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Oxidative stress is increased in sarcopenia and associated with cardiovascular disease risk in sarcopenic obesity

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ABSTRACT

Objectives: To define whether circulating markers of oxidative stress correlate with sarcopenia in terms of glutathione balance and oxidative protein damage, and whether these biomarkers are associated with risk of cardiovascular disease (CVD).

Study design: Population-based cross-sectional study. 115 out of 347 elderly subjects were classified as non-sarcopenic non-obese (NS-NO), sarcopenic non-obese (S-NO), non-sarcopenic obese (NS-O), and sarcopenic obese (S-O).

Main outcome measurements: Sarcopenia was defined as a relative skeletal muscle mass index (RASM) < 7.25 kg/m^2 for men or < 5.67 kg/m^2 for women, while obesity was diagnosed in those presenting with% fat > 27 for men or > 38 for women. The CVD risk was estimated by the carotid intima-media thickness (IMT) and the Framingham score. Blood reduced glutathione (GSH), oxidized glutathione (GSSG), plasma malondialdehyde-(MDA) and 4-hydroxy-2,3-nonenal-(HNE) protein adducts were analyzed.

Results: Significantly greater blood GSSG/GSH ratio and plasma MDA/HNE protein adducts were observed in sarcopenic than in non-sarcopenic patients. A logistic regression model showed a close relationship between serum HNE and MDA adducts and sarcopenia (OR = 1.133, 95% CI 1.057–1.215, and OR = 1.592, 95% CI 1.015–1.991, respectively). Linear and logistic regression analysis evidenced strong associations between the IMT or the Framingham CVD risk category and blood GSSG/GSH or serum HNE protein adducts in the S-O group.

Conclusion: Circulating markers of oxidative stress are increased in sarcopenia and related to CVD risk in sarcopenic obesity, suggesting that redox balance analysis would be a useful part of a multidimensional evaluation in aging. Further research is encouraged to support interventional strategies to correct redox imbalance, which might contribute to the prevention or at least limitation of sarcopenia and its co-morbidities.

1. Introduction

Sarcopenia can be defined as an age-related loss of muscle mass and function associated with poor quality of life and high mortality [1]. Sarcopenia is frequently combined with an increase in body fat, a condition termed sarcopenic obesity [2]. There is no consensus definition of both sarcopenia and obesity in aged subjects; as a consequence, prevalence of sarcopenia and sarcopenic obesity is significantly variable [3]. The co-presence of both sarcopenia and obesity is predictive of worse outcomes than either condition alone [4].

Sarcopenia and obesity share several pathophysiological mechanism which may potentiate each other, synergistically increasing their effect on metabolic disorders, cardiovascular disease and mortality [5]. Thus, the early and correct identification of patients with sarcopenic obesity is extremely important in order to target preventive and therapeutic strategies for those at greatest cardiovascular risk.

Clinical diagnosis of sarcopenia is performed by the assessment of skeletal muscle mass using anthropometry, bioelectrical impedance analysis (BIA) or dual energy X-ray absorptiometry (DEXA). While anthropometric measures and BIA have limited accuracy and validity,

Abbrevations: BIA, bioelectrical impedance analysis; DEXA, dual energy X-ray absorptiometry; BMI, Body Mass Index; HNE, 4-hydroxy-2-nonenal; MDA, malondialdehyde; CVD, cardiovascular disease; LBM, lean body mass; ASM, appendicular skeletal muscle mass; RSMI, relative skeletal muscle mass index; EWGSOP, European Working Group on Sarcopenia in Older People; GSH, oxidized glutathione; GSSG, reduced glutathione; TNF-α, tumor necrosis factor alpha; IMT, intima-media thickness; SDM, standard deviation of the mean; ANOVA, analysis of variance; OR, odds ratio; CI, confidence interval; MNA, Mini Nutritional Assessment

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DEXA provides valid estimates of appendicular skeletal muscle mass, and skeletal muscle measures with DEXA are associated with physical disability [6]. Obesity is defined as Body Mass Index (BMI) ≥ 30 kg/m^2, and central obesity as a waist circumference > 102 cm in men and 88 cm in women, but these criteria are not appropriate in the elderly [7]. Currently, little is known about biological markers of sarcopenia and sarcopenic obesity.

