

Original Research Article

## Inflammatory and Redox Markers in Overweight and Obese Menopausal Women

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### ABSTRACT

The aim of this work was to determine nutritional status, metabolic, oxidative stress and inflammatory markers in postmenopausal women with overweight or obesity.

Serum glucose, lipids, interleukin-6, C-reactive protein, erythrocyte and plasma redox markers were investigated in 40 overweight, 38 obese and 40 normal weight postmenopausal women and were compared to 80 regularly menstruating women. Daily nutrient consumption was determined by the 24-h dietary recall technique. Our findings showed that low energy, fat and carbohydrate intakes prevented weight gain in menopausal women. These normal weight menopausal women presented micronutrient deficiency, lipid alterations and oxidative stress (high NO, O<sub>2</sub><sup>-</sup>, MDA, carbonyl proteins and low ORAC, vitamin C and GSH) without pro-inflammatory state. Overweight menopausal women had adequate dietary intake with minor micronutrient deficiency, lipid and redox abnormalities with a pro-inflammatory state (high CRP and IL-6). In obese menopausal women, high energy, fat and carbohydrate intakes, high sodium and calcium intakes and several micronutrient deficiencies were associated to an accentuation of lipid alterations, oxidative and pro-inflammatory states.

In conclusion, nutritional care to avoid overweight and obesity could contribute to the quality of life of menopausal women.

**Key words:** Menopause, obesity, overweight, oxidative stress, inflammation.

### INTRODUCTION

The menopause is associated with multiple chronic diseases such as coronary heart disease, stroke, breast cancer, osteoporosis with electrolyte and fluid dysregulation [1-3] and oxidative stress, [4,5] related to estrogen deficit. [6-8] Free radicals and oxidative stress have been implicated in the pathogenesis of various menopause-related symptoms and complications, [4,7] and they could be exacerbated in the

association of menopause with overweight or with obesity.

Obesity is an independent risk factor for more severe menopausal symptoms. Women with abdominal obesity have high vasomotor scores, personal life dissatisfaction, nervousness, memory loss, depression, flatulence, muscle pains, sleeping disorders and lack of energy. [9] Central obesity is a risk factor for cardiovascular disease and diabetes. Women commonly increase central fat deposition

during the menopausal transition. [4,10,11] Postmenopausal obesity increases the risk of metabolic alterations and of oxidative stress. [12-14] Obesity is associated with increased oxidative stress and chronic low-grade chronic inflammation. [15,16] Indeed, the reduction in circulating sex hormones that occurs with menopause is commonly associated with increased pro-inflammatory activity. [17,18] Close monitoring of weight gain and oxidant/antioxidant status with nutritional guidelines are recommended in menopausal women. Nutritional habits in menopausal women could be improved for reducing several menopause-related complications.

Today, growing interest is given to the association of specific components of human nutrition and oxidative stress. Previous study showed that fruit consumption or Mediterranean-style diet decreased the risk of reporting vasomotor menopausal symptoms, whereas consumption of a high-fat and -sugar diet increased this risk. [19] Magnesium deficiency was suggested as a risk factor for obesity and osteoporosis in menopause. [20] Nutritional antioxidant supplementation was shown to be an effective approach in improving menopausal symptoms. [21] A significantly higher intake of total fat and saturated fatty acids in postmenopausal obese women stimulates ROS production. [22] Furthermore, a high intake of cholesterol and low supply of PUFAs may increase the cardiovascular risk independently of plasma cholesterol levels. [23] Modest fish intake was associated with less progression of coronary atherosclerosis among postmenopausal women. [24] Effects of dietary macronutrients on atherosclerosis may be in part mediated by effects on body mass index, insulin sensitivity, or serum lipids. [25] Moreover, reductions in inflammatory molecules occurred when the diet was supplemented with 18C fatty acids in obese premenopausal women. [26]

However, relations between dietary and metabolic, inflammatory and oxidative markers in overweight or obese menopausal

women are still unclear. We therefore investigated the nutritional status, metabolic, redox and inflammatory markers in postmenopausal women with overweight or obesity.

