipid peroxidation [5].

Vitamins A and E were incubated at approximately 5 times the physiological concentration. Citrus extract was at a concentration of 3.60 mg mL<sup>-1</sup> which is equivalent to 0.65 mg mL<sup>-1</sup> bioflavonoids. All three of these substances gave significant inhibition of LPO in the high glucose medium when compared to the control.

Vitamin A and citrus extract were such effective inhibitors that they significantly decreased LPO in the high glucose media below that in the normal glucose control media. A

