

OXIDATIVE STRESS STATUS IN OBESITY ALGERIANS POPULATION

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Article Received on
22 July 2014,

Revised on 28 July 2014,
Accepted on 06 August 2014

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ABSTRACT

Methods: To realise this study, a survey of anthropometric measurement was carried out on 61 subjects with a BMI ≥ 25 , aged 20 to 67 years, were recruited at the city of Ain Fakroun, and located in eastern Algeria. Blood assays include at fasting blot biochemical assays, vitamin E, A, and antioxidant status. **Results:** Antioxidant serum levels of vitamins E/A, GSH and catalase activity is higher among overweight subject than in obese subject (BMI ≥ 30) with increase in the MDA levels ($P < 0.05$). Catalase activity is significantly ($P < 0.05$) lower in the obesity group; however the SOD activity was not decreased. Plasma levels of total cholesterol, triglycerides and fasting glucose were significantly higher in obese subjects ($P < 0.05$). A reduced antioxidant status in women serum is

associated with an increase in the percentage of physical inactivity ($P < 0.05$). According to these results, women are in the early stages of obesity against the latter persists long in men. Blood pressure was positively associated with waist circumference, obesity and age, obesity increases with age ($P < 0.05$). **Conclusion:** These preliminary results reveal that obesity reduces the serum levels of antioxidant status.

KEYWORDS: Obesity, Oxidative stress, clinical and biochemical characteristics.

INTRODUCTION

Obesity is a major public health problem the world over and its prevalence has more than doubled since 1980^{1,2}. According to the WHO, in 2010, there were 1.5 billion adults (20 years and above) who were overweight with 300 million females and 200 million males being obese¹. Obesity has several health consequences; it is a major risk factor for the global burden of non-communicable diseases including diabetes, heart diseases, hypertension, stroke and some cancers³. Obesity and overweight are the fifth leading causes of global deaths with about three million adults dying each year from being obese or overweight¹. Other health related problems associated with obesity include increasing disability adjusted life years, premature death, reduction in life expectancy⁴ and high cost of health care⁵.

The increase in body fat, especially intravisceral, insulin resistance, chronic inflammation, endothelial dysfunction and hypertension are the main factors of increased oxidative stress in patients with metabolic syndrome^{6,7,8}. Laboratory tests of overweight subjects (BMI > 25) indicate a higher oxidative stress than in subjects with BMI < 25⁹. Indeed, several studies have reported higher lipid peroxidation markers^{10,11} and lower antioxidant status^{12,13} for overweight and obese adolescents as compared to their non-obese counterparts.

In the general population, several groups at risk of deficiency antioxidant status were identified. Among the main causes of deficits, there are low dietary intakes of antioxidants and increasing the free radical production increases need (overweight subjects, insulin resistance, the elderly, smokers, or diseases such as diabetes, hypertension ...) ¹⁴.

So obesity and oxidative stress are closely linked, there are many arguments more convincing to associate the treatment of obesity and especially the prevention of metabolic and cardiovascular an effective fight against oxidative stress complications. From the perspective of reducing the incidence of obesity in our society, the present study aims to evaluate oxidative stress status in obesity patients.

MATERIALS AND METHODS

1. Subjects and anthropométric measurments

We recruited 61 subjects with a BMI \geq 25 for both sexes, higher age of 18 years (20-67), the city of Ain Fakroun, located in eastern Algeria. After taking anthropometric parameters (weight, height, waist circumference, blood pressure), an information sheet was prepared for

each individual in order to identify risk factors as: (age, sex, the presence or absence of personal and family history).

2. Samples collection

Blood samples were collected from an antecubital vein in two different heparin tubes at fasting state. The tubes were centrifuged at 3000 g for 10 minutes. The supernatant was collected in tubes without anticoagulant and stored at -80 ° C for further analysis: lipid profile (triglycerides, total cholesterol, high density lipoproteincholesterol [HDL-C], low density lipoproteincholesterol [LDL-C], glucose), vitamin E, A and antioxidant status.

3. HPLC estimation of vitamin E and A

The serum collected was used to explore the status of two antioxidant micronutrients; the vitamins E and A serum; was assayed by HPLC (high performance liquid chromatography) procedure according to the method of Steghens¹⁵. The chromatographic conditions were as follows: C18 column, 4,6 mm × 15 cm, 3µm; injection volume 20 µl, mobile phase: 100% methanol, rate 1.5 ml / min, retention time 11 min. The measuring wavelength: 295 nm for the vitamin E, 325 nm for vitamin A.

4. Biochemical characteristic and lipid profile

Fasting blood glucose and lipid profile were assayed using an automatic biochemistry (Eos Bravo plus).

5. Oxidative stress parameters

Reduced glutathione (GSH) level

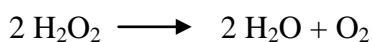
The assay principle is based on glutathione reduction of 5,5'-dithiobis (2 - acid) by nitrobenzoic acid (DTNB). The formation of 5-Thio-2 nitrobenzoate (TNB) is followed spectrophotometrically at 412 nm¹⁶.

Malondialdehyde (MDA) level

TBARS were measured by assaying the rate of MDA by the method of Lefevre¹⁷. The intensity of pink color measured at 532 nm by condensation with thiobarbituric acid in an acid medium, hot.

