Oxidative Stress in Normal-Weight Obese Syndrome

Laura Di Renzo[1,2], Fabio Galvano[3], Carmine Orlandi[1], Alessia Bianchi[1], Claudia Di Giacomo[3], Luca La Fauci[3], Rosaria Acquaviva[3] and Antonino De Lorenzo[1,2]

The normal-weight obese (NWO) syndrome was identified in women whose body weight (BW) and BMI are normal but whose fat mass (FM) is >30%. In these subjects, an early inflammatory status has been demonstrated. The aim was to verify whether oxidative stress occurs in NWO. Sixty age-matched white Italian women were studied and subdivided as follows: 20 normal-weight individuals (NW) (BMI <25 kg/m^2; FM% <30%); 20 NWO (BMI <25 kg/m^2; FM% >30%); 20 preobese-obese (OB) (BMI >25 kg/m^2; FM% >30%). Anthropometric, body composition (by dual-energy X-ray absorptiometry) variables, plasma levels of some cytokines, reduced glutathione (GSH), lipid hydroperoxide (LOOH), nitric oxide (NO) metabolites (NO_2^-/NO_3^-), antioxidant nonproteic capacity (ANPC) were measured and compared between groups. Glucose and lipid metabolism parameters were assessed. GSH and NO_2^-/NO_3^- levels resulted lower in OB and NWO compared to NW ($P < 0.01$). LOOH levels resulted higher in OB and NWO ($P < 0.01$). ANPC in NWO was lower than NW but higher with respect to OB ($P < 0.01$). Correlation analysis revealed strong associations between GSH levels and BW, BMI, FM% ($R = -0.45$, at least $P < 0.05$); waist circumference (W) ($R = -0.33$, $P < 0.05$); FFM% ($R = 0.45$, $P < 0.01$); IL-1α, IL-6, IL-10, IL-15 ($R = -0.39$, -0.33, -0.36 -0.34, respectively, $P < 0.05$); triglycerides ($R = -0.416$, $P < 0.05$). LOOH levels were negatively related to FFM% ($R = -0.413$, $P < 0.05$) and positively to FM%, IL-15, TNF-α, insulin, total cholesterol, low-density lipoprotein cholesterol, and triglycerides ($R = 0.408$, $R = 0.502$, $R = 0.341$, $R = 0.412$, $R = 0.4036$, $R = 0.405$, $R = 0.405$, respectively, $P < 0.05$). The study clearly indicates that NWO, besides being in early inflammatory status, are contextually exposed to an oxidative stress related to metabolic abnormalities occurring in obesity.

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INTRODUCTION

It was believed for a long time that simply an inert energy storage tissue with irrelevant metabolic activity, in the last years the adipose tissue, properly defined adipose organ (1), has been assuming a constantly growing metabolic relevance due to its pleiotropic functions. Indeed, besides its well-known fundamental function in regulating energy homeostasis, adipocytes also mediate many physiologic and pathologic processes by means of numerous secretory products.

In this regard, adipocytokines, such as leptin and adiponectin, and proinflammatory factors, such as tumor necrosis factor (TNF-α), interleukin (IL)-6 and 1, have been demonstrated to play an important role in the onset of the major obesity-related comorbidities (2,3).

Several lines of evidence suggest that obesity cannot be characterized only by BMI. Percentage fat mass (FM) and fat distribution may be different in subjects with the same BMI, and lean and obese subjects share different metabolic characteristics (4,5). Furthermore, ethnic differences in body composition are well known (6) and specific values for waist circumference (W) and central obesity definition have been therefore proposed (7). Moreover, in white women two conditions of normal weight and increased body fat composition have been recently characterized and have been described as metabolically obese normal weight (8) and normal-weight obese (NWO) (9).

The NWO syndrome, the distinctive characteristic of 33.7% of healthy female subjects studied, was characterized by a normal BMI (<25 kg/m^2), but high FM percentage (FM% >30%), and significantly higher values of proinflammatory cytokines (10).

NWO were similar to preobese-obese (OB) women not only for fat body mass distribution, but also for cardiovascular (CVD) risk index values. They also do not manifest the metabolic syndrome, despite few, if any, metabolic abnormalities (11).

