Oxidant/antioxidant status and endothelial dyfunction in obese and hypertensive models of insulin resistance

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ABSTRACT

We studied the oxidant/antioxidant status and nitric oxide (NO) production in healthy controls (group I), essential hypertensive (group II), normotensive obese (group III) and hypertensive obese (group IV) subjects. The levels of these blood parameters in the insulin resistant (IR) patients were also compared with those of insulin sensitive (IS) subjects. We measured serum malondialdehyde (MDA) (a marker of lipid peroxidation) and total plasma nitrites and nitrates (Nox) (an index for NO production). Erythrocytic reduced glutathione (GSH), glutathione peroxidase (GSH-PX), superoxide dismutase (SOD), blood vitamin C and plasma selenium were also estimated. Our results showed a significant increase in mean levels of MDA and reduction in Nox, GSH, vitamin C and SOD in all patients' groups. Significant reduction in GSH-PX was detected only in the obese groups III and IV. Significant increase in MDA and decrease in Nox, GSH and vitamin C were detected in IR versus IS patients. Significant positive correlation was found between MDA and MABP, BMI and WHR respectively; negative correlation was found between Nox, GSH and vitamin C and MABP, BMI and WHR. The correlations were higher with WHR than BMI. The F.G/I ratio was negatively correlated with MDA and positively correlated with Nox, GSH and vitamin C. By multiple regression analysis, the WHR was an independent variable concerning F.G/I ratio and MABP, suggesting that body fat distribution is related to IR and hypertension. In conclusion the present study showed that the activation of the lipid peroxidation processes and depression of some antioxidants (especially GSH and vitamin C) proceed more or less in parallel with declining NO bioavailability, the severity of hypertension, the extent of obesity, the distribution of body fat and the degree of IR.

INTRODUCTION

There is increasing evidence that oxidative stress, an excessive production of reactive oxygen species (ROS) outstripping antioxidant defense mechanisms, is implicated in pathophysiological conditions that affect the cardiovascular system such as cigarette smoking, diabetes and hypertension [1,2]. Hypertensive states accompanied by oxidative stress in animal models include spontaneous hypertension [3] obesity related hypertension [4] and others [5,6]. Both essential hypertension [7,8] and obesity [9] were reported to be associated with signs of insulin resistance. Also it was reported that persons with essential hypertension are more prone than normotensive to develop diabetes [10].

Oxidative stress has been suggested to be a contributing factor in the development of endothelial dysfunction (ED) and insulin resistance [11]. An important event in ED is derangement of nitric oxide (NO) production/action [12].

Thus the present study aimed at studying the oxidant/antioxidant status and nitric oxide production in essential hypertensive patients, comparing them to normotensive and hypertensive obese subjects. This study also aimed at comparing the forementioned parameters in the insulin resistant patients versus the insulin sensitive selected subjects.

SUBJECTS AND METHODS

Subjects. Patients were selected from the outpatient clinic at Kasr El-Aini Medical Hospital, Cairo University This study included 58 subjects classified into 4 groups. Group I (n=16): healthy normal subjects, normotensive and non-obese, age and sex matched to other groups. Group II (n=16): non-obese patients suffering from essential hypertension. Group III (n=16): obese normotensive subjects. Group IV (n=20): Obese hypertensive subjects. Patients were further classified according to the fasting glucose insulin ratio (F.G/I) (an easily obtainable, highly specific and sensitive test for insulin resistance) into: insulin sensitive patients (IS) (F.G/I $\geq$ 4.5) and insulin resistant patients (IR) (F.G/I < 4.5) [13,14]. For essential hypertensive patients (group II) the inclusion criteria were: (i) essential hypertension defined according to the criteria of the VI Joint National Committee [15]; (ii) age from 30-55 years; (iii) never previously treated for hypertension or off medication for at least 1 month before the beginning of the study; (iv) Body mass index (BMI) $\leq$ 25 kg/m^2. Over weight and obese patients (groups III & IV) were selected to have a BMI > 25 kg/m^2 [16]. Pregnant females or females taking contraceptive pills were excluded from the study. Patients with a history of diabetes mellitus, a fasting glucose > 120 mg/dl, with a total cholesterol > 240 mg/dl or cigarette consumption were also excluded. The following investigations were performed for all subjects. Detailed history and physical examination. Blood pressure (BP) measurements: patients with BP > 140/90 were considered hypertensive [15)]; mean arterial blood pressure (MABP) was calculated as DBP+1/3 pulse pressure. Body Mass Index (BMI) measurement, expressed as weight in kilograms

divided by square of height in meters (Kg/m^2). Patients with a BMI > 25 kg/m^2 and 30 kg/m^2 were considered overweight and obese respectively [16]. Body fat and central fat accumulation evaluation by waist to hip ratio (WHR) according to the recommendations of WHO [17].