The development of sarcopenic obesity might recognize an intricate interplay of factors including insulin resistance, altered dietary energy, and inflammation [8]. Recent studies suggest pathophysiological links between sarcopenia, sarcopenic obesity and oxidative stress [9]. Oxidative metabolism in skeletal muscle cells produces potentially toxic free radicals which are neutralized by the intracellular antioxidant system. Glutathione plays a central role in detoxification reactions and in the regulation of cellular thiol-disulfide status [10]. One of the indices of systemic oxidative stress is the depletion of circulating glutathione as an index of decreased antioxidant systems in several tissues [11]. A further consequence of oxidative stress is the peroxidation of membrane lipids, a process that leads to alterations in the biological properties of the membrane and to amplification of cellular damage. Lipid peroxidation produces a variety of relatively stable decomposition end products, mainly reactive aldehydes such as 4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA), which in turn may produce aldehyde-protein adducts measurable in serum as indirect oxidative stress markers [12]. Increasing evidence associates biomarkers of oxidative stress to several human conditions such as obesity, inflammation, aging and cardiovascular diseases [13]. Nevertheless, to date no studies have reported variations of these markers in human sarcopenia as well as sarcopenic obesity. Thus, the present study was designed in order to define whether circulating oxidative stress correlates to sarcopenic obesity in terms of glutathione balance and oxidative protein damage, and whether these biomarkers are associated with cardiovascular disease (CVD) risk in this special sub-population.

2. Methods

2.1. Patients

115 out of 347 elderly outpatients attending the geriatric clinic of the “Casa Sollievo della Sofferenza” hospital in San Giovanni Rotondo (Foggia, Italy) were enrolled. Subjects < 65 years old or with previous diagnosis of ischemic heart disease, stroke, liver cirrhosis, chronic kidney disease, and active cancer were excluded. Further exclusion criteria were chronic bedridden conditions, physical handicap, severe neuro-muscular disease, use of drugs affecting body composition or redox balance, and daily alcohol intake > 40 g. The study was performed according to the Declaration of Helsinki. All patients gave written informed consent.

2.2. Anthropometric and body composition measurements

Baseline evaluation included subjects’ demographics, co-morbidities and socio-economic factors. Height, body weight, and waist circumference were measured according to standardized procedures. Body mass index (BMI) was calculated as the ratio between weight in kilograms and the square of height in meters. The whole-body dual-energy X-ray absorptiometry (DEXA) scan Lunar iDXA™ (GE-Healthcare, Wisconsin, USA) was used for the measurement of fat-free lean body mass (LBM) and percentage of fat mass. Appendicular skeletal muscle mass (ASM) was calculated as the sum of LBM from both arms and legs, according to the method of Heymsfield et al. [14]. Relative skeletal muscle mass index (RSMI) was defined as ASM divided by height (in meter) squared.

2.3. Diagnostic criteria for sarcopenia and obesity

According to the European Working Group on Sarcopenia in Older People (EWGSOP) criteria, after the assessment of gait speed and grip strength, each patient underwent a DEXA measurement and sarcopenia was diagnosed in subjects presenting with a RSMI < 7.25 kg/m^2 (men) or < 5.67 kg/m^2 (women) [1]. Obesity was diagnosed in patients presenting with% fat mass > 27 (men) or > 38 (women).

2.4. Laboratory measurements

Standard laboratory measurements included serum glucose, glycated haemoglobin, insulin, total cholesterol, LDL- and HDL-cholesterol, tryglycerides, creatinine, albumin, uric acid, vitamin D, and microalbuminuria.

A specific enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) was used to measure plasma tumor necrosis factor alpha (TNF-α), as previously reported [15].

Oxidized (GSH) and reduced (GSSG) glutathione were determined in whole blood as previously described [15].

Plasma fluorescent adducts formed between peroxidation-derived aldehydes (HNE and MDA) and proteins were measured by spectrofluorimetry as previously reported [16].

2.5. Carotid ultrasonography

Bilateral carotid arteries in longitudinal projections were investigated by an experienced operator using an ultrasound instrument (Philips Affiniti 70, Amsterdam, the Netherlands) equipped with a high-resolution broadband width linear array transducer. The participants were examined in the supine position. Each participant had intima-media thickness (IMT) measured on the far wall of the common carotid artery by longitudinal view [17].

2.6. Cardiovascular disease risk assessment

The CVD risk was estimated by the Framingham risk score, based on the Framingham Heart Study (National Heart, Lung, and Blood Institute in Bethesda, MD, USA). The risk score was calculated based on categorical values of age, sex, total and HDL cholesterol, systolic blood pressure, smoking, and diabetes [18]. The participants were then stratified into the following three groups: low risk, presenting with less than 10% CVD risk at 10 years; intermediate-risk, presenting with 10–20% CVD risk at 10 years; high risk, presenting with more than 20% CVD risk at 10 years.

