



## Serum Malondialdehyde (MDA) Level in Healthy North-Central Nigerian Adults after Six Months Nutraceuticals and Functional Foods Intervention

Carole Chibuzo Nweze<sup>1,\*</sup>, Mariam Solomon<sup>2</sup> and Alqasim Abdullahi Mustapha<sup>1</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi, Nigeria.

<sup>2</sup>Department of Biochemistry, University of Jos, Nigeria.

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### ABSTRACT

To assess the long-term effects of antioxidants in individuals according to age and sex distribution. We studied the effects of antioxidant functional foods and nutraceuticals on Malondialdehyde (MDA) level. The studied subjects included a total of 150 healthy adults of 96 men and 54 women aged between 30 and 74 years. At the baseline visit, eligible candidate were randomized to either 1 capsule per day of antioxidant nutraceutical or antioxidant functional foods or placebo, and the first dose was dispensed and followed up for six months. The results were grouped based on gender and age ranges, positive decrease in the MDA after antioxidant dietary intervention and lower concentration in men than in female at some age ranges was observed. We suggest the use of antioxidant functional foods as an alternative to antioxidant nutraceuticals/functional foods in reducing the effects of MDA in either healthy or stressed individuals.

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### Introduction

Antioxidants are essential for normal metabolic processes such as physiological functions (Lucas, Morales & Velando, 2014). Moreover, small amount of certain non-energetic macronutrients are essential for normal metabolic processes (Senar *et al.* 2010). Antioxidants such as vitamin C and E respectively are however cannot be synthesized naturally by vertebrates, and must be obtained through dietary sources (Evans & Halliwell, 2001; Surai, 2002). Deficiencies in either of the antioxidants have been linked to an increased risk of many diseases (Ames, 2006; Christian & Stewart, 2010; Isaksson, Sheldon & Uller, 2011).

There is a critical balance in the generation of oxidative stress and immune functions via antioxidants defense system under normal physiological conditions (Yang *et al.* 2008). However, the rat of damages caused by oxidative stress increases during aging processes (Okoduwa *et al.* 2015) as the efficacy of antioxidants defense and repair mechanisms decreases (Gil *et al.* 2006). The protective effect of antioxidants against several chronic conditions such as cardiovascular disaesas has been known (Dauchet, Amouyel & Dallongeville, 2006; Sasazuki, 2001; Law & Morris, 1998; Knekt *et al.* 1994) and also reported to reduce malondialdehyde (MDA) level thereby leading to reduction of inflammatory-related diseases (Keli *et al.* 1996; Steinmetz & Potter, 1996; Hertog *et al.* 1993). However, little is known on the effect of antioxidants nutraceuticals and functional foods on MDA. This study is aimed at investigating the effects of sex and age on serum MDA level in healthy North-Central Nigerian adults after six months nutraceuticals and functional foods intervention.

### Materials and Methods

#### Study Area

The study was conducted in Nasarawa State, Nigeria. Nasarawa State is located in the north central geopolitical zone of Nigeria. It lies between latitude 8°35'N and longitude 08°36'E. It is bounded to the North-west by Federal capital territory (FCT), Abuja, and to the North-east by plateau state, to

the South-east by Taraba state and to the North by Kaduna state, to the South by Benue state and to the South-west by Kogi state. It has a land mass of 21,117 square kilometre with a population of 2, 100, 000 making it the 10<sup>th</sup> largest state in Nigeria according to Nigerian 2006 population census.

#### Nutraceutical and Functional Foods Intervention

The volunteers were randomly assigned to three groups. Group 1 (control group) received oral antioxidant nutraceutical (Forever living product) 1 capsule per day (containing vitamin E 10mg, vitamin C 60mg and β- carotene 2,000mcg of vit A). Group 2 (treatment group) received antioxidant functional foods of equivalent vitamin composition oranges, carrots, and soybean).

#### Study Population

The study subjects included a total of 150 healthy adults of 96 men and 54 women aged between 30 and 74 years. All volunteers are staff of Nasarawa State University, Keffi.

#### Study Design, Inclusion and Exclusion Criteria

A randomized, prospective, parallel group, comparative, open dose and single centre study was undertaken by the 150 healthy subjects (96 men and 54 women). The inclusion and exclusion criteria were that the subjects had no history of gastrointestinal surgery, or other significant pathology, were non-smokers, had no history of alcohol or drug abuse, were non-diabetic, were not on a calorie-reduced or vegetable diet nor were taking antioxidant/vitamin supplement, female were not pregnant or lactating. No concomitant medication was allowed throughout the study except contraceptive pill.