## **MATERIALS AND METHODS**

### **Patients**

The protocol was approved by the Tlemcen Hospital Committee for Research on Human Subjects. The purpose of the study was explained to all participants and investigation was carried out with their written consent. A total of 118 postmenopausal women were recruited in the department of obstetrics and gynecology at Tlemcen Hospital, Tlemcen. Menopause status was established after 1 year since the last menstrual period. The gynecological interview, physical examination and hormonal profile, including the measurement of follicle stimulating hormone (FSH) levels, confirmed the postmenopausal period. A group of 80 regular menstruating women in the reproductive age with normal weight was included in the study as a control. Women with heart disease, diabetes melitus (DM), any neoplasia, arthritis or any other inflammatory disease, endocrine, cardiovascular or infectious diseases, malignancy, hepatic or renal dysfunction, hypertension, hypolipidemic treatment and women taking hormonal replacement therapy were excluded from the study.

We selected a homogenous group of 40 postmenopausal overweight women (body mass index, BMI between 25 and 29 kg/m<sup>2</sup>), 38 postmenopausal obese women (BMI  $\geq$ 30 kg/m<sup>2</sup>) and 40 age -matched postmenopausal healthy lean women (BMI <25kg/m<sup>2</sup>).

Participants were asked to complete a questionnaire with epidemiologic information on demographic and lifestyle factors, family history of any diseases, physical activity and menopause symptoms. Physical activity was assessed using the Seven-Day Physical Activity Recall interview. [27] A structured questionnaire

reported the time spent sleeping and doing moderate, hard, and very hard physical activity and the intensity and duration of each activity.

Energy expenditure (kilocalories per kilogram per day) was estimated from the time spent in each activity multiplied by the metabolic equivalent (1 metabolic equivalent is 1 kcal/kg per hour) for each activity. Dietary questionnaires were administered to participants at enrollment by trained interviewers. The 24-h dietary recall technique was applied, collected on 2 non-consecutive weekdays and 1 weekend day, and analyzed using the nutritional analysis program with database of food composition (REGAL Windows, France), regarding both macronutrients and micronutrients.

#### **Blood samples**

Fasting venous blood samples were collected in two tubes, EDTA tubes and dry tubes, from each patient, and were centrifuged. Serum was separated for glucose, lipid and inflammatory parameters. Plasma was separated for oxidant/antioxidant determinations.

The remaining erythrocytes were washed, hemolyzed by the addition of cold distilled water (1/4) and the cell debris was removed by centrifugation (2000g for 15 min). The hemolysates were assayed for antioxidant enzyme activities and GSH contents.

#### **Determination of biochemical parameters**

Serum glucose, triglycerides and cholesterol contents were determined by enzymatic methods (Kits Sigma Chemical Company, St Louis, MO, USA).

#### **Determination of markers of the oxidant/antioxidant status**

Plasma malondialdehyde (MDA, marker of lipid peroxidation) was estimated by the method of Draper and Hadley et al. [28] using thiobarbituric acid (TBA).

Plasma carbonyl proteins (marker of protein oxidation) were assayed by 2, 4-dinitrophenyl hydrazine reaction.

Vitamin C levels were determined in plasma by using the method of Roe and

Kuether. [29] Nitric oxide (NO) was determined by the method of Guevara et al. [30] using Griess reagent.

The determination of the superoxide anion ( $O_2^-$ ) was based on Nitro Blue Tetrazolium (NBT) reduction in monofarmazan by  $O_2^-$ . [31]

Catalase (CAT EC 1.11.1.6) activity was measured by spectrophotometric analysis of the rate of hydrogen peroxide decomposition at 240 nm. [32]

Superoxide dismutase (EC 1.15.1.1) activity was measured by the NADPH oxidation procedure. [33]

Erythrocyte reduced glutathione (GSH) levels were assayed by a colorimetric method using 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB), according Sigma Aldrich Kit (Saint Louis, USA).

The oxygen radical absorbance capacity (ORAC) reflecting the total antioxidant capacity of plasma was measured using the fluorescent allophycocyanin (APC), as we have previously described. [34]

#### **Determination of inflammatory markers**

Serum IL-6 contents were assayed by using a commercially available ELISA kit (Cayman Chemicals ACE, EIA Kit, USA).

Serum C-reactive protein (CRP) levels were measured according to Sigma-Aldrich kit (St Louis, MO).

#### **Statistical analysis**

The results are presented as means and standard deviations. A priori power analysis was performed to determine the sample size, using power and sample size calculator (Statistical solutions, Sigma). The results were tested for normal distribution using the Shapiro-Wilk test. The comparison of means between the four groups of women is performed by ANOVA one factor. This analysis is completed by the Tukey's test to locate the source of significant difference. The Means indicated by different superscript letters (a, b, c, d) are significantly different ( $P < 0.05$ ). All tests were performed using STATISTICA 4.1 program (StatSoft, Tulsa, OK).