[1]Division of Human Nutrition, Department of Neuroscience, University of Rome Tor Vergata, Rome, Italy; [2]I.N.Di.M., National Institute for Mediterranean Diet and Nutrigenomic, Reggio Calabria, Italy; [3]Division of Medical Chemistry and Molecular Biology, Department of Biological Chemistry, University of Catania, Catania, Italy. Correspondence: Antonino De Lorenzo (delorenzo@uniroma2.it)

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Overall, in obese subjects an inflammatory status is accompanied by oxidative stress as recently underlined by two valuable reviews (12,13). According to Grattagliano *et al.*, the associations between increased abdominal fat and systemic oxidative stress, the diminished concentration of nitric oxide (NO) derivatives and antioxidant vitamins, and the endothelial oxidative damages, observed in subjects with the metabolic syndrome, definitively support oxidative stress, as the common second-level event, in a unifying pathogenic view. de Ferranti and Mozaffarian efficaciously defined as the *perfect storm* the vicious circle linking obesity, oxidative stress, inflammation, and metabolic disorders.

The objective of the present study was to verify the hypothesis that in NWO women early inflammation is accompanied by oxidative stress. With this aim, we evaluated four oxidative stress markers in plasma: glutathione (GSH), lipid hydroperoxide (LOOH), NO metabolites (NO_2^-/NO_3^-) levels, and antioxidant nonproteic capacity (ANPC) on the same NWO women previously investigated (10). Moreover, we examined the relationship among markers of oxidative stress and body composition, and cytokines level, and metabolic parameters, in all the study population.

METHODS AND PROCEDURES

Subject characteristics

The study population comprised 60 white Italian women (aged 20–35 years) previously selected in the study of De Lorenzo *et al.* (10). Subjects were divided into three groups: (i) 20 women with a normal weight and a BMI <25 kg/m^2 (control group, NW); (ii) 20 NWO women with a normal weight, a BMI <25 kg/m^2, and a FM% >30%; and (iii) 20 OB women with a BMI >25 kg/m^2 and a FM% >30%. The subjects were classified as OB according to a World Health Organization Technical Report and a World Health Organization Technical Report Series (14,15). All of the women were free of hypertension and CVD, had regular 28-day menstrual cycles, were in generally good health, did not assume antioxidant supplementations, did not smoke or abuse alcohol, and did not take any hormonal contraceptives or any other drug.

All of the subjects provided consent to take part in the study, which was conducted according to the guidelines of the "Tor Vergata" University Medical Ethical Committee, Rome, Italy.

Anthropometric measurements

After a 12-h overnight fast, all subjects underwent anthropometric evaluation. Anthropometric parameters of all the participants were measured according to standard methods (body weight (BW), height and W) (16). Subjects were instructed to take off their clothes and shoes before performing all the measurements. BW (kg) was measured to the nearest 0.1 kg, using a balance scale (Invernizzi, Rome, Italy). Height (cm) was measured using a stadiometry to the nearest 0.1 cm (Invernizzi, Rome, Italy). W was measured with a flexible steel metric tape to the nearest 0.5 cm, at the horizontal plane that corresponds with the narrowest point between the crest iliac and the bottom rib. BMI was calculated using the formula: BMI = BW (kg)/height (m)^2.

Dual-energy X-ray absorptiometry

The total body composition was assessed by dual-energy X-ray absorptiometry (Lunar DPX; GE Medical Systems, Milwaukee, WI), according to the previously described procedure (11). The average measurement time was 20 min. The effective radiation dose from this procedure is about 0.01 mSv. The coefficient of variation (CV% = 100 × s.d./mean) intra- and intersubjects ranged from 1 to 5%. The coefficient of variation for bone measurements is <1%; coefficient of variations on this instrument for five subjects scanned six times over a 9-month period were 2.2% for FM, and 1.1% for lean body mass. The expected and reference values for FM% in NWO women were 30.1–48.3%.