2.7. Statistical analysis

Data were expressed as count and percentages for qualitative values, and as mean ± standard deviation of the mean (SDM) for quantitative variables. Gaussian distribution of the samples was evaluated by Kolgomorov–Smirnov test. The significance of differences between 2 groups (sarcopenic vs non-sarcopenic) was assessed by student’s *t*-test (continuous variables) or in contingency tables by Pearson’s Chi-squared test and Fisher’s exact test (categorical variables). The significance of differences between more than 2 groups was assessed by the one-way analysis of variance (ANOVA) after ascertaining normality by the Kolgomorov-Smirnov test; the Tukey-Kramer was applied as post hoc test. The odds ratio (OR) and the 95% confidence interval (CI) were calculated. Here, ORs > 1 imply a higher chance for SVR relative to the reference category. Linear regression models were used to analyse the association between carotid IMT and GSSG/GSH ratio or serum HNE-protein adducts. Logistic regression models were used to analyse the association between redox measurements and sarcopenia or sarcopenic obesity, as well as between the CVD risk categories according to the Framingham risk score (along with each individual score component)

Table 1
Baseline characteristics of the patients included in the study according to the presence or the absence of sarcopenia. Statistical differences were assessed by student's *t*-test or Pearson's Chi-squared test.

	Non-sarcopenic (N = 67)	Sarcopenic (N = 48)	P
Age (years)	76.49 ± 6.97	77.96 ± 6.22	0.206
Gender (M/F)	33/44	28/20	0.336
BMI (kg/m^2)	31.94 ± 5.86	24.62 ± 5.45	**< 0.001**
Waist circumference (cm)	118.1 ± 19.90	102.1 ± 18.60	**0.001**
Tricipital fold (cm)	19.91 ± 14.67	26.40 ± 9.32	0.209
Carotid intima-media (cm)	0.92 ± 0.10	1.01 ± 0.13	**< 0.001**
Diabetes mellitus (n, %)	33 (49%)	11 (23%)	**0.003**
Hypertension (n, %)	55 (82%)	31 (64%)	**0.028**
Smokers (n, %)	26 (39%)	23 (48%)	0.329
Glucose (mg/dL)	106.9 ± 32.65	99.42 ± 21.93	0.167
HbA1c (%)	6.74 ± 1.88	5.86 ± 0.86	**0.003**
Insulin (μU/mL)	6.86 ± 2.57	6.84 ± 3.09	0.984
Total Cholesterol (mg/dL)	160.7 ± 37.47	144.6 ± 50.49	0.052
LDL-Cholesterol (mg/dL)	96.67 ± 33.36	83.46 ± 36.64	**0.047**
HDL-Cholesterol (mg/dL)	43.15 ± 11.11	41.50 ± 15.83	0.513
Tryglycerides (mg/dL)	120.7 ± 51.14	105.3 ± 52.69	0.119
Creatinine clearance (ml/min)	73.75 ± 25.89	56.23 ± 22.22	0.307
Albumin (g/dL)	3.83 ± 0.43	3.66 ± 0.58	0.074
Microalbuminuria (mg/24 h)	98.31 ± 23.26	60.39 ± 90.58	0.545
Uric Acid (mg/dL)	5.69 ± 1.64	4.95 ± 1.98	**0.028**
Vitamin D (ng/mL)	10.11 ± 8.31	13.01 ± 15.04	0.507

and blood GSH, GSSG, GSSG/GSH ratio, serum HNE- and MDA-protein adducts. All tests were 2-sided, and P values < 0.05 were considered to be statistically significant. Statistical analysis was performed with the Statistical Package for Social Sciences version 23.0 (SPSS, Inc., Chicago, IL) and the package Graph-Pad Prism 6.0 for Windows (GraphPad Software, Inc., San Diego, CA).

3. Results

Baseline characteristics of the subjects grouped according to the absence or presence of sarcopenia are reported in Table 1. The two groups were comparable in terms of age and gender; nevertheless, the sarcopenic group showed lower BMI and waist circumference, and a reduced prevalence of diabetes mellitus and hypertension, as compared to non-sarcopenic. Accordingly, the mean serum HbA1c value was lower in sarcopenic rather than non-sarcopenic subjects, as well as LDL-cholesterol and uric acid levels. The carotid IMT was higher in the sarcopenic rather than the non-sarcopenic patients (Table 1).