## RESULTS

### Population Characteristics

As shown in Table 1, BMI value was significantly higher in overweight and obese menopausal women compared to controls. Age at menarche and childbirths were similar in all women selected. All women selected have either no family history of diseases (40% to 60%) or a family history of cardiovascular disease and osteoporosis, with the highest prevalence in obese menopausal women. Menopausal symptoms were observed in all menopausal women regardless of their BMI. Physical activity was significantly decreased in all menopausal women compared to young control women; the lowest value was noted in obese menopausal women.

### Daily consumption of macro-nutrients and micro-nutrients

Obese menopausal women reported a higher median energy intake, while menopausal women with normal weight had a lower energy intake than did young control women (Table 2). Nevertheless, overweight menopausal women showed similar energy intake to that of controls.

Carbohydrate and lipid intakes, expressed as percentages of total energy intake, were significantly decreased in menopausal women and significantly increased in obese menopausal women compared to young controls. Variations in complex carbohydrate, saturated fatty acid and polyunsaturated fatty acid intakes were similar to those observed for total carbohydrate and total lipid intakes. Protein, simple carbohydrate and cholesterol intakes were only enhanced in obese menopausal women. Monounsaturated fatty acid intake was significantly decreased in menopausal women compared to other groups. Nevertheless, overweight menopausal women showed similar macronutrient intakes to that of controls. Fiber intake was reduced in all menopausal women.

Obese menopausal women had higher sodium and calcium intakes and lower vitamin A intake than the other groups (Table 3). Menopausal women with

normal weight had reduced magnesium and iron intakes compared to control women. There was no significant difference in phosphorus, potassium, vitamin C, D and E intakes between menopausal and control women. Concerning vitamin B intakes, the differences were not significant except for riboflavin intake which was low in all menopausal women, and for folate intake which was reduced in overweight and obese menopausal women.

### Biochemical characteristics

Significant differences were found between obese menopausal women and the other subjects for serum glucose levels. The highest glucose concentrations were apparent in obese menopausal women (Figure 1).

All menopausal women demonstrated significantly higher serum levels of total cholesterol and triglycerides compared with controls; the highest values were observed in obese menopausal women.

### Oxidative stress biomarkers

Plasma oxidant markers (carbonyl proteins, MDA, O<sub>2</sub><sup>-</sup>, NO) were significantly increased in all menopausal women compared to young controls; the obese menopausal women's values being the highest (Table 4). However, antioxidant markers (Plasma vitamin C, erythrocyte GSH) were significantly decreased in these menopausal women; with the lowest values in obese ones.

Total antioxidant status (ORAC) was significantly reduced in menopausal women, inversely proportional to BMI (Figure 2). Erythrocyte antioxidant enzyme activities (catalase and SOD) were significantly decreased in only obese menopausal women compared to the other groups (Figure 2).

### Inflammatory biomarkers

In menopausal women with normal weight, CRP as well as IL-6 levels were similar to those found in young control women. However, CRP and IL-6 concentrations were significantly increased in overweight and obese menopausal

women compared to controls, with the highest values in obese ones (Table 5).

**Table 1: Population characteristics**

Parameters	Young control women	Menopausal women	Overweight menopausal women	Obese Menopausal women
Number	80	40	40	38
Age (years)	28 ± 4	56 ± 3 **	59 ± 4 **	57 ± 3.50 **
BMI (kg/m <sup>2</sup> )	22.18 ± 1.04	22.80 ± 1.27	27.67 ± 1.42 *	33.12 ± 2.73 **
Duration of menopause (years)	-	6 ± 3	8 ± 4	7 ± 4
Age at menarche	12 ± 2	13 ± 1.50	13 ± 1.45	14 ± 2
Number of children	4 ± 2	4 ± 1	5 ± 2	6 ± 3
<b>Physical activity:</b>				
Active	80 %	75 %	40 %	30 %
Sedentary	20 %	25 %	60 %	70 %
<b>Family history:</b>				
+ Cardiovascular disease	35 %	40 %	45 %	60 %
+ Osteoporosis	5 %	0 %	15 %	15 %
+ None	60 %	60 %	55 %	40 %
<b>Symptoms:</b>				
+ Hot flash	-	80 %	85 %	80 %
+ Sleep disturbance	-	40 %	45 %	60 %
+ Mood disturbances	-	40 %	45 %	55 %
+ Weight gain	-	45 %	60 %	75 %
+ The tired	-	55 %	65 %	75 %
+ Joint aches	-	70 %	75 %	80 %
<b>Physical activity (METs)</b>	58.65 ± 3.44	43.50 ± 2.67 *	35.33 ± 3.51 **	26.25 ± 2.16 **