Hematological sampling and measurements

Heparinized venous blood was collected after overnight fasting and between days 8 and 12 of the preovulation phase. Standard serum laboratory tests of fasting glucose, insulin, cholesterol, and triglycerides were carried out by the accredited Clinical Chemical Laboratories of the "Tor Vergata" Polyclinic (PTV) of Rome, Italy. Plasma for GSH, LOOH, NO_2^-/NO_3^- and ANPC assays was separated by centrifugation at 800 *g* for 10 min. Levels of total GSH were measured, in 200 µl of plasma, using Miao Lin's method (17). Plasmatic LOOH levels were measured following the oxidation of Fe^{2+} to Fe^{3+} in the presence of xylenol orange at λ = 560 nm (18). Plasmatic NO_2^-/NO_3^- concentrations were determined with Griess reagent at λ = 540 nm (19). ANPC of human plasma was evaluated measuring its free-radical scavenging ability. Superoxide anion was generated *in vitro* as described by Russo *et al.* (20).

Immunological assay

Blood samples (5 ml) were collected between days 8 and 12 of the preovulation phase into sterile tubes containing EDTA (evacuated tubes), via venipuncture early in the morning, after an overnight fast (12 h). All materials were immediately placed in ice, and plasma was separated by centrifugation at 1,600 *g* for 10 min at 4 °C. Plasma samples were stored at −70 °C in 1-ml aliquots until assayed. Plasma concentrations of IL-1α, IL-1β, IL-6, IL-10, IL-15, TNF-α cytokines were determined in duplicate using a high sensitivity commercial sandwich enzyme-linked immunosorbent assay kit (SearchLight Human Inflammatory Cytokine Array 1; Endogen, Perbio, Brebières, France). All assay procedures were performed as described by the manufacturer. The lower limit of cytokine's detection was 0.02 pg/ml.

Statistical analysis

Data are presented as group means ± s.d. Data were analyzed by nonparametric methods to avoid assumptions about the distribution of the measured variables. The Mann–Whitney analysis of variance test was used to compare groups. Associations between parameters were assessed using the Spearman's rank correlation test. All tests were considered significant at $P < 0.05$. Statistical analysis was performed using a computer software package (SPSS for Windows, version 13.0; SPSS, Chicago, IL).

RESULTS

All the 60 enrolled individuals completed the study and their results were eligible for data analysis.

The anthropometric and body composition characteristics, i.e., BMI and FM%, of the studied groups are presented in **Figures 1** and **2**. As expected, BMI and FM% values of NWO were found in an intermediate position between NW and OB. Significant differences in FM% between NW and NWO, NW and OB were observed ($P < 0.05$), but not between NWO and OB. The *z*-score BMI was calculated for each group; NW: −0.89 ± 0.40; NOW: −0.24 ± 0.34; OB: 1.16 ± 0.95. Significant difference in BMI between each group was obtained ($P < 0.05$). Moreover, the three groups differed also for BW (NW: 51.8 ± 4.6 kg; NWO: 59.6 ± 7.2 kg; OB: 70.9 ± 10.3 kg; $P < 0.05$) and for W (NW: 65.1 ± 3.9 cm; NWO: 72.3 ± 4.9 cm; OB: 85.9 ± 10.2 cm; $P < 0.05$; data not shown).

The mean values of GSH, ANPC, LOOH, NO_2^-/NO_3^- for the NW, NWO, and OB are given in **Table 1**. Plasma GSH levels were lower in OB and NWO than in NW ($P < 0.01$). The present

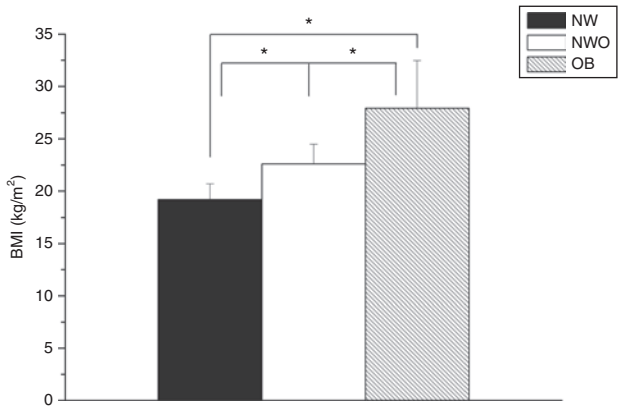

Figure 1 Characterization of the studied groups through BMI. All values are mean ± s.d.; BMI *z*-score NW: –0.89 ± 0.40; NWO: –0.24 ± 0.34; OB: 1.16 ± 0.95. *Significantly different, $P < 0.05$ (Mann–Whitney test); $n = 20$ for each group. NW, normal weight; NWO, normal-weight obese; OB, preobese-obese.