Analytical Procedures. 12 mL fasting blood samples were drawn from the antecubital vein of every subject. 4 mL were added to a tube containing heparin for determination of erythrocytic reduced glutathione, glutathione peroxidase, superoxide dismutase, vitamin C, plasma selenium and total plasma nitrites and nitrates (Nox) (as an index for NO production). 2 mL of blood were added to flouride in a second tube for determination of fasting blood glucose. Serum was separated from the remaining blood to measure insulin, malondialdehyde, lipid profile, ALT, AST, creatinine and uric acid. Total nitrite and nitrate levels (Nox) were estimated colorimetrically using the Greiss reaction, after reduction of nitrate to nitrite by incubation with NADPH, FAD and nitrate reductase (aspergillus species) as described by Moshage et al [18]. Serum malondialdehyde (MDA) was estimated by acid-catalysed thermal decomposition of lipid peroxide to MDA which reacts with thiobarbituric acid to form a colored adduct [19]. Erythrocytic reduced glutathione (GSH) was measured after red cell lysis and deproteinization (sulfuric acid and sodium tungstate) by reacting with 5'-5' dithiobis (2-nitrobenzoic acid) (DTNB) to yield a yellow anion [20]. Erythrocytic glutathione peroxidase (GSH-PX) was assessed using the commercially available Ransel kit (Antrim, U.K.), based on the method of Paglia and Valentine [21]. Plasma Selenium (Se) was measured by the atomic absorption spectrophotometer (UNICAM, 939/959). Vitamin C was estimated according to colorimetric technique described by Jagota and Dani [22]. Erythrocytic superoxide dismutase (SOD) was assessed using the Ransod kit (Antrim, U.K.) [23]. Haemoglobin, lipid profile, serum creatinine, uric acid, ALT, AST, fasting blood glucose and insulin were estimated by conventional methods using commercially available kits.

Statistical Analysis. Values were expressed as mean ± SD. For comparison of means between groups, ANOVA with post-hoc test was used. For comparison of means of insulin resistant versus insulin sensitive patients student t test was used. Simple correlation (r value) was performed. Multiple regression analysis was used to assess correlation between different variables to determine independent risk factors [24].

RESULTS

Results of physical and anthropometric measurements in the different groups are shown in Table 1; routine biochemical data are listed in table 2. Fasting insulin (FI) and fasting glucose / insulin ratio (F.G/I) showed significant differences in groups II, III and IV versus the control group I. Also significant differences were found between group II and each of groups III and IV (Table 2). As regards the lipid profile, although still within normal values, the mean levels showed a significant increase in total cholesterol and LDL-c and a significant decrease in HDL-c in all groups versus the control group (I). No significant differences in lipid profile between groups II, III & IV were detected (Table 2). Hemoglobin, serum ALT, AST, creatinine and uric acid showed no significant differences between the studied groups.

The mean levels of plasma Nox was significantly decreased and serum MDA increased in all groups versus the controls. There was a further decrease in Nox and increase in MDA in group IV versus groups II and III (Table 3). There was also a significant decrease in Nox and an increase in MDA in insulin resistant (IR) versus the insulin sensitive (IS) groups (Table 4).

Erythrocytic GSH mean levels were significantly lower in all groups versus the control group. A significant decrease was also found in GSH in group IV versus group II (Table 3). There was also a significant decrease in the mean levels of GSH in the IR versus the IS groups (Table 4). A significant reduction of mean values of erythrocyte glutathione peroxidase (GSH-PX) was found in the obese hypertensive group IV versus groups I and II; and in obese group III versus group I (Table 3). No significant differences was found between the IR and IS groups (Table 4).

No significant differences in the mean levels of plasma Se was detected between the studied groups (Tables 3 &

Table 1
Age, blood pressure and anthropometric data in the study groups. Values are given as mean ± standard deviation.