Values are means ± SD or number (percentage). BMI: body mass index, weight/height<sup>2</sup>. MET: metabolic equivalent (1 MET = 1 kcal/kg/h). The significance of the differences between two groups was determined by Student's t test: Menopausal women versus controls: \* p <0.05; \*\* p<0.01.

**Table 2: Daily consumption of nutrients in control and menopausal women**

	Young control women	Menopausal women	Overweight menopausal women	Obese menopausal women	P (ANOVA)
Energy (Kcal/day)	1845.50 ± 124 <sup>b</sup>	1490.86 ± 80.84 <sup>c</sup>	1835 ± 105.92 <sup>b</sup>	2604.91 ± 116 <sup>a</sup>	0.007
Proteins (g/day)	65.67 ± 5 <sup>b</sup>	63.48 ± 4.38 <sup>b</sup>	65.03 ± 4.15 <sup>b</sup>	75.17 ± 2.15 <sup>a</sup>	0.008
Total Carbohydrate (g/day)	288.11 ± 25 <sup>b</sup>	235.89 ± 19.92 <sup>c</sup>	305.40 ± 26.31 <sup>b</sup>	351 ± 24.23 <sup>a</sup>	0.005
Simple carbohydrate (g/ day)	94 ± 6.57 <sup>b</sup>	87.21 ± 7.88 <sup>b</sup>	97.27 ± 6.04 <sup>b</sup>	122.42 ± 5.38 <sup>a</sup>	0.008
Complex carbohydrate(g/ day)	194 ± 18.34 <sup>b</sup>	127.94 ± 12.82 <sup>c</sup>	208.14 ± 20.53 <sup>b</sup>	228.58 ± 22.71 <sup>a</sup>	0.005
Fibers (g/ day)	35.80 ± 2.33 <sup>a</sup>	17.98 ± 1.09 <sup>c</sup>	22.29 ± 2.52 <sup>b</sup>	25.43 ± 3.66 <sup>b</sup>	0.006
Total lipids (g/ day)	50.44 ± 2.53 <sup>b</sup>	32.61 ± 1.77 <sup>c</sup>	47.02 ± 2.41 <sup>b</sup>	68.12 ± 2.56 <sup>a</sup>	0.004
Saturated fatty acids (g/ day)	24.04 ± 2.04 <sup>a</sup>	12.90 ± 1.73 <sup>b</sup>	23.29 ± 2.25 <sup>a</sup>	24.09 ± 2.39 <sup>a</sup>	0.030
Mono-unsaturated fatty acids (g/ day)	19.20 ± 2.32 <sup>a</sup>	13.52 ± 1.32 <sup>b</sup>	16.35 ± 2.43 <sup>a</sup>	19.89 ± 2.74 <sup>a</sup>	0.020
Polyunsaturated fatty acids (g/ day)	7.27 ± 0.57 <sup>b</sup>	6.39 ± 0.44 <sup>c</sup>	7.33 ± 0.43 <sup>b</sup>	9.55 ± 0.65 <sup>a</sup>	0.030
Cholesterol (mg/ day)	182.25 ± 21.12 <sup>b</sup>	192.87 ± 23.69 <sup>b</sup>	211.32 ± 27.41 <sup>b</sup>	302.85 ± 24.46 <sup>a</sup>	0.020

Values are means ± SD. After checking the normal distribution of variables (test Shapiro - Wilk), the comparison of means between the four groups of women is performed by ANOVA one factor. This analysis is completed by the Tukey test to locate the source of significant difference. The Means indicated by different superscript letters (a. b. c ..... ..) are significantly different (P <0.05).