Table 1 Plasma oxidative markers of the studied groups

	Groups		
Parameters	NW ($n = 20$)	NWO ($n = 20$)	OB ($n = 20$)
GSH (μmol/ml)	0.56 ± 0.05	0.44 ± 0.05*	0.43 ± 0.04*
LOOH (μmol/ml)	38.61 ± 1.94	66.76 ± 4.73*	71.50 ± 6.94*
NO_2^-/NO_3^- (nmol/ml)	91.94 ± 4.60	63.09 ± 4.41*	66.43 ± 3.98*
ANPC (%)	99.25 ± 2.03	75.47 ± 4.11*	54.61 ± 5.52*,**

All values are mean ± s.d.
ANPC, antioxidant non proteic capacity (%); GSH, reduced glutathione; LOOH, lipid hydroperoxide; NO_2^-/NO_3^-, nitrite/nitrate; NW, normal weight; NWO, normal weight obese; OB, preobese-obese.
*Significantly different from NW group, $P < 0.01$ (Mann–Whitney test). **Significantly different from NWO group, $P < 0.01$ (Mann–Whitney test).

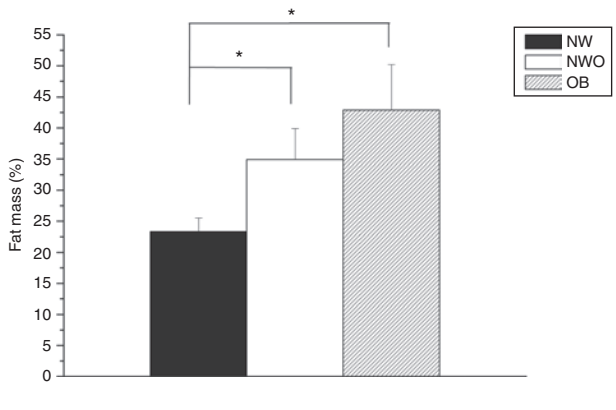

Figure 2 Characterization of the studied groups through fat mass content (in percentage). All values are mean ± s.d. *Significantly different, $P < 0.05$ (Mann–Whitney test); $n = 20$ for each group. NW, normal weight; NWO, normal-weight obese; OB, preobese-obese.

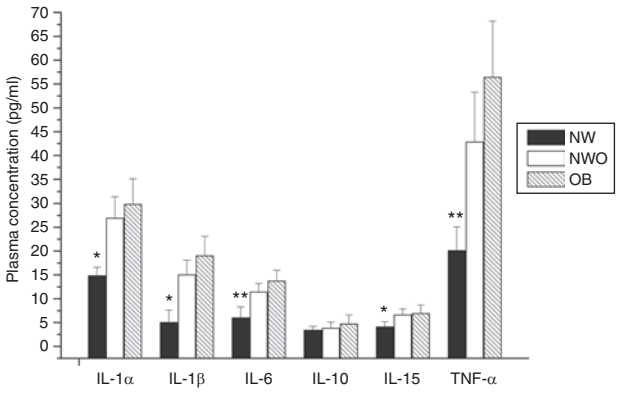

Figure 3 Plasma cytokine levels in all studied groups. All values are mean ± s.d. *Significantly different from other groups. $P < 0.05$ (Mann–Whitney test); **significantly different from other groups, $P < 0.001$ (Mann–Whitney test); $n = 20$ for each group. NW, normal weight; NWO, normal-weight obese; OB, preobese-obese.

study highlighted a significant reduction of ANPC values in NWO with respect to controls ($P < 0.01$); moreover, ANPC in OB was significantly lower ($P < 0.01$) than in NWO and NW. Coherently with low plasmatic GSH and ANPC levels, LOOH concentration resulted higher in OB and NWO than in NW ones ($P < 0.01$). Additionally, the concentration of NO_2^-/NO_3^- resulted lower in OB and NWO than in NW ($P < 0.01$).