Catalase (Cat) activity estimation

The catalase activity was determined by the method described by Aebi¹⁸. This method is based on measuring disappearance of hydrogen peroxide due to the catalase activity according to the following reaction:



The serum was added to a cuvette containing phosphate buffer prepared in 0.1 M (pH 7.4) and a solution of H_2O_2 in 0.5M. Catalase activity was measured at 240 nm for 1 min using the spectrophotometer (UV 6715 / Vis. JENWAY Spectrophotometer). The blank is made on the same solution to which was added 20 μL of phosphate buffer instead serum. The molar extinction coefficient of H_2O_2 0.043 $\mu\text{M}/\text{ml}$ was used to determine the catalase activity. One unit of activity is equal to one micromole of H_2O_2 degraded per minute and is expressed as micromol $\text{H}_2\text{O}_2/\text{mn}/\text{ml}$.

Superoxide dimutase (SOD) activity estimation

SOD activity was estimated by Marklund¹⁹ methods. A standard range of SOD is performed under the same conditions. One unit of SOD activity is 50% inhibition of the rate of autooxidation of pyrogallol as determined by change in absorbance / min at 420 nm. SOD activity is expressed as units / min / ml.

Electrophoresis in polyacrylamide gel and revelation of antioxidant enzymes

Native Polyacrylamide gels were prepared essentially according to the procedure of Laemmli²⁰, under no denaturing conditions (without SDS). Each gel was composed of a stacking gel and a separation gel of respectively 5% and 10% acrylamide. The migration is done at 4 ° C under constant amperage of 40 mA. Gels were first incubated in the dark for 30 minutes in a freshly prepared solution containing 50 mM potassium phosphate (pH 7.0), 0.1 mM EDTA, and 0.5 mM nitroblue tetrazolium. The gels were after that transferred to a second fresh solution (50 mM potassium phosphate (pH 7.0), 0.1 mM EDTA, 5 mM H_2O_2 , and 10 mM ascorbic acid) in light until appearance of white bands on purple background (Figure 1) characteristics of the catalase activity in the serum of patients. The gels were finally rinsed in deionised water several times.

Statistical analysis

Data were analyzed using Epi Info Version 3.5.3 for the calculation and comparison of means. $P < 0.05$ was considered statistically significant.

RESULTS

Anthropometric measurements

Table 1 shows the general characteristics of the subjects recruited.

Table 1. Anthropometric measurements of study participants

variables	Men Mean \pm SEE	Women Mean \pm SEE	Total sample Mean \pm SEE
Ages (years)	41,27 \pm 11,75	36,85 \pm 12,11	37,66 \pm 12,07
Sex (%)	18,03	81,97	
Weight (kg)	94,00 \pm 12,58	80,00 \pm 14,59	82,56 \pm 15,16**
Height (cm)	171,27 \pm 5,10	160,20 \pm 5,20	162,23 \pm 6,71**
BMI (kg/m ²)	32.01 \pm 3.80	31.1 \pm 5.04	31.26 \pm 4.82
Waist circumference (cm)	106,54 \pm 6,63	104,85 \pm 10,44	105,16 \pm 9,83
Mean Blood Pressure (mm Hg)	100,90 \pm 15,13	96,32 \pm 11,40	97,16 \pm 12,15
Overweight (%)	10.71	89.29	45.9
Obesity (%)	24.24	75.76	54.1
Family history (%)	11.54	88.46	86.66
Tobacco (%)	72.73	00	13 .33
Alcohol (%)	00	00	00
Sedentarity (%)	54.55	93.88	86.7**

Abbreviation: BMI: body mass index, SEE, Standard error of estimate, * Significant at $p < 0.05$, ** Significant at $p < 0,004$.

Distribution of subjects according to the type of disease:

In our study population, we found 83.05% of normal individuals and only 16.95% of individuals have a disease. Among these, we include:

1. Hypertensive individuals (10%)
2. Hypertensive diabetics (10%)
3. The diabetic individuals (30%)
4. Dyslipidemia (20%)

86.44% of those recruited have a family history; 80% are women.

Oxidative stress parameters

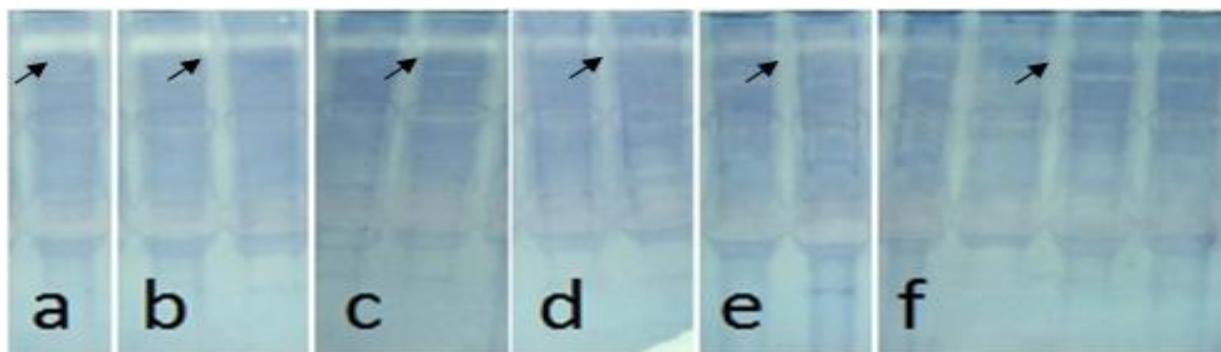


Fig. 1. Representative native gels stained for the activity of catalase.