**Table 3: Daily consumption of micronutrients in control and menopausal women**

	Young control women	Menopausal women	Overweight menopausal women	Obese menopausal women	P (ANOVA)
Sodium (mg/day)	2882.21 ± 148 <sup>b</sup>	2941.31 ± 141.69 <sup>b</sup>	2967.4 ± 124.76 <sup>b</sup>	3237.27 ± 191.45 <sup>a</sup>	0.040
Magnesium (mg/ day)	276.95 ± 22.17 <sup>a</sup>	198.51 ± 23.98 <sup>b</sup>	277.44 ± 26.27 <sup>a</sup>	286.12 ± 23.04 <sup>a</sup>	0.050
Phosphorus (mg/ day)	1025 ± 65	881.18 ± 42.93	939.71 ± 51.62	1085.51 ± 62.60	0.127
Potassium (mg/ day)	2251 ± 89.56	2197.02 ± 91.64	2238 ± 75.67	2247 ± 93.95	0.204
Calcium (mg/ day)	560 ± 30.92 <sup>b</sup>	539.60 ± 35.68 <sup>b</sup>	540.66 ± 38.78 <sup>b</sup>	730.62 ± 25.84 <sup>a</sup>	0.050
Iron (mg/ day)	14.23 ± 2.60 <sup>a</sup>	9.97 ± 1.78 <sup>b</sup>	11.02 ± 1.58 <sup>a</sup>	11.59 ± 1.03 <sup>a</sup>	0.040
Vitamin A (µg/ day)	1402.91 ± 58.38 <sup>a</sup>	1493.39 ± 50.37 <sup>a</sup>	1403.65 ± 64.52 <sup>a</sup>	702.39 ± 39.12 <sup>b</sup>	0.030
Vitamin D (Ug/ day)	1.45 ± 0.25	1.57 ± 0.27	1.39 ± 0.29	1.54 ± 0.36	0.138
Vitamin E (mg / day)	6.11 ± 1.50	5.52 ± 1.08	6.26 ± 1.19	6.38 ± 1.63	0.276
Vitamin C (mg/ day)	98.57 ± 4.62	95.29 ± 4.54	95.03 ± 4.03	93.48 ± 6.35	0.147
Thiamine (mg/ day)	1.20 ± 0.35	0.97 ± 0.13	0.94 ± 0.21	1.14 ± 0.25	0.135
Riboflavin (mg/ day)	12 ± 2 <sup>a</sup>	3.25 ± 0.33 <sup>b</sup>	3.02 ± 0.57 <sup>b</sup>	2.82 ± 0.43 <sup>b</sup>	0.040
Niacine (mg/ day)	14.50 ± 2.25	12.05 ± 2.52	12.27 ± 2.65	13.83 ± 2.28	0.209
Pantothenic acid (mg/ day)	4.35 ± 0.50	4.14 ± 0.62	4.20 ± 0.46	4.56 ± 0.52	0.144
Vitamin B6 (mg/ day)	1.22 ± 0.20	1.17 ± 0.26	1.33 ± 0.24	1.45 ± 0.25	0.176
Vitamin B12 (Ug/ day)	4.25 ± 0.25	4.44 ± 0.22	4.28 ± 0.27	4.65 ± 0.39	0.132
Folates (Ug/ day)	345 ± 38 <sup>a</sup>	327.62 ± 43 <sup>a</sup>	250.67 ± 22.75 <sup>b</sup>	247 ± 27.14 <sup>b</sup>	0.040

Values are means ± SD. The Means indicated by different superscript letters (a. b. c ..... ..) are significantly different (P <0.05).



