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Oxidative stress status in vegetarians and non-vegetarians

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ABSTRACT
Oxidative tress has been implicated in the cause of many diseases as well as having impact on the body's aging process. This led nutritionists to suggest that vegetarian diet rich in antioxidants may provide a variety of health benefits and also, that vegetarians enjoy greater longevity than non-vegetarians. The present study was aimed at investigating the differences in the oxidative stress status between vegetarians and non-vegetarians using antioxidant enzymes level as biomarkers; hematological parameters were also examined. Vegetarians and non-vegetarians (15 volunteers for each group) were recruited for this study. Venous blood samples were collected before and two hours after meal for biochemical analysis. Result of the hematological examination showed no significant difference (P>0.05) in the packed cell volume (PCV) and white blood cell (WBC) count between the vegetarians and non-vegetarians. However, the plasma protein concentration in vegetarians was higher than those of non-vegetarians after meal. Furthermore, the plasma antioxidant enzymes; catalase (CAT) and superoxide dismutase (SOD) activity after meal in non-vegetarian group were significantly higher (P < 0.05) than before meal while the vegetarian group recorded no significant difference in both CAT and SOD activity after meal compared with before meal. From this study, it may be concluded that the vegetarian group had lowered oxidative stress status than non-vegetarian group.

KEYWORDS: *Antioxidants, Free Radicals, Oxidative Stress, Vegetarians, Non-Vegetarians*

INTRODUCTION

Oxidative stress (OS) is the term referring to a shift towards the pro-oxidant in the pro-oxidant/antioxidant balance that can occur as a result of an increase in oxidative metabolism (Ravindra *et al.,* 2004). It results in massive cell damage inducing cellular mutations, tissue breakdown and immune compromise (Valko *et al.,* 2005). Endogenous antioxidant defenses are inadequate to completely minimize the ongoing oxidative damage to DNA, lipids, proteins and other biological molecules. Hence diet-derived antioxidants may be particularly important in protecting against diseases resulting from cellular damages. The best way to ensure adequate intake of the antioxidant nutrients is through a balanced diet consisting of 5-8 servings of fruits and vegetables per day (Berg *et al.,* 2002). Furthermore, research had also shown that vegetarian diet which excludes meat, poultry, game and fish provides a number of health benefits in conditions related to heart disease, high blood pressure, adult-onset diabetes, obesity, osteoporosis, and certain cancers (Shils, 2005). It has also been observed that vegetarians in general, have lower serum total cholesterol and low density lipoprotein-cholesterol (Cariappa *et al.,* 2005). Therefore, this present study aims to investigate the plasma SOD and Catalase activities in vegetarians and non-vegetarians.

MATERIALS AND METHODS

A total of thirty healthy adults (vegetarians and non vegetarians) ages from 20-45yrs from Babcock University Community were recruited for this study. Whole blood samples (5 ml) were drawn with syringe into an EDTA bottle, before and after meal from the study group and biochemical analysis was done within 2hr.

Plasma-protein-concentration was determined by means of the biuret reaction as described by Gornal *et al.* (1949) with some modification: potassium iodide was added to the biuret reagent to prevent the precipitation of Cu^{2+} ions as cuprous oxide. The

resulting violet solutions were read in a spectrophotometer at 540nm. Bovine serum albumin (BSA) was used as standard.

Superoxide Dismutase (SOD): SOD activity in plasma was determined by the method of Mistra and Fridovich (1972). 0.5 ml plasma was diluted in 4.5 ml of distilled water (1:10) dilution factor. An aliquot of 0.2 ml of diluted plasma sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in a spectrophotometric curvette and the reaction was started by addition of 0.3 ml of freshly prepared 0.3 mM epinephrine. The reference curvette contained 2.5 ml of carbonate buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of distilled water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

Increase in absorbance per minute

$$= \frac{A_1 - A_0}{2.5}$$

Where A_0 = absorbance after 30 seconds

A_1 = absorbance after 150 seconds

$$\% \text{ inhibition} = 100 - 100 \times \frac{\text{Increase in absorbance for substrate}}{\text{Increase in absorbance of blank}}$$

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline and SOD levels were expressed as units/mg protein.