Plasma IL-1α, IL-2β, IL-6, IL-10, IL-15 and TNF-α levels of the three groups are shown in **Figure 3**. Plasma cytokines concentration was higher in OB and NWO, with respect to NW (TNF-α and IL-6, both $P < 0.001$; IL-1α, IL-2β, IL-15, all $P < 0.05$). Instead, the anti-inflammatory IL-10 was not altered both in NWO and OB with respect to NW. No significant differences in cytokines levels between NWO and OB were obtained. Spearman's correlation between inflammatory cytokines concentration and body composition parameters revealed significant associations of BW ($R = 0.436$, $P < 0.01$), BMI ($R = 0.520$, $P < 0.01$), free FM percentage (FFM%) ($R = -0.651$, $P < 0.001$), FM% ($R = 0.630$, $P < 0.001$), W ($R = 0.508$, $P < 0.01$), only with IL-15 (data not shown).

Spearman's correlation was performed to evaluate the relationship between oxidative stress, inflammation, and body composition parameters (**Table 2**). GSH concentration was negatively related to weight, BMI, W, FM% ($P < 0.01$), IL-1α, IL-6, IL-10, IL-15 ($P < 0.05$); and positively related to FFM% ($P < 0.01$). LOOH was negatively associated with FFM% ($P < 0,01$) and positively with FM% ($P < 0.01$), TNF-α and IL-15 ($P < 0,05$ both). A strong negative correlation between GSH and LOOH levels was also found ($R = -0.762$, $P < 0.01$). NO_2^-/NO_3^- concentration was negatively related to IL-15 ($P < 0.05$). Any correlation between ANPC% and body composition, inflammatory parameters was obtained. A significant negative correlation between ANPC% and GSH was highlighted ($P < 0.05$).

Spearman's correlation between inflammation, oxidative stress, and glucose/lipid metabolism blood parameters was performed (**Table 3**). GSH levels were negatively related to triglycerides ($R = -0,4416$, $P < 0.05$). A multiple positive correlation was observed between LOOH and: insulin ($R = 0.414$, $P < 0.05$); total cholesterol ($R = 0.436$, $P < 0.05$); low-density lipoprotein cholesterol ($R = 0.405$, $P < 0.05$); and triglycerides ($R = 0.405$, $P < 0.05$). No significant correlations between inflammation and glucose/lipid metabolism blood parameters were observed.

DISCUSSION

Oxidant stress may be an important pathogenic mechanism in the obesity-associated metabolic syndrome (21), and it plays a critical role in the pathogenesis of various diseases such as atherosclerosis cancer, CVDs, and diabetes mellitus (22,23). Oxidant stress results when free-radical formation is greatly increased or protective antioxidant mechanisms are compromised. Several research studies have suggested that obesity is associated with increased oxidant stress (24,25). However, recently, Brown *et al.* demonstrated that only obesity, and not moderate overweight, elevates LOOH levels (26).

In this article, we demonstrate, for the first time, that in NWO syndrome (12–14) overall the inflammatory status and the FM in excess are accompanied by oxidative stress. Indeed, in NWO, all the investigated markers of oxidative stress were comparable to those of OB except for ANPC that remains at an intermediate position between NW and OB.

GSH represents about 90% of nonproteic thiol groups and has a protective role against oxidative and free radical-mediated injury. An overall decrease in GSH levels has been associated with chronic inflammatory diseases, and obesity does not seem to be an exception (27,28). Our data not only confirmed a significant lower GSH level in OB than NW, but also evidenced a comparable concentration of GSH between NWO and OB. The negative correlations between GSH and body composition parameters, IL-lα, IL-6, and the anti-inflammatory IL-10, IL-15 further support the association between oxidative stress and inflammation. Moreover, the negative correlation with triglyceride levels highlights the implication of a reduced antioxidant defense on metabolic abnormalities occurring in obesity.

Lipid peroxidation is a well-known example of early oxidative damage in cell membranes, lipoproteins, and other lipid-containing structures. Our data clearly add further evidences of the increased LOOH level in OB, comparable to NWO. The significant association between LOOH levels and body composition, i.e., positive with FM% and negative with FFM%, gives efforts to the relationship between LOOH and some inflammatory cytokines herein observed. The significant correlation between LOOH and most of the glucose/lipid metabolism blood parameters finally stresses the direct effect of oxidative stress on metabolic abnormalities, involved in CVDs. The increase of lipid peroxidation in OB is coherent with other studies (29,30). However, to our best knowledge, no literature data are available about lipid peroxidation in NWO. Recently, Brown *et al.* (26) confirmed that LOOH was increased in obese subjects but not in overweight subjects, and

Table 2 Correlation analysis between oxidative stress, inflammation and body composition parameters in all the study population