**Table 4: Oxidative stress markers in control and menopausal women**

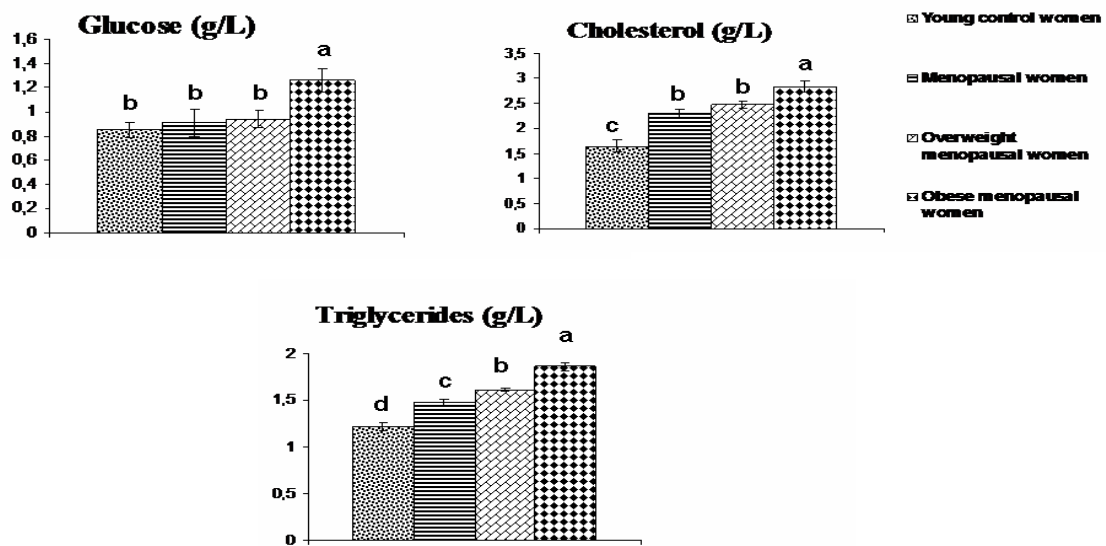
Parameters	Young control women	Menopausal women	Overweight menopausal women	Obese menopausal women	P (ANOVA)
NO (μmol/L)	8.61 ± 1.34 <sup>d</sup>	11.31 ± 1.61 <sup>c</sup>	14.482 ± 1.14 <sup>b</sup>	20.18 ± 2.77 <sup>a</sup>	0.010
O <sub>2</sub> <sup>-</sup> (μmol/L)	4.27±0.41 <sup>c</sup>	8.52 ± 0.30 <sup>b</sup>	8.66 ± 0.45 <sup>b</sup>	11.83 ± 0.59 <sup>a</sup>	0.010
MDA (μmol/L)	1.48 ± 0.06 <sup>c</sup>	3.71 ± 0.41 <sup>b</sup>	3.78± 0.35 <sup>b</sup>	4.81± 0.46 <sup>a</sup>	0.040
CP (nmol/mg protein)	4.79±0.50 <sup>d</sup>	8.76 ± 0.47 <sup>c</sup>	13.37 ± 1.11 <sup>b</sup>	18.78 ± 1.02 <sup>a</sup>	0.007
Vitamin C (μmol/L)	41.05 ± 2.42 <sup>a</sup>	34.05 ± 1.14 <sup>b</sup>	31.326 ± 2.18 <sup>b</sup>	26.218 ± 1.18 <sup>c</sup>	0.020
GSH (μmol/gHb)	4.04 ± 0.12 <sup>a</sup>	2.17 ± 0.20 <sup>b</sup>	2.04 ± 0.23 <sup>b</sup>	1.74 ± 0.06 <sup>c</sup>	0.030

Values are means ± SD. CP: carbonyl proteins; GSH: reduced glutathione; MDA: malondialdehyde; NO: nitric oxide; O<sub>2</sub><sup>-</sup>: superoxide anion. The Means indicated by different superscript letters (a. b. c ..... ..) are significantly different (P <0.05).

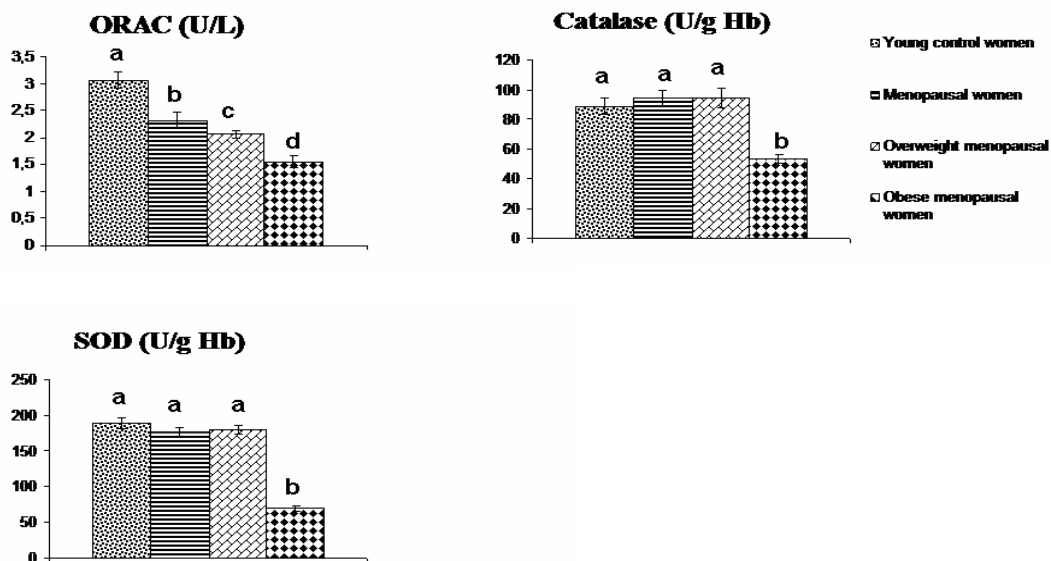
**Table 5: Inflammatory factors in control and menopausal women**