Bands (a), (b), (c) and (d) represent individuals with a BMI < 30 kg / m², the bands (e) and (f) correspond to individuals with a BMI ≥ 30 kg / m². The analysis of the electrophoretic profile shows the presence of Cat activity in the majority of overweight individuals (figure (a) - Figure (d)), Quantification of the band intensities revealed that bands (a) and (b) are more intense than the bands (C), (d), and (e); so enzyme activity decreases with increasing BMI, there bands very low intensity in Part (F) in obese individuals (BMI ≥ 30 kg / m²). Variations in lipid profile are set out in Figure 2. As shown in Figure 2, we found an increase in fasting blood glucose and lipid profile (total cholesterol, triglycerides, LDL) in obesity group of both sexes with a decrease in plasma HDL. Plasma levels of triglycerides and glucose were significantly higher in this group.

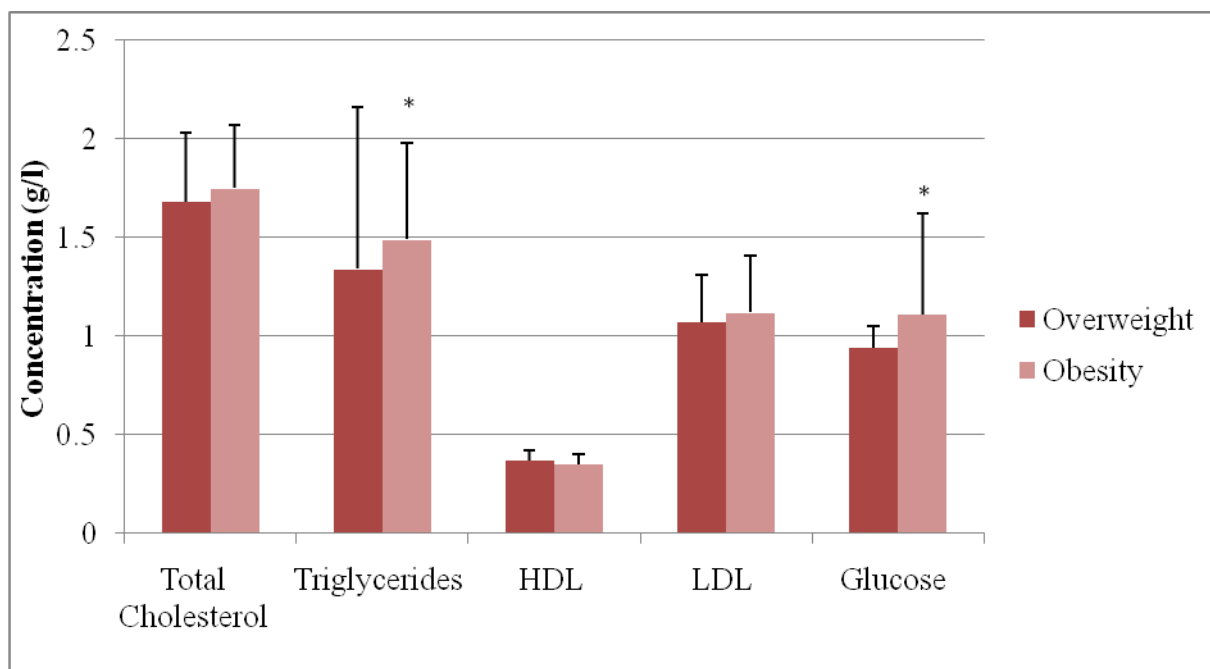


Fig. 2. Lipid profile in subjects according to BMI.

The comparison of the pro-oxidant and antioxidant markers between overweight and obesity groups are shown in Table 2. This assessment shows that overweight women ($25 \leq \text{BMI} \leq 30$) have decreased plasma concentrations of antioxidant vitamins E / A, GSH, SOD and catalase compared with overweight men and a high plasma concentration of MDA. The difference is statistically significant at $p < 0.05$ between the two sexes for serum levels MDA, triglycerides and glucose. The antioxidant enzyme catalase activity is almost 3times more decreased in obese men with a BMI ≥ 30 kg / m².

Table 2. Oxidative stress parameters and lipid profile in subjects according to BMI and sex

	25 ≤ BMI ≤ 29.9		BMI ≥ 30 kg/m ²	
	Man Mean ± SEE	Woman Mean ± SEE	Man Mean ± SEE	Woman Mean ± SEE
Vitamin E (mg/l)	13,20 ± 1,75	10,60 ± 3,25	11,03 ± 3,85	11,01 ± 2,95
Vitamin A (mg/l)	0,77 ± 0,25	0,45 ± 0,13	0,53 ± 0,17	0,51 ± 0,13
GSH (µg/ml)	560,51±116,15	531,63±118,03	582,30 ± 109,45	516,11± 101,93
MDA (nmol/ml)	35,02 ± 1,37	41,83± 15,59	37,58 ± 9,15	46,97 ± 13,58*
SOD (Unit)	8,86 ± 2,35	7,97 ± 4,63	7,25 ± 3,25	10,11 ± 4,14*
Cat (Unit)	12,21 ± 10,62	5,52 ± 3,51	3,84 ± 1,15	6,72 ± 5,86
Total cholesterol (g/l)	1,88 ± 0,35	1,61 ± 0,29	2,07 ± 0,43	1,70 ± 0,30
Triglycerides (g/l)	1,71 ± 0,62	1,07 ± 0,49	2,72 ± 0,82*	1,40 ± 0,41*
HDL (g/l)	0,33 ± 0,07	0,38 ± 0,04	0,32 ± 0,05	0,36 ± 0,05
LDL (g/l)	1,19 ± 0,32	1,04 ± 0,23	1,23 ± 0,31	1,07 ± 0,27
Glucose (g/l)	0,99 ± 0,19	0,93 ± 0,09	1,17 ± 0,31	1,08 ± 0,57*

* Significant at $p < 0.03$.