	GSH	LOOH	NO_2^-/NO_3^-	ANPC%
BW	–0.453**	0.091	–0.100	0.257
BMI	–0.459**	0.249	–0.174	0.249
FFM%	0.450**	–0.413*	0.172	–0.280
FM%	–0.445*	0.408*	–0.179	0.276
W	–0.326*	0.106	–0.191	–0.243
IL-1α	–0.391*	0.078	0.168	0.266
IL-1β	–0.216	–0.018	0.143	0.051
IL-6	–0.325*	0.048	0.109	0.261
IL-10	–0.357*	0.343	–0.073	0.088
IL-15	–0.341*	0.502*	–0.405*	0.059
TNFα	0.059	0.341*	–0.245	–0.203
GSH	—	–0.762**	–0.183	–0.490**
LOOH	–0.762**	—	–0.133	0.210
NO_2^-/NO_3^-	–0.183	–0.133	—	0.279
ANPC%	–0.490*	0.210	0.279	—

Data are correlation coefficients of Spearman analysis.
ANPC, antioxidant non proteic capacity (%); BW, body weight; FFM, fat free mass; FM, fat mass; GSH, reduced glutathione; IL, interleukin; LOOH, lipid hydroperoxide; $n = 60$, total population; NO_2^-/NO_3^-, nitrite/nitrate; TNF-α, tumor necrosis factor-α; W, waist circumference.
*$P \le 0.05$; **$P \le 0.01$.

Table 3 Correlation analysis between oxidative stress, inflammation and glucose/lipid metabolism blood parameters in all the study population

	Fasting glucose	Fasting insulin	Total cholesterol	LDL cholesterol	HDL cholesterol	Triglycerides
IL-1α	–0,152	0,081	–0,097	–0,106	0,151	–0,036
IL-1β	–0,162	–0,058	0,146	0,098	0,321	0,032
IL-6	–0,186	0,140	–0,118	–0,117	0,120	–0,142
IL-10	–0,165	–0,084	0,139	0,058	0,278	0,195
IL-15	–0,041	0,074	–0,062	0,068	–0,238	0,006
TNF-α	0,000	0,148	0,115	0,276	–0,152	0,061
GSH	0,029	–0,031	–0,291	–0,229	–0,049	–0,416*
LOOH	0,037	0,412*	0,436*	0,405*	–0,061	0,405*
NO_2^-/NO_3^-	–0,068	0,065	0,105	0,074	0,031	0,163
ANPC%	–0,088	0,249	–0,083	–0,051	–0,160	0,217

Data are correlation coefficients of Spearman analysis.
ANPC, antioxidant non proteic capacity (%); GSH, reduced glutathione; HDL, high-density lipoprotein; IL, interleukin; LDL, low-density lipoprotein; LOOH, lipid hydroperoxide; $n = 60$, total population; NO_2^-/NO_3^-, nitrite/nitrate; TNF-α, tumor necrosis factor-α.
*$P \le 0.05$.

that, in these latter subjects, some markers of oxidative stress were not different from NW, concluding that oxidative stress is not present in overweight subjects. However, in this latter study, overweight subjects had a higher BMI and a lower FM% than those of NWO of the present study. Moreover, the study of Brown *et al.* also included subjects of both sexes. These differences, in our opinion, are crucial when comparing results of the two studies (31). However, taking into account the whole experimental differences, the two studies would suggest that FM, and FFM, rather than BMI, could be predictive of oxidant status.

Most studies on vascular functions in obese subjects demonstrated impaired endothelial function often associated with the pivotal role exerted by NO. The reduction in NO_2^-/NO_3^- blood levels might be due to the chronically elevated oxidant stress typical of obesity and related pathologies (32). In our study, NO_2^-/NO_3^- plasma levels resulted lower in OB and NWO than in NW, thus confirming recent results (33). We also observed a significant negative association between NO_2^-/NO_3^- and IL-15 levels, thus confirming the relationship between endothelial dysfunction and inflammatory state, depending also on IL-15Rα polymorphism (34).

Coherently with previous results, significantly low ANPC levels both in OB and NWO were found. Indeed, ANPC of NWO was significantly lower than that of NW, but still higher than OB, suggesting the beginning of a systemic oxidative stress in NWO.