Parameter	Group			
	I (controls) n=16	II (essential hypertension) n = 16	III (normotensive obese) n = 16	IV (hypertensive obese) n = 16
Age (years)	39.9 ± 7.5 (a)	41.9 ± 7.9 (a)	38.9 ± 11.9 (a)	41.8 ± 7.2 (a)
SBP (mmHg)	121.1± 4.6 (a)	175.6 ± 19.7 (b)	124.0 ± 9.3 (a)	178.2 ± 11.3 (b)
DBP (mmHg)	74.6 ± 4.5 (a)	90.6 ± 7.3 (b)	83.4 ± 7.9 (a)	104 ± 4.5 (b)
MABP	93.3 ± 4.5 (a)	130.8 ± 3.8 (a)	96.9 ± 7.9 (a)	128.8 ± 5.7 (b)
BMI (Kg/m^2)	22.4 ± 1.1 (a)	23.4 ± 1.1 (a)	31.9 ± 2.1 (b)	32.9 ± 2.4 (b)
WHR	0.8 ± 0.1 (a)	0.9 ± 0.1 (a)	0.9 ± 0.1 (b)	1.0 ± 0.1 (b)

Same letters (a or b) under each group indicate non-significant differences between groups; difference in letters (a or b) designate significant differences (P<0.05).

Table 2
Routine biochemical data in the study groups. Values are given as mean ± standard deviation. FBS: fasting blood glucose; F.G/I: fasting glucose/insulin ratio.

Parameter	Group			
	I (controls) n=16	II (essential hypertension) n = 16	III (normotensive obese) n = 16	IV (hypertensive obese) n = 16
FBG (mg/dl)	82.8 ± 11.2 (a)	88.3 ± 19.3 (a) (b)	92.5 ± 8.9 (b) (c)	98.1 ± 11.7 (c)
Fasting insulin (μIU/mL)	14.8± 2.1 (a)	21.6 ± 3.4 (b)	25.8 ± 5.2 (c)	27.6 ± 6.2 (c) (d)
F.G/I ratio	5.6 ± 0.9 (a)	4.0 ± 0.9 (b)	3.7 ± 0.8 (c)	3.6 ± 0.8 (c) (d)
Haemoglobin (g/dL)	13.1 ± 0.9 (a)	13.3 ± 0.6 (a)	13.0 ± 0.7 (a)	13.1 ± 0.8 (a)
Cholesterol (mg/dL)	180.9 ± 16.9 (a)	191.3 ± 17.23 (b)	191.3 ± 14.8 (b)	196.6 ± 16.3 (b)
HDL-C (mg/dL)	52.2 ± 6.3 (a)	43.5 æ 4.9 (b)	43.7± 6.1 (b)	43.1 ± 4.9 (b)
LDL-C (mg/dL)	108.6 ± 17.8 (a)	124.8 æ 14.5 (b)	124.1 ± 14.9 (b)	132.0 ± 14.8 (b)
Triacylglycerol (mg/dL)	99.6 ± 24.3 (a)	114.8 ± 32.9 (a)	117.5 ± 32.5 (a)	111.4 ± 29.4 (a)
Creatinine (mg/dL)	0.7 ± 0.1 (a)	0.8 ± 0.2 (a)	0.8 ± 0.1 (a)	0.8 ± 0.2 (a)
ALT (IU/L)	28.7 ± 8.3 (a)	25.3 ± 6.9 (a)	27.4 ± 5.1 (a)	26.2 ± 7.9 (a)
AST (IU/L)	26.8 ± 4.6 (a)	25.7 ± 6.3 (a)	24.1 ± 5.5 (a)	24.0 ± 4.8 (a)
Uric acid (mg/dL)	4.2 ± 0.6 (a)	4.12 ± 0.6 (a)	4.1 ± 0.5 (a)	4.1 ± 0.4 (a)

Same letters (a, b or c) under each group indicate non-significant differences between groups; difference in letters designate significant differences (P<0.05).

Table 3
Total plasma nitrites and nitrates (Nox), Malondialdehyde (MDA), and antioxidants in the study groups. Values are given as mean ± SD. GSH: erythrocyte reduced glutathione; GSH-PX: glutathione peroxidase; SOD: superoxide dismutase.