The Supplementary Tables S1 and S2 report differences in measures obtained by DEXA assessment and by the multidimensional evaluation in the two groups, respectively. Of note, the Mini Nutritional Assessment (MNA) score was lower in sarcopenic rather than non-sarcopenic subjects. The Framingham CVD score was not significantly different between the two groups (data not shown).

3.1. Sarcopenic patients show increased circulating oxidative stress

To assess the correlation between sarcopenia and oxidative stress, we measured the redox balance in terms of circulating glutathione balance and serum proteins oxidatively modified by lipoperoxidative reactions. A significant decrease in the blood GSH levels was found in patients with sarcopenia when compared to non-sarcopenic (Fig. 1A). No difference was observed between the two groups with regard to GSSG levels (Fig. 1B), nevertheless when the glutathione balance was expressed in terms of GSSG/GSH, patients with sarcopenia exhibited an increase in the ratio as compared to non-sarcopenic subjects (Fig. 1C).

To explore the inflammatory pattern of sarcopenia, we measured plasma TNF-α and reported significantly higher levels in sarcopenic with respect to non-sarcopenic patients (Fig. 1D).

To verify whether the circulating glutathione imbalance was related to oxidative stress in sarcopenia, the level of protein oxidation was measured in terms of serum HNE- and MDA-protein adducts and the results are reported in Fig. 1E, F. A marked increase of aldehyde-protein adducts was evident in sarcopenic as compared to non-sarcopenic group, significantly for HNE-protein adducts.

To evaluate whether the redox balance could contribute to explain the presence of sarcopenia, we performed a binary logistic regression model where sarcopenic/non-sarcopenic was the dependent variable, and blood GSSG/GSH ratio, serum HNE- and MDA-protein adducts were the independent variables. As shown in Table 2, the oxidative stress variables were associated with the presence of sarcopenia: in particular, the serum HNE- and MDA-protein adducts predicted the presence of sarcopenia (OR = 1.133, 95% CI 1.057–1.215, and OR = 1.592, 95% CI 1.015–1.991, respectively).

3.2. Circulating oxidative stress markers are associated with CVD risk in sarcopenic obesity

51 subjects were found obese when the BMI was applied for the diagnosis of obesity: of these, 42 were non-sarcopenic but 9 were included in the sarcopenic group. Since the BMI could not be appropriate to diagnose obesity in the elderly [7], we decided to identify the obese subjects as presenting with% fat mass > 27 (men) or > 38 (women). According to these criteria, the four following groups of patients were defined: no sarcopenia-no obesity (NS-NO, n = 8, 7%), no sarcopenia-obesity (NS-O, n = 59, 51%), sarcopenia-no obesity (S-NO, n = 23, 20%), and sarcopenia-obesity (S-O, n = 25, 22%).

Both BMI and waist circumference were higher in the obese as compared with non-obese groups; nevertheless, while we did not report any significant difference in the waist circumference between NS-O and S-O, the BMI was higher in NS-O as compared to S-O (Supplementary Fig. S1). Interestingly, blood GSSG/GSH ratio, plasma TNF-α and serum HNE-/MDA-protein adducts were higher in the S-O group with respect to the other three groups (Fig. 2A–F).

Notably, Pearson analysis showed that there was a strong correlation between the carotid intima-media thickness and blood GSSG (r = 0.763, p < 0.001), serum HNE-protein adducts (r = 0.756, p < 0.001), serum MDA-protein adducts (r = 0.471, p < 0.001). A logistic regression model correlating S-O with circulating oxidative stress markers showed a close relationship between GSSG/GSH ratio (OR = 1.4, 95% CI = 1.1–2.3) as well as serum HNE-adducts (OR = 2.3, 95% CI = 1.3–2.7) to sarcopenic obesity. Finally, linear regression analysis evidenced a strong association between the carotid intima-media thickness and blood GSSG/GSH (r^2 = 0.6294, p < 0.0001) or serum HNE-protein adducts (r^2 = 0.625, p < 0.0001) in the S-O group (Fig. 2G, H); similarly, a logistic regression analysis associated the Framingham CVD risk category to both blood GSSG/GSH (r^2 = 0.662, p < 0.001) and serum HNE-protein adducts (r^2 = 0.606, p < 0.001) in sarcopenic obese patients (Supplementary Fig. S2). To gain insight into the individual components of the Framingham CVD risk score, further regression analyses were performed in sarcopenic patients, showing that GSSG/GSH associated with total cholesterol (r^2 = 0.660, p < 0.0001), HDL-cholesterol (r^2 = 0.662, p = 0.003) and hypertension (r^2 = 0.549, p < 0.02), and HNE-protein adducts associated with total cholesterol (r^2 = 0.408, p < 0.027) and HDL-cholesterol (r^2 = 0.436, p = 0.018).