The concentration of the inflammatory cytokines was significantly increased in NWO, hypothesizing the occurrence of an early inflammatory state, as well as in OB (13). Although for the first time the co-presence of oxidative stress and early inflammation is observed in NWO, if attributed to obesity this latter finding *per se* is not surprising. On the whole of our studies, we observed a contemporary and independent association between FM% and oxidative stress, and inflammation. According to our previous results on body composition and IL-6 promoter (−174G/C) polymorphism relationship (35), genetic pattern could affect the correlation between cardiometabolic parameters, body composition, and the rate of pro- and anti-inflammatory cytokine production. Finally, the genetics could explain the results of correlation analysis herein observed (34–36). Indeed, besides determining an overall inflammatory status, obesity also promotes chronic increase in reactive oxygen species and/or a decrease in cellular antioxidant defense. Both the events coparticipate in metabolic abnormalities occurring in obesity, in a vicious cycle, that is not clearly understood. A conceptual view of events put, at the first level of obesity-related metabolic changes, the hyperglycemia and the lipid accumulation, that in turn deregulate adipose tissue, liver, and other tissues balance. Hyperglycemia, lipid accumulation, and insulin resistance can induce oxidative stress by several mechanisms independently associated (12). Inflammatory signalling pathways are also triggered by lipids. Free fatty acids bind innate immune receptors such as TLR4 and, thus, are directly linked to the development of inflammation in states of hyperlipidemia, such as obesity. It remains unclear whether insulin resistance precedes the development of inflammation or *vice versa*. Otherwise, a series of studies revealed impaired insulin action after the administration of TNF-α, as well as through IL-6 pathway (37). The development of hypoxic conditions in the expanded adipose tissue during obesity results in an increased production of reactive oxygen species and the activation of kinases, that in turn induce the expression of proinflammatory cytokines, and insulin resistance via dependent or independent by cytokines (38).The cytokine profile may shift toward a prooxidant state in obesity and toward a balanced prooxidant–antioxidant state with optimal weight. It has been proposed that with obesity a vicious cycle of adipocyte initiated macrophage recruitment and cytokine/reactive oxygen species production by macrophages occurs, which could potentially lead to oxidative damage and disease processes such as atherosclerosis (37). Chronic excess reactive oxygen species production may result in mitochondrial dysfunction in liver and also skeletal muscle, which may cause lipid accumulation in these tissues and further contribute to the vicious cycle of insulin resistance (13).

The oxidative stress appears to possess, at least in part, the credentials to mechanistically explain the perpetuation of insulin resistance, the altered energy production, the endothelial dysfunction and the appearance of vascular complications in this condition. In fact, oxidative stress plays an important role in the pathogenesis of vascular alterations by either triggering or exacerbating the biochemical processes accompanying the metabolic syndrome. It has been closely related to atherosclerotic processes and is believed to be an important secondary consequence of inflammation associated with the atherosclerotic process and its CVD complications (12).

Our data couple with our previous observations (9), and demonstrate that the triggering of the vicious circle inflammation-oxidative stress occurs largely before the onset of metabolic syndrome. To a better understanding the relationships between oxidative stress, inflammation and body composition, further investigation exploring the genetic profile is needed. Despite the validation and reliability of oxidative stress markers herein chosen, in order to support our findings, isoprostane measurement as a gold-standard biomarker of oxidative stress, in future investigation will be assessed.

Finally, on the basis of the present and the previous outcomes (4,9–11,31,34–36,39), it is possible to affirm that in NWO contextually occurred oxidative stress and inflammation, comparable to those observed in OB, as a consequence of the higher FM. According to other points of view (12,13,37,38), we agree with the paradigmatic hypothesis that the trigger factor of CVD complications and metabolic disorders could be the oxidative stress occurring in the vicious cycle of obesity, depending at the first level on inflammation and genetic susceptibility.

Consequently, warnings arise concerning the vulnerability of NWO. Indeed, our data confirm that the misclassification of obesity based on BMI likely expose NWO to an underevaluated risk of developing obesity-related diseases. Finally,

it is underlined the opportunity to accurately evaluate both FM% and the FM distribution and, eventually, adopt suitable strategies (i.e., diet and physical acivity) to reverse the obesity-associated inflammation and oxidant stress.

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DISCLOSURE

The authors declared no conflict of interest.

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