#### Ethical Review and Independent Monitoring

The scope, nature, aim and objectives of this study were thoroughly explained to voluntary participants for their consent, and all of them were made to sign an informed consent letter and a questionnaire. The protocol was reviewed and approved by the Chairman Ethical committee, Federal Ministry of Health Abuja through the Chief Medical Director, Hospital Management Board Lafia Nasarawa State and the Medical Director, Nasarawa State University Medical Clinic, Keffi. The study was

independently monitored by an Ethical committee desk officer from Federal Ministry of Health, Abuja according to Quality Assurance programme such that Good clinical practice were followed throughout the one year study.

#### Specimen Collection and Laboratory Analysis

Three hundred (300) volunteers' venous blood samples were taken by local physicians from the university staff clinic in 3rd week of June, 2012 and clinical records were taken. Volunteers with desirable health status were chosen. Most of the people not chosen have either HIV+ve, hepatitis B or C, very high/low blood sugar ( $\geq 7.77$  and  $\leq 3.33$ mmol/L), extremely low/high blood pressure ( $< 100/60$  mmHg and  $> 140/95$ mmHg), those that are not sure of their date of birth. Some due to personal reasons refuse to participate. At the end we had 180 assumed healthy participants.

#### Six Months Dietary Intervention and Samples Collection and Evaluation

Baseline samples were collected six months ago and the blood parameters analysed as reported in these six months antioxidants dietary intervention. Six months blood samples were drawn from the volunteers who are assumed healthy according to their groups, after a 12-14 hour fast, in a 0.1% EDTA tubes, Lithium hyperinized bottles, and sterile bottles for biochemical analysis. Volunteers were six months ago randomly assigned to groups of three. Control group volunteers were given antioxidant nutraceuticals ((Forever living product) 1 capsule per day of vitamin E (10mg), vitamin C (60mg) and  $\beta$ - carotene (2,000mcg of vitamin A)). The treatment group volunteers were given antioxidant functional foods of equivalent vitamin composition (oranges (100g), carrots (100g), and soybean drink (75cl) and 1 heaped table spoon of soybean powder (35g/day)). The placebo group volunteers were giving clean drinking water (ordinary Swan table water (75cl) with no antioxidants).

#### Analysis of Immunological Status

Lipid peroxidation (Malondialdehyde, MDA) level in serum were estimated spectrophotometrically using Thiobarbituric acid- reactive substances (TBARS) method as described by Ohkawa, Ohishi & Yagi, 1979).

#### Statistical Analysis

Pair-wise comparisons of the means were validated using Analyze-It for Microsoft Excel Version 10, where a p-value  $< 0.05$  was considered statistically significant. Further Post hoc test like the Fischer's least significant difference (LSD) was used together in the analysis of variance (ANOVA).

Post hoc test (LSD) are designed for situations in which researcher has obtained a significant omnibus F-test with a factor that consists of three or more means and additional exploration of the differences among means so as to provide specific information on which means are significantly different from each other.

#### Results

Serum malondialdehyde (MDA) results after six months intervention of antioxidants nutraceuticals and antioxidant functional foods are shown in table 1. MDA level in serum showed a positive decrease after antioxidant intervention. MDA in blood serum showed that there was a significant decrease in different sexes after treatment with LSD-Male (p-value) 0.44 (0.0003) and LSD-Female (p-value) 0.28 (0.0002) except for age with LSD-Gender (p-value) 0.94 (0.8778) with no significant decrease. The results of MDA after treatment also showed that their concentrations in the blood are lower in younger males at age range of 30-39 (Male =  $0.90 \pm 0.03$ ) and 40-49 (Male =  $0.10 \pm 0.05$ ) than in females (Female =  $1.10 \pm 0.04$ ) and (Female =  $1.12 \pm 0.05$ ) of the same age ranges respectively. However,