4. Discussion

To our knowledge, this is the first study demonstrating that circulating markers of oxidative stress are increased in elderly subjects

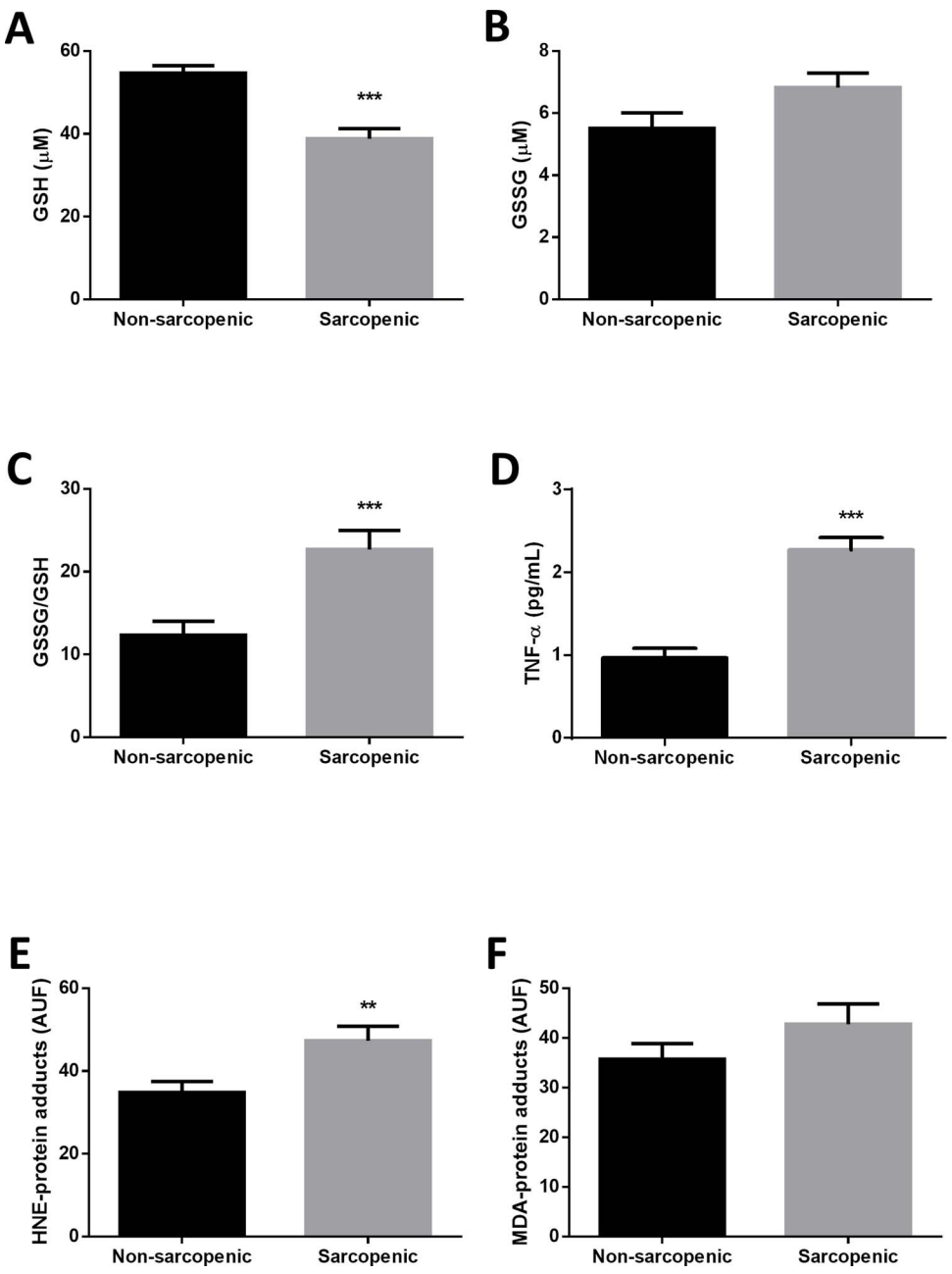

Fig. 1. Changes in oxidized (GSH, panel A), reduced (GSSG, panel B) blood glutathione levels and GSSG/GSH ratio (panel C), in plasma Tumor Necrosis Factor-alpha (TNF-α) levels (panel D), and in serum levels of fluorescent hydroxynonenal- (HNE, panel E) and malondialdehyde- (MDA, panel F) protein adducts, in the patients included in the study according to the presence or the absence of sarcopenia. Data are expressed as mean ± SDM, statistical differences were assessed by student's *t*-test for independent samples. ** = $p < 0.01$; *** = $p < 0.001$. AUF, arbitrary units of fluorescence; OR, odds ratio; CI, confidence interval.