It appears from the Table 2 that the serum levels of vitamins E/A and antioxidant enzyme activity is higher in men with a $25 \leq \text{BMI} \leq 30 \text{ kg} / \text{m}^2$ as obese men ($\text{BMI} \geq 30 \text{ kg} / \text{m}^2$) who also have elevated serum MDA and GSH versus overweight ($25 \leq \text{BMI} \leq 29.9$). However in women, the serum levels for both vitamins are close for overweight and obesity. Serum GSH and catalase activity are decreased in obese women ($\text{BMI} \geq 30 \text{ kg} / \text{m}^2$) as they have a statistically significant elevated serum ($p < 0.05$) of MDA and SOD.

Correlations between the different parameters of subjects

It is apparent that the serum vitamin E increases with the mean arterial pressure (+0.451), serum vitamin A (+0.417), age (+0.365), TC (+0,726) and TG (+0,519); waist circumference increases with age (+0.406); blood pressure is positively associated with obesity (+0.402), waist circumference (+0.374) and age (+0.385); Obesity increases with age (+0.338), The plasma TG level is positively associated with total cholesterol (+0,571).

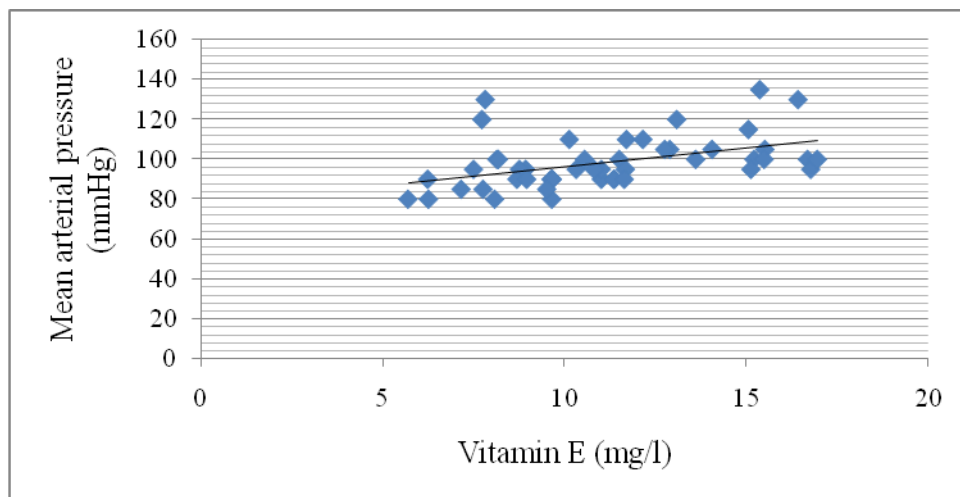


Fig. 3. Correlation of blood pressure with vitamin E ($R^2 = 0,204$, $p = 0,001$).

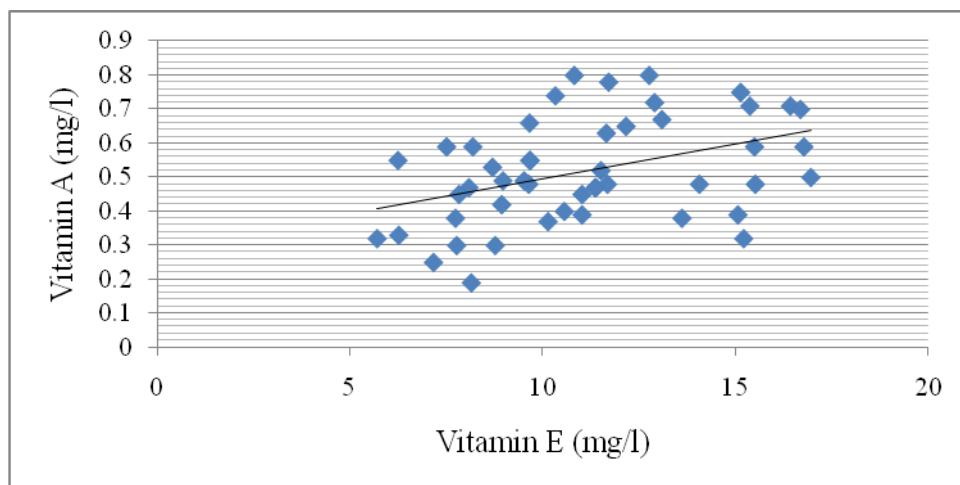


Fig. 4. Correlation of vitamin A with vitamin E ($R^2 = 0,174$, $p = 0,003$).