Parameters	Young control women	Menopausal women	Overweight menopausal women	Obese menopausal women	P (ANOVA)
CRP (mg/L)	1.89 ± 0.22 <sup>c</sup>	1.78 ± 0.31 <sup>c</sup>	2.35 ± 0.44 <sup>b</sup>	5.64 ± 0.86 <sup>a</sup>	0.030
IL-6 (pg/mL)	30.27 ± 1.45 <sup>c</sup>	32.51 ± 2.77 <sup>c</sup>	38.87 ± 1.24 <sup>b</sup>	45.11 ± 2.06 <sup>a</sup>	0.020

Values are means ± SD. CRP: Serum C-reactive protein; IL-6: interleukin 6. The Means indicated by different superscript letters (a. b. c ..... ..) , are significantly different (P <0.05).



**Figure 1:** Serum glucose and lipids in control and menopausal women, Values are means ± SD. The Means indicated by different superscript letters (a. b. c ..... ..) are significantly different (P <0.05).



**Figure 2:** Total antioxidant status (ORAC) and erythrocyte antioxidant enzyme activities (catalase and superoxide dismutase, SOD) in control and menopausal women, Values are means ± SD. The Means indicated by different superscript letters (a. b. c ..... ..) are significantly different (P <0.05).

## DISCUSSION

Menopausal women in our study displayed an altered nutritional status associated to several metabolic and redox abnormalities which were worsened in the presence of obesity. In women's life, menopausal transition is often significantly associated with deleterious changes in body composition with weight gain and increase in fat mass. [35] Other studies showed that the physical activity was significant predictor of the greater body weight in postmenopausal women. [36] In our study, overweight and obese menopausal women were less active with lower physical activity score than young control women. Menopausal women with normal weight were more active than overweight and obese ones. This physical activity could be responsible for maintaining normal weight in these menopausal women. However, compared to young women, physical activity score was significantly reduced in these menopausal women despite normal weight. This was balanced by a lower energy intake associated to a lower consumption of either dietary carbohydrate or lipids including saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in normal weight menopausal women compared to young controls. Obese menopausal women reported a higher energy intake compared with the control group. Other researchers have reported that excessive energy intake is the primary cause of obesity. [37] Other scientists looked at the composition of the diet and reported that excessive consumption of dietary fat may be a more important determinant of obesity than excessive consumption of either carbohydrate or protein. [38] Our findings showed that obese menopausal women had significantly higher protein, carbohydrate and fat intakes, especially PUFA than control groups. Dietary cholesterol intake was also found to be higher in these obese menopausal women than in controls. In a previous study, PUFA intake was positively

associated with atherosclerotic progression in postmenopausal women. [25]

Obese menopausal women had higher intakes of sodium and calcium and lower intakes of vitamin A, riboflavin and folate than controls. Riboflavin and folate intakes were also reduced in overweight menopausal women compared to young controls. Normal weight menopausal women reported lower magnesium, iron and riboflavin intakes than young controls. In previous studies, low calcium intake in menopausal women was an independent risk factor for postmenopausal osteoporosis. [39] Epidemiologic studies showed an inverse correlation between adiposity and calcium intake. [40] Distinctive finding of our study was that calcium intake was significantly increased in obese menopausal women. These findings could be linked to hormonal changes and to deterioration of calcium metabolism at menopause. A high intake of sodium was associated with high prevalence of obesity. [41] Low magnesium intake should be considered as a possible factor in depressed immune function, osteoporosis and hyperlipidemia in older women. [42] Iron deficiency due to low dietary intake of iron is the most common cause of anemia in older adults. [43] Vitamin A intake was found to be low in obese adults. [44] Low riboflavin intake was associated with fracture risk in menopausal women. [45] Folate deficiency was noted in overweight and obese patients, in agreement with our results. [46,47]

On the other hand, the results of the present study showed that menopausal status alone caused significant lipid and redox alterations, and that overweight or obesity worsened them. Serum glucose concentrations were significantly higher in obese menopausal women compared to young controls, reflecting probably an insulin resistance state.