Table 2
Binary logistic expression analysis testing the association between oxidative stress, expressed as blood GSSG/GSH ratio, serum HNE- and MDA-protein adducts, and sarcopenia. Statistical differences were assessed by the Chi-square test.

	Sarcopenic vs Non-sarcopenic		
	OR	95% CI	p
Blood GSSG/GSH	0.964	0.906–1.025	0.242
Serum HNE-protein adducts	1.133	1.057–1.215	< **0.001**
Serum MDA-protein adducts	1.592	1.015–1.991	< **0.001**

OR, odds ratio; CI, confidence interval; GSSG, reduced glutathione; GSH, oxidized glutathione; HNE, hydroxynonenal; MDA, malondialdehyde.

presenting with sarcopenia and associate with cardiovascular risk in sarcopenic obesity.

Ageing is associated with important modifications in body composition, characterized by a substantial reduction in muscle mass and an increase in visceral fat mass, but no significant changes in total body weight [2]. The loss in lean mass decreases energy expenditure leading to increased obesity risk, and the gain in fat mass induces inflammation contributing to the development of sarcopenia [19]. The identification of obesity in sarcopenic subjects is a major concern, since BMI and waist circumference could not estimate the muscle mass loss in elderly population. Indeed, cardiovascular mortality is increased in aged subjects presenting with a normal as compared to high BMI, a phenomenon defined as "obesity paradox" [20]. In the current investigation, both BMI and waist circumference were significantly reduced in the sarcopenic group; thus, we decided to use the% fat mass obtained by DEXA measurement to identify elderly subjects with obesity. Even though computerized tomography and magnetic resonance represent the gold standard to estimate visceral fat, recent studies indicate that DEXA represents a valuable alternative quantification method [21].

Both sarcopenia and obesity are associated with metabolic alterations leading to increased disability, morbidity and mortality [22]. It has previously been suggested that sarcopenia is associated with risk