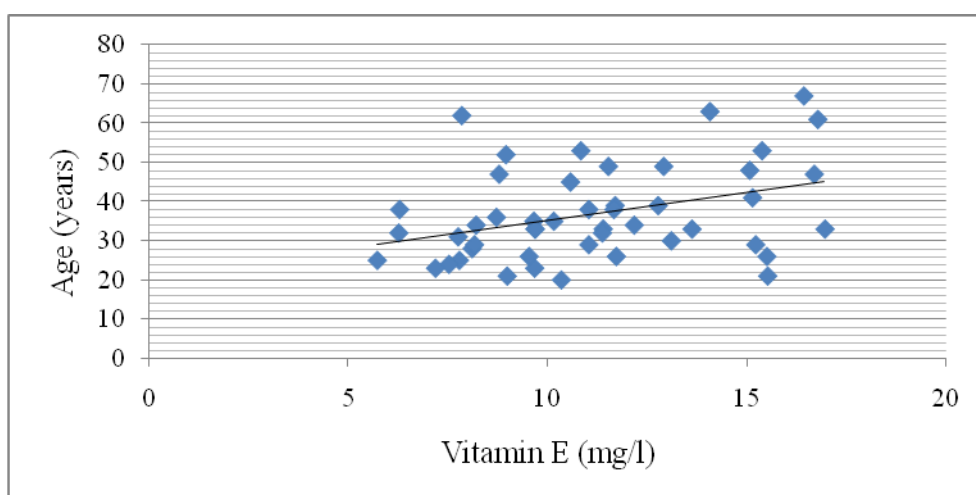


Fig. 5. Correlation of age with vitamin E ($R^2 = 0,133$, $p = 0,01$).

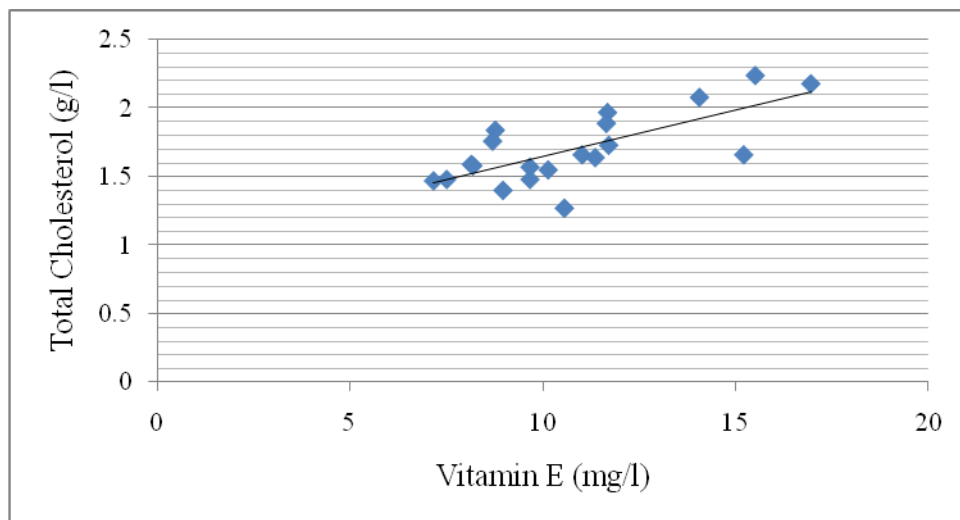


Fig. 6. Correlation of total cholesterol with vitamin E ($R^2 = 0,513$, $p = 0,0006$).

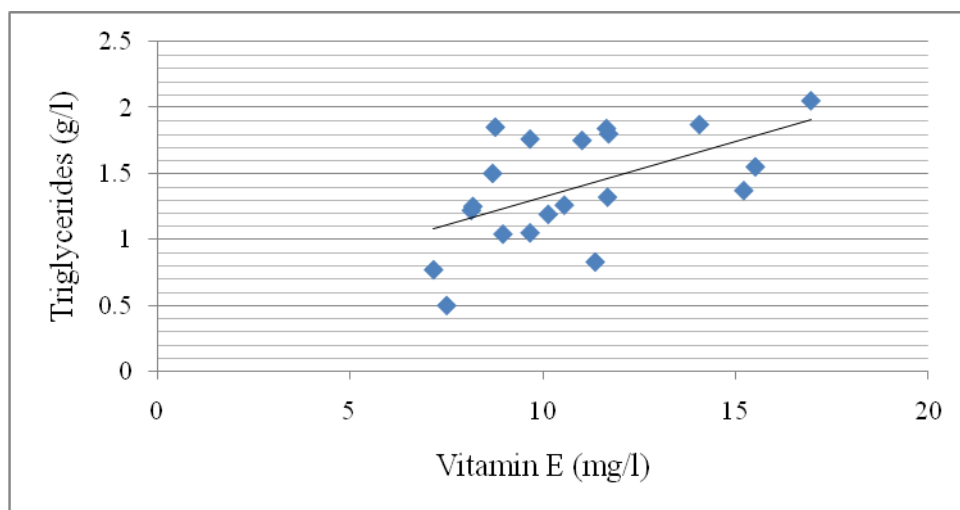


Fig. 7. Correlation of triglycerides with vitamin E ($R^2 = 0,309$, $p = 0,027$).