Catalase (CAT): Catalase activity was determined according to the method of Sinha (1972). The assay mixture contained 4 ml of H_2O_2 solution and 5 ml of phosphate buffer, pH 7.0 in a 10 ml flat bottom flask. 1 ml of diluted plasma sample was rapidly mixed with the reaction mixture by a gently swirling motion at room temperature. Immediately, 1 ml portion of the reaction mixture was withdrawn and blown into 2 ml dichromate/acetic acid reagent at 60s interval and optical density was measured using SpectrumLab 752S uv-visible spectrophotometer at 570 nm. CAT activity was expressed as moles of H_2O_2 consumed/min/mg protein.

Statistical Analysis

This was carried out with the aid of SPSS for Windows (Version 11, SPSS inc. Chicago, IL). All results were expressed as mean ± standard deviation. Student t test was used to test the significance of difference and the rejection criteria for the null hypothesis were fixed at probability level of 0.05.

RESULTS

The plasma protein concentration of the vegetarians increased significantly (P< 0.05) after meal when compared with the concentration before meal while there was no significant difference in the plasma protein content of non vegetarians before and after meal (Fig. 1). The assay result for antioxidant enzymes: superoxide dismutase (SOD) activity revealed an increased activity in the plasma of non vegetarians compared to vegetarians (Fig. 2). Catalase (CAT) activity in the plasma of non-vegetarians increased compared with that of vegetarians; however the CAT activity in the plasma of vegetarians before meal when compared to after meal was higher as shown on Fig. 3. The hematological data revealed no significant difference in packed cell volume (PVC) and white blood cell count (WBC) among the study groups (Table 1).

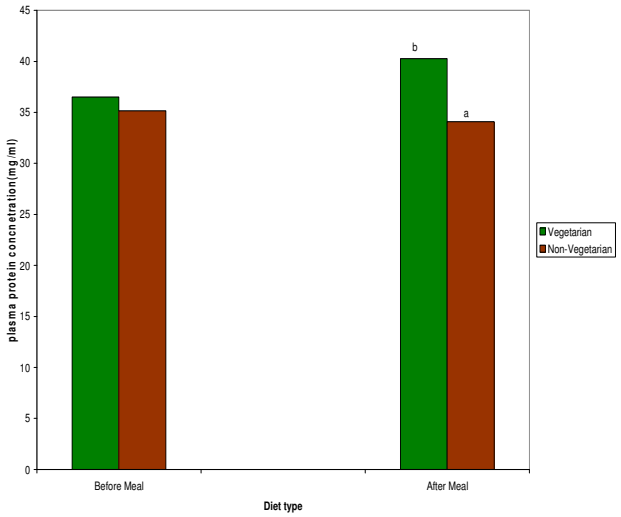

Fig 1: Plasma protein content of vegetarians and non vegetarians

[a] = significant difference at p<0.05 compared with vegetarians in after meal.

[b] = significant difference at p<0.05 compared with before meal in only vegetarians

Table 1: Data for Hematological analysis (PCV and WBC) before meal

Diet Type	% Packed cell volume (PCV)	White blood cell count (WBC)
Vegetarians	42.00 ± 2.64[ab]	4800 ± 888.82[ab]
Non-Vegetarians	42.13 ± 3.86[ab]	5287.5 ± 1379.00[ab]