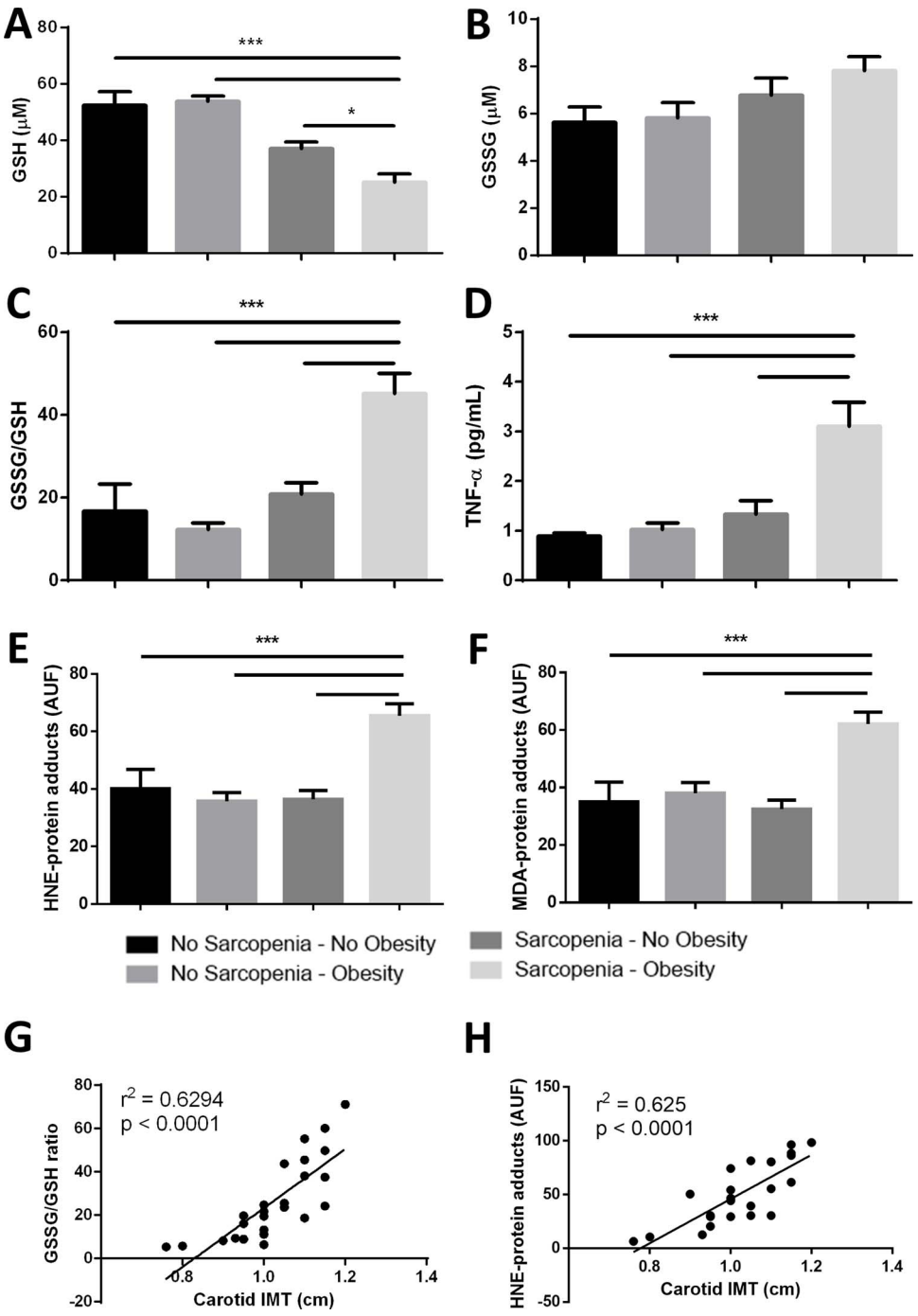

Fig. 2. Changes in oxidized (GSH, panel A), reduced (GSSG, panel B) blood glutathione levels and GSSG/GSH ratio (panel C), plasma Tumor Necrosis Factor-alpha (TNF-α, panel D), serum hydroxynonenal- (HNE, panel E) and malondialdehyde- (MDA, panel F) protein adducts in the patients included in the study according to the presence or the absence of sarcopenia and obesity. Data are expressed as mean ± SDM, statistical differences were assessed by one-way ANOVA for independent samples, applying the Tukey-Kramer as post hoc test. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Panels G-H: linear regression analysis between the carotid intima-media thickness (IMD) and blood GSSG/GSH ratio (G) or serum HNE-protein adducts (H) in the patients affected by sarcopenic obesity included in the study. AUF, arbitrary units of fluorescence.

factors for CVD and may itself be an individual risk factor for CVD [23]. We did not observe any significant changes in the parameters used to calculate the Framingham CVD risk score between non-sarcopenic and sarcopenic patients. Of note, the prevalence of diabetes and hypertension, as well as LDL-cholesterol and glycated haemoglobin, were lower in sarcopenic rather than non-sarcopenic group. Nevertheless, we described a higher carotid IMT in sarcopenic with respect to non-sarcopenic subjects. Several studies have shown an association between carotid IMT and future CVD events [24]. Recent observations associate sarcopenic obesity to cardiovascular disease, however the lack of a unified definition for sarcopenic obesity contributes to inconsistent findings about this association [5]. A British Regional Health Study reported a greater cardiovascular mortality in older men presenting with sarcopenia and central adiposity [25]. Our data show that sarcopenic patients with increased fat mass present with a higher CVD risk compared to non-sarcopenic and to sarcopenic non-obese subjects, suggesting a possible synergic interaction between these two conditions.