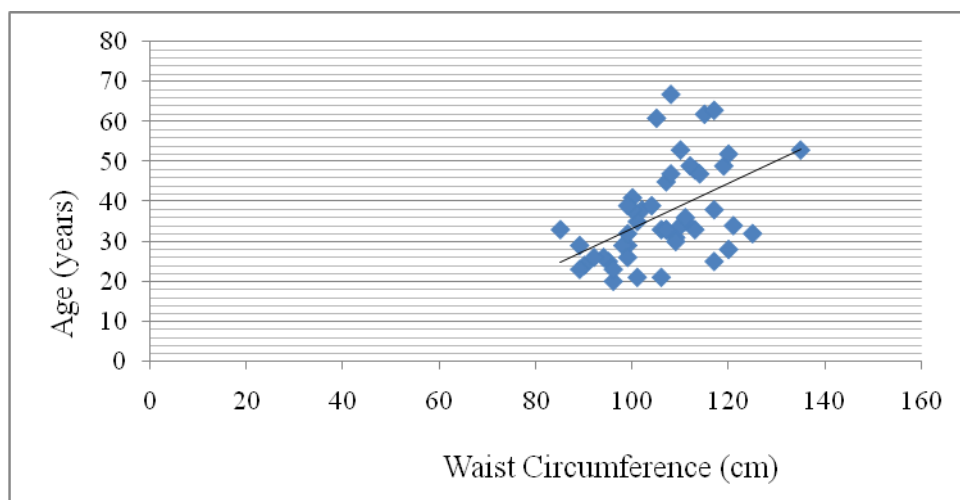


Fig. 8. Correlation of age with waist circumference ($R^2 = 0,241$, $p = 0,001$).

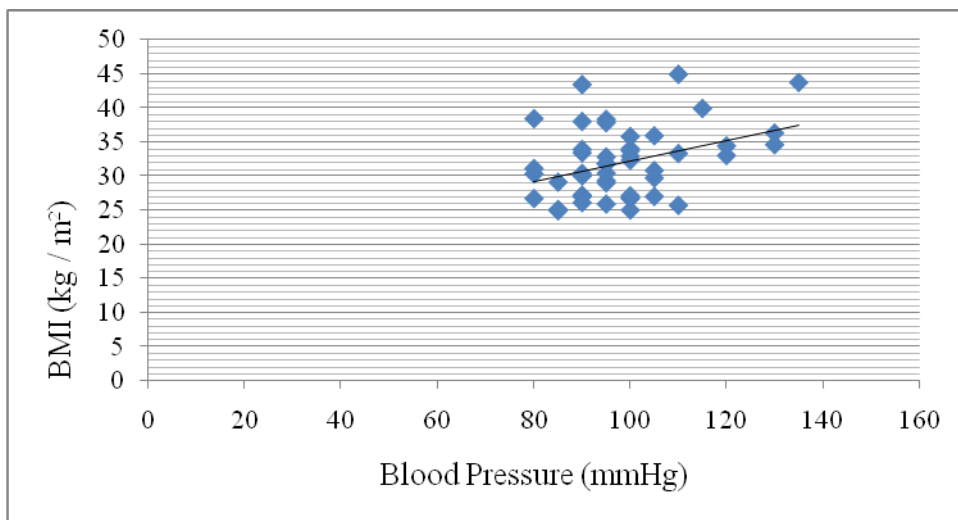


Fig. 9. Correlation of obesity with blood pressure ($R^2 = 0,143$, $p = 0,001$).

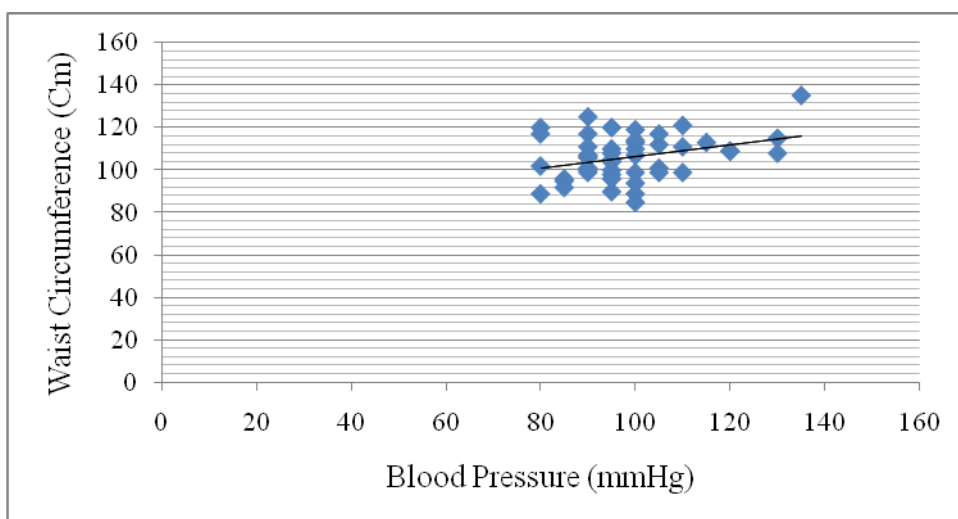


Fig. 10. Correlation of waist circumference with blood pressure ($R^2 = 0,112$, $p = 0,003$).