Parameter	Group			
	I (controls) n=16	II (essential hypertension) n = 16	III (normotensive obese) n = 16	IV (hypertensive obese) n = 16
Nox (μIU/mL)	24.3 ± 2.4 (a)	16.1 ± 2.9 (b)	15.8 ± 3.1 (b)	13.4 ± 3.4 (c)
MDA	3.7± 0.4 (a)	5.8 ± 1.0 (b)	6.1 ± 0.9 (b)	6.9 ± 1.0 (c)
GSH (μmol/gHb)	5.5 ± 0.4 (a)	3.9 ± 0.6 (b)	3.9 ± 0.6 (b) (c)	3.5 ± 0.4 (c)
GSH-PX (μ/gHb)	25.9 ± 3.6 (a)	24.1 ± 3.3 (a) (b)	23.4 ± 3.1 (b) (c)	21.7 ± 3.1 (c)
Selenium (μg/L)	116.6 ± 11.5 (a)	109.6 ± 104.5 (a)	110.0 ± 17.1 (a)	109.7 ± 14.4 (a)
Vitamin C (mg/dL)	1.4 ± 0.2 (a)	0.9 æ 0.3 (b)	0.8± 0.4 (b)	0.8 ± 0.3 (b)
SOD (μ/gHb)	1051 ± 141 (a)	801.0 æ 97.7 (b)	811.3 ± 104.6 (b)	766.5 ± 72.6 (b)

Same letters (a, b or c) under each group indicate non-significant differences between groups; difference in letters designate significant differences (P<0.05).

4). Mean levels of vitamin C and superoxide dismutase (SOD) were significantly decreased in all groups versus the control group (Table 3). A significant decrease in the mean levels of vitamin C but not SOD was found in the IR versus the IS groups.

By simple correlation analysis, the MABP was significantly positively correlated with MDA (r=0.683) and negatively correlated with the fasting G/I ratio (r=-0.707), GSH (r=-0.688), Nox (r=-0.698) and vitamin C (r=-0.809). The BMI and WHR were positively correlated with the fasting insulin levels (r=0.518 and r=0.566 respectively) and MDA (r=0.613 & r=0.783 respectively). They were negatively correlated with the fasting G/I ratio (r=-0.577 & r=-0.638, respectively), Nox (r=-0.708 and r=-0.80), GSH (r=-0.521 & r=-0.575) and vitamin C (r=-.524 and r=-668). The correlation were more significant with WHR more than BMI. No significant correlation was detected between MABP, BMI, WHR and the other parameters.

Significant positive correlation was detected between fasting G/I ratio and each of Nox (r=0.515), GSH (r=0.553) and vitamin C (r=0.493). A negative correlation between F.G/I ratio and MDA was found (r=-0.466). No significant correlations were detected between F.G/I ratio and each of lipid profile, SOD, Se and GSH-PX. A positive correlation was found between blood pressure levels and markers of obesity being more significant with WHR than BMI (table 5).

By multiple regression analysis BMI, WHR, F.G/I ratio were found to be independent variables for MABP (p<0.001; p < 0.0001 and p < 0.01) respectively. Other

Table 4
Parameters studied in insulin-resistant (IR) and insulin-sensitive (IS) patients. Values are expressed as mean ± SD.

Parameter	Group		Significance
	IR (G/I ratio < 4.5) (n = 36)	IS (G/I ratio ≥ 4.5) (n = 36)	
SBP (mmHg)	164.8 ± 29.48	151.5 ± 23.71	S
DBP (mmHg)	99.5 ± 10.23	91.8 ± 12.09	S
MABP	123.1± 17.76	111.6 ± 15.78	S
BMI (kg/m^2)	30.3± 4.68	27.1 ± 4.58	S
WHR	0.99± 0.114	0.87 ± 0.115	S
Total cholesterol (mg/dL)	132.5 ± 13.94	193.7 ± 20.55	NS
LDL-c (mg/dL)	126.2± 13.60	127.5 ± 19.06	NS
HDL-c (mg/dL)	43.8 ± 5.3	43.6 ± 5.88	NS
Triacylglycerol (mg/dL)	114.0± 31.94	114.1 ± 29.24	NS
Fasting insulin (μIU/mL)	27.4± 5.03	19.6 ± 2.82	S
F.G/I ratio	3.36± 0.54	4.75 ± 0.48	S
Fasting glucose (mg/dL)	93.9 ± 12.14	91.9 ± 10.74	NS
Nox (μmol/L)	13.7± 3.04	17.7 ± 2.4	S
MDA (nmol/ml)	6.6± 0.84	5.36 ± 0.89	S
GSH (μmol/gHb)	3.59 ± 0.47	4.12± 0.53	S
GSH-PX (μ/gHb)	22.7± 3.14	23.5 ± 3.59	NS
Selenium (μg/L)	108.8 ± 14.6	112.0 ± 12.44	NS
Vitamin C (mg/dL)	0.79± 0.28	1.11± 0.24	S
SOD (μ/gHb)	770.6 ± 81.3	837.4 ± 99.1	NS

S = significant; NS = non-significant

Table 5
Correlation between SBP, DBP,MABP; and body mass index (BMI) and waist hip ratio (WHR) respectively.