In the present study, serum total cholesterol and triglycerides showed a significant rise in all menopausal women compared to young controls, in accordance with previous studies. [48] These lipid

abnormalities had been explained by estrogen deficiency. [49] These findings suggested increased cardiovascular risk in menopausal women regardless of their body weight. [23] These lipid abnormalities were accentuated with obesity. The association between obesity and hyperlipidaemia is well established.

Our data revealed that plasma total antioxidant activity (ORAC) was decreased in all menopausal women in favor of an oxidative stress. These results are in agreement with previous studies showing that oxidative stress is involved in the pathogenesis of menopausal symptoms. [5] Oxidative stress was characterized by an imbalance between pro-oxidants (MDA, carbonyl proteins, superoxide anion and nitric oxide) and antioxidants (superoxide dismutase, catalase, glutathione and vitamin C) in all menopausal women studied. The reduction of ORAC was associated with increased oxidative stress markers such as superoxide anion, MDA and protein carbonyl levels in menopausal women.

All these redox abnormalities were worsened in obese menopausal women. Elevated levels of oxidant markers could result from estrogen deficiency, insulin resistance state, hypercholesterolemia, abnormal metabolism and metabolites in adipose tissue and/or excessive proinflammatory and inflammatory cytokines release. [6,7,15,16] Low plasma levels of vitamin C despite normal vitamin C intake could reflect high utilization rate, suggesting that this vitamin may be used to reduce oxidative stress in menopausal women irrespective of BMI.

Increased oxidative markers were associated with reduced erythrocyte antioxidant SOD and catalase activities in obese menopausal women suggesting a lower antioxidant defense due to enzyme depletion, lower metabolic rate, and mitochondrial dysfunction and reduced oxygen consumption. [14,50] Additionally, glutathione content was low in menopausal women irrespective of their body weight. With acute oxidative stress, as would be

seen with the association menopause - obesity, one may expect an overproduction of free radicals and resultant severe depletion of glutathione. The reduction in GSH, the most important cellular antioxidant involved in glutathione dependent enzyme reactions, would lead to increased numbers of free radicals and this could thereafter be responsible for the increased lipid and protein oxidation in menopausal women, in agreement with previous studies. [50,51]

Our results showed that nitric oxide levels were increased in menopausal women irrespective of their body weight. Menopausal status, by itself and not as a consequence of aging, is associated with increased serum nitric oxide concentrations. [52] Both nitric oxide and superoxide anion concentrations were increased in menopausal women. Several pathological conditions, including ischemia/reperfusion and inflammation, may induce the simultaneous production of superoxide anion and nitric oxide radicals, generating peroxynitrite, which has a much greater oxidizing potential than the nitric oxide and superoxide anion radicals alone. [53]

Biomarkers of inflammation, C-reactive protein (CRP) and interleukin-6 (IL-6) were enhanced in overweight and in obese menopausal women. Positive correlation between BMI and CRP levels was found in menopausal women. [48] It has been suggested that plasma CRP concentrations reflect the levels of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 and IL-6 which are implicated in the process of atherosclerosis. CRP synthesis in the liver is controlled by IL-6 which is secreted by adipose tissue. [54] Adiposity is then a significant predictor of plasma CRP in menopausal women. Furthermore, menopause with increased adiposity contributes to systemic inflammation because adipose tissue secretes a variety of pro-inflammatory adipokines including IL-6 and CRP. [17] Despite the existence of an oxidative stress in normal weight menopausal women, inflammation was not



present. However, the pro-inflammatory state worsened the oxidative stress in overweight and obese menopausal women.

## CONCLUSION

In conclusion, low energy, fat and carbohydrate intakes prevented weight gain in menopausal women. These normal weight menopausal women presented micronutrient deficiency, lipid alterations and oxidative stress without pro-inflammatory state. Overweight menopausal women had adequate dietary intake with minor micronutrient deficiency, lipid and redox abnormalities with a pro-inflammatory state. In obese menopausal women, high energy, fat and carbohydrate intakes, high sodium and calcium intakes and several micronutrient deficiencies were associated to an accentuation of lipid alterations, oxidative and pro-inflammatory states. It is important to control and monitor the nutritional status in menopausal women to prevent nutritional alterations, weight gain and obesity and associated metabolic alterations such as strong lipid abnormalities, oxidative stress and inflammation. Menopausal women should also be encouraged to enhance their physical activity exerting beneficial metabolic changes.

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