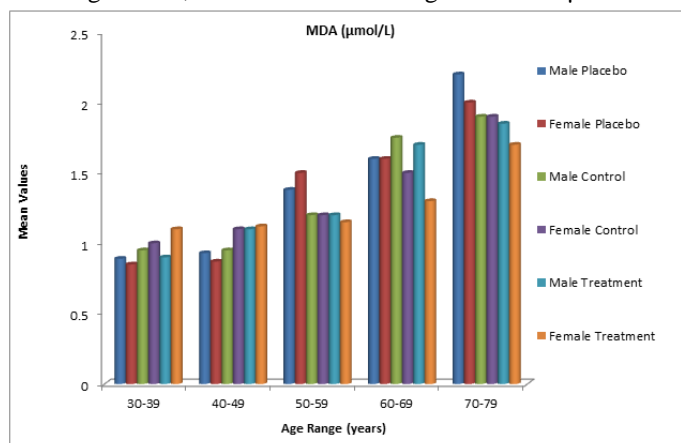
higher concentration of MDA in older males was seen at 50-59 (Males =  $1.20 \pm 0.07$ ), 60-69 (Males =  $1.70 \pm 0.03$ ) and 70-79 (Males =  $1.85 \pm 0.01$ ) than in females (Female =  $1.15 \pm 0.06$ ), (Female =  $1.30 \pm 0.02$ ) and (Female =  $1.70 \pm 0.00$ ) of the same age ranges respectively.

**Table 1. Effect of antioxidant nutraceuticals and functional foods on Serum Malondialdehyde (MDA) ( $\mu\text{mol/L}$ ) after six months treatment**

PLACEBO (n=31)	CONTROL (n=32)	TREATMENT (n=31)	PLACEBO (n=19)	CONTROL (n=18)	TREATMENT (n=19)
Male			Female		
0.89 $\pm$ 0.04	0.95 $\pm$ 0.02	0.90 $\pm$ 0.03	0.85 $\pm$ 0.03	1.00 $\pm$ 0.03	1.10 $\pm$ 0.04
0.93 $\pm$ 0.09	0.95 $\pm$ 0.05	0.10 $\pm$ 0.05	0.87 $\pm$ 0.08	1.10 $\pm$ 0.04	1.12 $\pm$ 0.05
1.38 $\pm$ 0.07	1.20 $\pm$ 0.04	1.20 $\pm$ 0.07	1.50 $\pm$ 0.06	1.20 $\pm$ 0.06	1.15 $\pm$ 0.06
1.60 $\pm$ 0.06	1.75 $\pm$ 0.03	1.70 $\pm$ 0.03	1.60 $\pm$ 0.02	1.50 $\pm$ 0.03	1.30 $\pm$ 0.02
2.20 $\pm$ 0.02	1.90 $\pm$ 0.00	1.85 $\pm$ 0.01	2.00 $\pm$ 0.00	1.88 $\pm$ 0.01	1.70 $\pm$ 0.00

The LSD- Gender (p-value): 0.94 (0.8778\*); LSD-Male (p-value): 0.44 (0.0003) and LSD-Female (p-value): 0.28 (0.0002);

\* Not Significant; Normal/reference range: 0.88-1.61 $\mu\text{mol/L}$



**Figure 1. Effect of antioxidants on MDA after six months treatment**

#### Discussion

This randomized, prospective, parallel group, comparative, open dose and single centre study was conducted to determine the effects of antioxidant nutraceuticals and functional foods intervention on Malondialdehyde MDA of healthy individuals in relation to age and sex distribution.

In the present study, the MDA level was evaluated. Table 1 showed that MDA increases with age. The result showed that the younger males have lower malondialdehyde than younger females. Figure 1 showed a significant decreased on the MDA level with antioxidant dietary intervention with the antioxidant functional foods given better results than the antioxidant nutraceuticals.

Malondialdehyde (MDA) is a secondary product of lipid peroxidation and is used as an indicator of tissue damage. MDA results agreed with a previous work that plasma thiobarbituric acid reacting substances increases with age, indicating increases lipid peroxidation (Coudray *et al.* 1997). Previous studies showed that patients with immune diseases have higher increase in MDA with increase in ageing than healthy ageing adult group (Prasher, Pandev & Gupta, 1993; Cohen, Olin & Feur, 1994). The raw result also showed that males have higher MDA concentration than females. The plasma level of MDA is a reliable and common biomarker of the overall lipid peroxidation. Nielsen, Mikkelsen & Nielsen (1997) showed that increased plasma MDA levels in ageing is not only consistent with the role of oxidative stress in ageing but also supports the idea that plasma MDA levels may be used as a marker of oxidative stress on immunological studies.

In conclusion, data of the present study suggest that the dietary intervention of antioxidant nutraceuticals and functional

foods intervention is likely to reduce the level of MDA. These findings also showed possible effects of age and gender on MDA of healthy individuals treated with antioxidant diets and supplements and this should open a way for more research on antioxidants functional foods and nutraceuticals. Findings from this study showing lower levels of MDA among men, point out to an advantage of males in relation to females. We recommend more intake of antioxidant functional food as an alternate to antioxidant nutraceuticals for both healthy and stressed individuals.

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#### Conflicts Of Interest

The authors declare no conflict of interest.

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