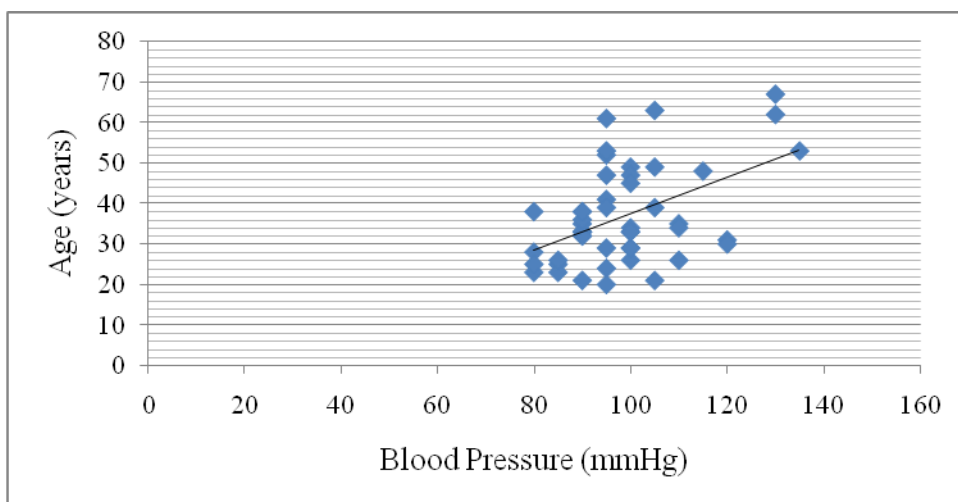


Fig. 11. Correlation of age with blood pressure ($R^2 = 0,234$, $p = 0,002$).

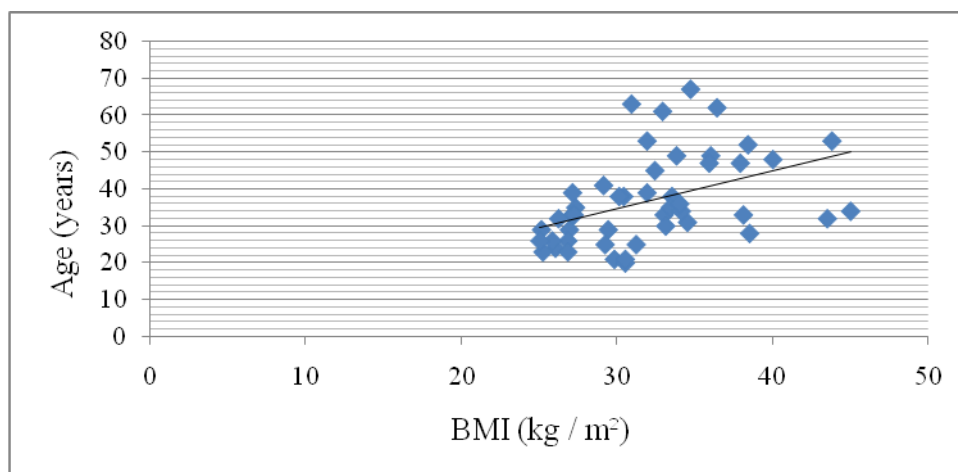


Fig. 12. Correlation of age with obesity ($R^2 = 0,196$, $p = 0,008$).

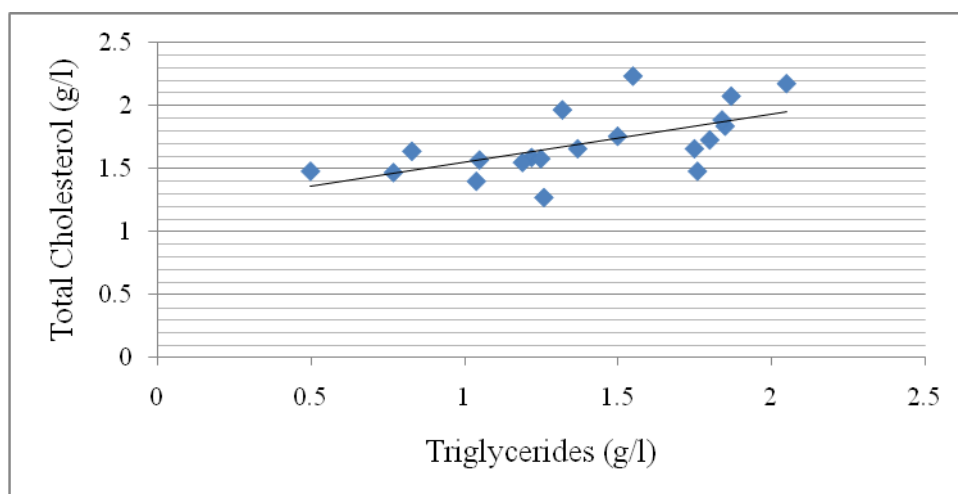


Fig. 13. Correlation of total cholesterol with triglycerides ($R^2 = 0,374$, $p = 0,001$).

DISCUSSION

Scientists attach more importance to a diet rich in fruits and vegetables and/or impact on the prevention of diseases in terms of taking antioxidants, the consumption of fruit and vegetables increases the vitamins E and A serum that helps fight against oxidative stress^{21,22}. In this context, the Italian epidemiological study EPIC showed that a diet rich in antioxidants is clearly associated with a significant decrease in lymphocyte DNA oxidized compared with subjects with a diet low in antioxidants²³.

It is constantly reported in the literature: other factors, such as excess of lipid availability^{24,25}, dietary mistakes²⁶, and chronic inflammation^{6,7}, make obesity an important target for oxidative stress. Indeed, some studies indicate a weakening of the plasma antioxidant status (vitamins and trace elements) both in young^{27,28,29,30}. Or obese adults^{31,32,33,34,35}. For example, concentrations of retinol, vitamin C, vitamin E and β -carotene are 15-37% lower in

obese women compared to their healthy counterparts³¹. In our study, plasma concentrations of antioxidant vitamins decreased with increasing BMI (13.20 versus 11.03 mg / l for vitamin E and 0.77 versus 0.53 mg / l for vitamin A in men respectively, for overweight and obesity); similar results were found in these studies^{31,28}. In addition, the total antioxidant status also appears to be lower in obese subjects^{36,37}.