Correlation	II (essential hypertension) n = 16	III (normotensive obese) n = 16	IV (hypertensive obese) n = 16
SBP/BMI	R=0.407*	R=0.311	R=0.478*
SBP/WHR	R=0.357	R=0.691*	R=0.697*
DBP/BMI	R=0.326	R=0.683*	R=0.442*
DBP/WHR	R=0.384	R=0.881*	R=0.420*
MABP/BMI	R=0.444*	R=0.562*	R=0.365
MABP/WHR	R=0.517	R=0.825*	R=0.525*

*Significant correlation

parameters that showed significant correlation by simple (r) correlation were not independent risk factors for hypertension. High BMI & low F.G/I ratio were independent variables for WHR (p=0.0089 and P=0.0043 respectively). WHR was also an independent variable for F.G/I ratio.

DISCUSSION

Several studies have associated oxidative stress with endothelial dysfunction (ED) and insulin resistance (IR) [1,7,8,9]. Since IR was reported to be associated with hypertension [7,8] and obesity [9], ED and the oxidant-antioxidant status were studied in these cases either alone or combined.

As regards ED, nitric oxide (NO) production was studied. Plasma nitrates and nitrites (Nox) were evaluated as an index for NO production [18]. A significant reduction in Nox was detected in essential hypertensive patients, and obese normotensive ones with further significant reduction when hypertension was accompanied by obesity (group IV). This is in accordance with other studies on essential hypertension [25,26], obesity without hypertension [27]

and hypertensive obese [28]. Also Nox was significantly lower in IR subjects versus IS ones. The decrease in plasma Nox was accompanied by a significant increase in MDA (a lipid peroxide end product). This is in accordance with other studies on obesity [29] and hypertension [25]. NO is known to be produced by nitric oxide synthases (NOS). Endothelial NOS (eNOS) constitutively produces both NO and superoxide ($O2^{\cdot-}$) suggesting that the effective release of NO from the vascular endothelium depends on the relative concentrations of the two species. The uncoupled eNOS is reported to exist in two states: uncoupled eNOS (producing more $O2^{\cdot-}$ than NO) and dimeric eNOS (producing more NO than $O2^{\cdot-}$). The uncoupled form was hypothesized to prevail in the IR states due to the decrease in the cofactor tetrahydrobiopterin that stabilizes the dimeric form of eNOS [30]. This may partly explain the oxidative stress (increased MDA) in the IR patients in the present study.

There are two theories proposed to explain the relation between obesity, IR and hypertension. One is that hyperinsulinemia results in increased sodium renal reabsorption, leading to increased intravascular volume. The other is that hyperinsulinemia results in vascular over activity due to sympathetic stimulation. Hyperinsulinemia and IR have been postulated to contribute to the dyslipidemia in these patients especially low HDL-c [3]. HDL-c protects against lipid peroxidation largely due to the enzymatic activity of paraoxonase associated with HDL-c. Paraoxonase activity is reported to decrease in some cardiovascular diseases [32]. Thus the significant decrease in HDL-c in the patients of the present study may partly account for the oxidative stress observed in these patients.

Whether oxidative stress is a cause or consequence of hypertension or I.R is contraversial. Studies reported that there are 3 key enzymes which besides the proton leakage across the mitochondrial membrane account for the majority of reactive oxygen species (ROS) generation: NADPH oxidase, xanthine oxidase and uncoupled eNOS [33]. There is accumulating evidence that hypertension and angiotensin II (ANG-II) increase vascular oxidative stress (i.e. oxidative stress is a consequence of the disease). This may be by increasing the vascular activity of NADPH and NADH oxidase. This in turn enhances ROS by several pathways including the increased activation of xanthine oxidase and the inactivation of SOD. Enhanced production of ROS, in addition to decreasing the bioavailability of NO and moreover, reaction of ROS with NO to produce the potent oxidant peroxynitrite further contributes to vasoconstriction and vascular injury [34].

On the other hand, oxidative stress, as a cause of the disease was studied in rats and found to induce IR by activating nuclear factor-kappa B pathway and disrupting normal subcellular distribution of phosphatidyl 3-kinase [35]. It was also reported that oxidative stress, in hypertensive and IR patients, partly mediates the inhibitory effect of ANG-II-ANG-II receptor on insulin action in the vascular and skeletal muscle tissues. This is by interfering with the insulin signalling through phosphatidyl 3-kinase and its downstream protein kinase B (Akt) signalling pathways. This leads to decreased and endothelial NO, increased myosin light chain kinase activation with vasoconstriction and reduced muscle glucose transport [10].