a : - mean ± standard deviation

b :- no significant difference (P> 0.05) between vegetarians and non-vegetarians

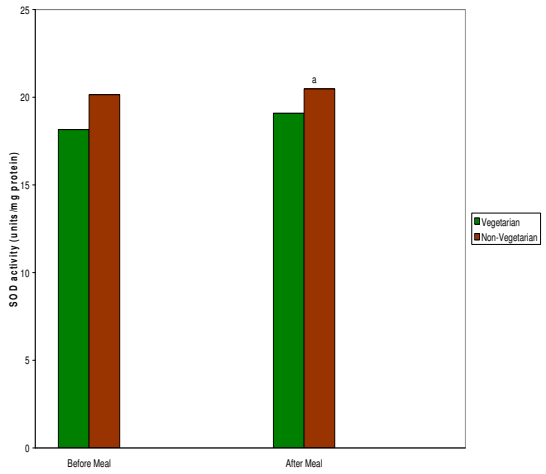

Fig. 2: Plasma superoxide dismutase activity in vegetarians and non vegetarians

[a] = significance difference at $p<0.05$ compared with vegetarians in after meal.

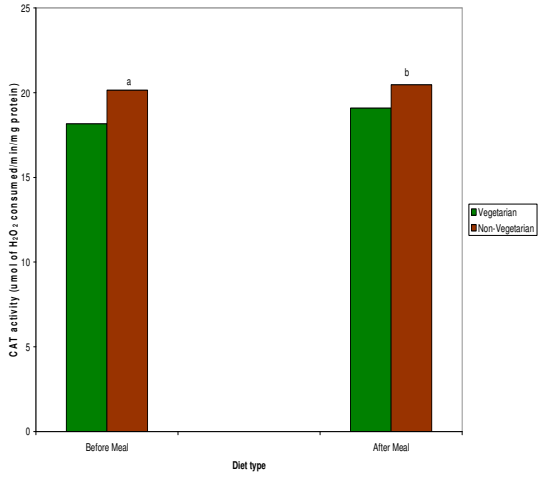

Fig.3: Plasma catalase activity in vegetarians and non vegetarians

[a] = significance difference at $p<0.05$ compared with vegetarians in before meal.

[b] = significance difference at $p<0.05$ compared with before meal in non-vegetarian

DISCUSSION

Epidemiological studies often report an inverse association between fruits and vegetables intake and disease risk (Khaw & Barrett-Cannor, 1987; Tribble, 1999). In the same vein, activities of antioxidant enzymes and the levels of free radical scavengers have been found to correlate with various physiological or pathological conditions, including oxidative stress (Czene *et al.,* 1997; Wojtaszek, 1997). It is well known that oxidative stress leads to a series of biochemical, physiological and behavioral changes, thus, altering normal body homeostasis (Boveris *et al.*, 1972). SOD is the antioxidant enzyme catalyzing the dismutation of the highly reactive superoxide anion to $O_2^{\cdot-}$ and to the less reactive species H_2O_2 (Teixeria, 1998) and Catalase catalyses the conversion H_2O_2 to form water and molecular oxygen (Urso & Clarkson, 2003).

The present study showed no significant difference in the superoxide dismutase activity before meal between vegetarians and non-vegetarians. However, an increase in SOD activity in non-vegetarians was observed after meal when compared to before meal as well as vegetarian. While there was no significant difference in catalase activity before and after meal of vegetarians, the catalase activity after meal in non-vegetarians was found to have increased significantly. These indicated an induced capacity of the antioxidant enzymes to scavenge superoxide anion and hydrogen peroxide produced in the blood cells in response to oxidative stress. A persistent oxidative stress which likely may be a compensatory mechanism to get rid of excess reactive oxygen species (Schlorff, 1999) may portends a danger to cell integrity. This is also in agreement with the results of collaborative meta-analysis which suggest that vegetarians may have a lower mortality than comparable non-vegetarians (Key *et al.,* 1999; Appleby, 2002). The hematological examination showed no significant difference in the packed cell volume (PCV) and white blood cell (WBC) count between the vegetarians and non-vegetarians (Table 1), and the values are within the accepted values of PCV and WBC for healthy individuals (Satish, 2004) before the commencement of study. In conclusion, the present data indicate that oxidative stress as measured by activities of superoxide dismutase and catalase may be lower in vegetarians than non vegetarians.

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