Concerning the enzymes antioxidant, Erdeve and coll have distinguished a significant increase in activity (including SOD and GPx) in the early stages of obesity, by increasing their substrate³⁸. As against, chronic obesity induced a decrease in enzymatic antioxidant activity even if free radicals are still present. According^{39,40}, the sources responsible for the enzymatic activity would be depleted during chronic obesity. Thus, in animals, several authors^{41,42,43} reported an increase in erythrocyte enzyme activity (GPx, SOD) after a short period (7-10 weeks) a high fat and / or carbohydrate diet but reported a decrease after a long period (7months)⁴⁴. In humans,³⁹ confirm the decreased activity of SOD and GPx in obese. These authors conclude that early obesity, antioxidant enzyme activity is stimulated. However, when obesity persists long (7 months in rats or humans in general), sources of antioxidant enzyme is significantly reduced leading to a lower level of activity.

In our study, we observed an increase in the enzymatic activity of SOD and Cat in obese women (7.97 versus 10.11 Unit for the enzymatic activity of SOD and 5.52 versus 6.72 Unit for the enzymatic activity of the Cat), by comparing our results with those of³⁸, so we concluded that women are in the early stages of obesity. By cons, in obese men, there was a decrease in enzyme activity including SOD and Cat, respectively, for overweight and obesity (8.86 versus 7.25 Unit and 12.21 versus 3.84 Unit) therefore obesity persists long in men. This is consistent with results of^{39,40} confirming the decrease in activity of SOD and GPx in obese after a long period.

However, the deficiency of dietary intake is not always the cause of the weakening of the antioxidant system in obese. Indeed, in obese children and adults the plasma vitamin status may be lower than that of healthy subjects without filler difference between the two groups^{28,34,45}. This result indicates that plasma antioxidants are consumed by the state of stress induced by obesity. The obesity subjects presented an oxidative stress, since the serum levels of antioxidant status: GSH, vitamin E and A, were lower in this group as previously established by⁴⁶. Indeed lipid peroxidation was considered as a representative of oxidative stress, this result could be justified by the depletion of the GSH and vitamin E, which is well

known to be involved in lipid peroxidation removal⁴⁷. It is well admitted that the antioxidant enzyme activity is stimulated in response to an oxidative stress state; this is for catalase activity is significantly lower in the obesity group as in the early days of the development of obesity, supporting the earlier studies on human models³⁹. Contrastingly, the SOD activity was not decreased in the obesity group. This is not surprising, since as demonstrated by³⁹, the SOD activity did not show a significant depletion below BMI 40 kg/m².

Additionally, the increase in fasting blood glucose and lipid profile make the obesity group presented an oxidative stress which is constantly reported in the literature: other factors, such as excess of lipid availability^{24,25}, dietary mistakes²⁶, make obesity an important target for oxidative stress. Table 2 shows that serum levels of vitamins E/A and antioxidant enzyme activity is higher in men with a $25 \leq \text{BMI} \leq 30$ as obese men ($\text{BMI} \geq 30$) suggesting that there exists a negative correlation between BMI and antioxidant status. Our results are comparable to those reported in the literature: biological assessments of overweight subjects ($\text{BMI} > 25$) indicate a higher oxidative stress than in subjects with $\text{BMI} < 25$ ⁹.

It is therefore necessary to assess the potential importance of intervention strategies to reduce obesity in the population⁴⁸. In general, the serum levels of antioxidant status are significantly higher ($p < 0.05$) in men than in women; it comes to the percentage of physical inactivity; 93.88% of women do not practice any physical activity. Then it should be noted that the practice of moderate physical activity reduces oxidative stress, while a sustained exercise leads to an overproduction of reactive oxygen species (ROS), not offset by the increase in defense systems, this is therefore not a desirable approach⁴⁹. We found that serum antioxidant vitamins in hypertensive individuals is in the standards However the study of^{50,51} in patients suffering from hypertension, oxidative stress has been shown, there may be a deficit of antioxidant defenses⁵⁰ as an excess of ROS production⁵¹. Our result may be explained by adaptation developed by these individuals to oxidative stress and / or diet varied.

CONCLUSION

At the end of this survey, which aims to evaluate oxidative stress status of some patients with a $\text{BMI} > 25$, it appears that, including: Serum levels of antioxidants status: vitamin, GSH and catalase activity is higher in overweight individuals than in obese individuals ($\text{BMI} \geq 30$) suggesting the involvement of reduced glutathione, vitamins and antioxidant enzyme activity in the mechanisms of compensation of repair of damage caused by oxidative stress.

Catalase activity is significantly lower in the obesity group; however the SOD activity was not decreased. Alteration in fasting glucose and lipid profile confirmed oxidative stress in the obesity group. Reduced antioxidant serum in women is associated with an increase in the percentage of inactivity while the practice of moderate physical activity reduces oxidative stress. According to these results, women are in the early stages of obesity against the latter persists long in men. Blood pressure was positively associated with waist circumference, obesity and age, obesity increases with age. Our study confirmed the decrease in antioxidant status of the subject obese with concomitant increase in the parameter of oxidant status.

ACKNOWLEDGMENTS

The authors appreciate and thank all the subjects who participated in the study. We thank all the staff of the Laboratory of Biochemistry of Establishment Public Hospital Ain Fakroun for their cooperation and valuable assistance.

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