As regards obesity, it was reported to cause oxidative stress which may contribute to obesity associated disease as hypertension [36]. The decrease of Nox in obesity may be due to the reported impaired stimulatory effect of leptin on NO production [37]. Weight reduction [27] and short term vigorous exercise [38] were found to improve ED, BP, oxidative stress and NO bioavailability in hypertensive obese. These results suggest obesity as a cause of oxidative stress may be through the reported production of proinflamatory cytokines [27].

The association of obesity with hypertension can be explained by the finding of Jia et al [39] who reported an interaction between a mutation of exon G894T of eNOS gene and overweight. They reported that this played an important role in essential hypertension, and that controlling body weight in these patients could markedly decrease the risk of hypertension. This is in accordance with our finding of decreased Nox mean levels in the obese hypertensive group versus the obese or hypertensive alone. This is further confirmed by the multiple regression analysis showing the BMI and WHR to be independent risk factors for hypertension.

As regards the antioxidant status, a significant reduction in GSH, vitamin C and SOD were found in all groups versus the controls. This is in accordance to other studies [25,33,40] GSH-PX was found to decrease significantly in the obese group III, but not in the essential hypertensive group II versus controls. GSH was significantly decreased in the obese hypertensive versus group II (non-obese hypertensive) but not versus the obese normotensive patients (group III). This indicates more oxidant / antioxidant imbalance in obesity whether associated with hypertension or not. These results suggest that more attention should be paid to health study and nutritional problems for the obese population especially concerning vitamins and oxidative stress.

Whether decreased antioxidant levels are simply consequences of tissue damage or are strictly involved in the pathogenetic mechanism of the disease needs further evaluation. However, the fact that the low level included several antioxidant systems [GSH, vitamin C, SOD, and GSH-PX (in obese subjects)] points to the reduction being more a consequence than a cause. Even though the increment of ROS was reported by others [41] to upregulate GSH-PX, prolonged consumption by ROS can overcome the increased production leading to low anti-oxidant capacity.

The antioxidants that correlated significantly with MABP, BMI and F.G/I ratio were vitamin C and GSH. The inverse correlation was between vitamin C and blood pressure found in the present study was also reported by others [42]. Treatment with vitamin C was previously reported to improve blood pressure [43]. The importance of vitamin C and GSH was shown recently by Morgan et al [44], who reported that certain amino acids, peptides and proteins react with singlet oxygen within intact cells to

produce protein-related peroxide derivatives that can activate key cellular enzymes. Studies have shown that cells do not have efficient enzymatic defenses against protein peroxide with only thiols and ascorbic acid able to remove these materials [44]. Also when rats were given a glutathione synthase inhibitor insulin induced glucose uptake and GLUT-4 translocation in adipose tissues were decreased [35]. This is supported in the present study by the decreased GSH levels in IR patients and the correlation between F.G/I ratio and GSH levels. Thus the previous reports [35,43 and 44] in addition to the present study confirms the important roles of GSH and vitamin C in preventing ED and oxidative stress. Vitamin C improves ED and decrease ROS by several mechanisms reviewed by May [45]. This provides a rationale for the use of vitamin C supplements or searching for a mechanism to preserve or increase GSH production in these conditions. However, it is first necessary to ensure that undesirable toxic effects are not present.

The correlations of vitamin C and GSH (as well as Nox and MDA, MABP and F.G/I ratio) were higher in significance with WHR than BMI. This suggests that the body fat distribution and not only the total body fat is associated with the risk of hypertension and IR. It was previously reported that a WHR of greater than 1.0 in men and 0.8 in women is strongly correlated with abdominal obesity and that IR and confers an increase risk of cardiovascular disease [14]. This confirmed, in the present study, by the multiple regression analysis that showed that the WHR (and not BMI) is an independent variable for F.G/I ratio.

In conclusion the present study showed that the activation of lipid peroxidation processes and the depression of some antioxidants (GSH & vitamin C) in blood proceed more or less in parallel with declining NO production, the severity of hypertension, extent of obesity, distribution of body fat (WHR) and degree of IR. Early intervention of revealed disorders before the appearance of clinical symptoms may be promising in terms of prevention and treatment of cardiovascular disease.

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