Free radicals are produced in both inactive and contracting skeletal muscles, and when their production exceeds the antioxidant capacity, oxidative stress occurs [23]. A cross-sectional study reported that oxidative stress is associated with a decline in muscle strength during aging, suggesting that oxidative damage may contribute to sarcopenia in elderly subjects [26]. Oxidative stress leads to the formation of molecules that can be used as markers of injury, such as 4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA), which are lipid peroxidation products able to generate adducts with cellular and circulating proteins [16]. On the other side, reduced glutathione (GSH) is

considered to be one of the most important endogenous antioxidant compounds, since this molecule acts as a free radical scavenger [10]. Altered levels of GSH and GSSG are associated to compromised muscular function in several cellular and animal models of aging [11]. Moreover, we have previously described increased circulating GSSG/GSH, and serum HNE- and MDA-protein adducts to human frailty [15]. The present investigation reports increased circulating GSSG/GSH levels as well as HNE- and MDA-protein adducts in sarcopenic with respect to non-sarcopenic patients. The higher GSSG/GSH is dependent on a reduced GSH rather than increased GSSG, suggesting an antioxidant depletion by free radicals excess that is not counterbalanced by an adequate GSH synthesis, which favours the progression of oxidative damage. The impact exerted by oxidative stress in sarcopenia development was also investigated using a logistic regression model where redox balance was used as independent variable. A condition of high oxidative stress increases the risk of suffering from sarcopenia.

Circulating levels of oxidative stress markers are particularly increased in patients presenting with sarcopenic obesity as compared to the other groups. Oxidative stress plays a determinant role in the pathogenesis of atherosclerosis and consequently of major cardiovascular diseases, since endothelial dysfunction is characterized by free radicals overproduction and reduced NO availability [27]. Obesity is also associated to increased free radicals damage, since oxidative stress markers are increased and directly related to BMI and fat mass, while there is an inverse relationship between central adiposity and antioxidant capacity [28,29]. The present results support the hypothesis that circulating oxidative stress markers are linked to increased CVD risk in elderly subjects affected by sarcopenic obesity. We could argue that the simultaneous presence of sarcopenia and obesity, which is associated to both oxidative stress and a pro-inflammatory state (as indicated by higher levels of circulating TNF-α), is determinant in the raise of CVD risk.

This investigation presents several limitations. First, it is possible that sample size was not enough for drawing solid conclusions. Moreover, the fact that this study was performed in a single centre may have presented some bias. Nevertheless, although further multicentre studies will be required to verify our findings, this study represents an important step in understanding the association between oxidative stress and sarcopenia. Even though we could not address the role of oxidative stress as precipitant or progressive factor in sarcopenia, speculations can be proposed. An impairment of redox capacity was described as a component of multisystem efficiency reduction which occurs in aging [30]. In addition, several chronic diseases may reduce antioxidant defences. Since co-morbidity is often described in the elderly, the redox balance is frequently moved toward the oxidative state and additional factors such as obesity may increase the susceptibility of sarcopenic patients to oxidative injury and cardiovascular disease.

5. Conclusions

The present study reported that circulating oxidative stress markers are increased in sarcopenia and related to cardiovascular disease risk in sarcopenic obesity. These observations suggest that redox balance analysis may be considered as part of multidimensional evaluation in aging, since it is a fast and relatively low-cost method. Upcoming investigations should be designed to evaluate clinical markers of oxidative stress in biological fluids for the early diagnosis and follow-up of age-related conditions. Furthermore, it is conceivable that future interventional strategies aiming at the correction of redox imbalance might contribute to prevent or limit sarcopenia and its co-morbidities. Future studies are required to define redox-dependent molecular mechanisms underlying sarcopenia and sarcopenic obesity, in order to identify specific markers able to predict sarcopenia development in elderly subjects.

Contributors

Francesco Bellanti wrote the paper, was responsible for the study design, analyzed and interpreted the data.

Antonino D. Romano analyzed and interpreted the data, and contributed to the drafting of the manuscript.

Aurelio Lo Buglio collected the data and critically revised the manuscript.

Valeria Castriotta collected and analyzed the data.

Giuseppe Guglielmi collected the data and critically revised the manuscript.

Antonio Greco collected the data and critically revised the manuscript.

Gaetano Serviddio contributed to the study concept and design, interpreted the data and critically reviewed the manuscript.

Gianluigi Vendemiale contributed to the study concept and design, interpreted the data and critically reviewed the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical approval

Informed consent was obtained from each patient. The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association. All patients were informed of the aims and procedures of the study and gave their consent.

Provenance and peer review

This article has undergone peer review.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.maturitas.2017.12.002.

References

[1] A.J. Cruz-Jentoft, J.P. Baeyens, J.M. Bauer, Y. Boirie, T. Cederholm, F. Landi, F.C. Martin, J.P. Michel, Y. Rolland, S.M. Schneider, E. Topinkova, M. Vandewoude, M. Zamboni, Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People, Age Ageing 39 (2010) 412–423.

[2] R.N. Baumgartner, Body composition in healthy aging, Ann. N. Y. Acad. Sci. 904 (2000) 437–448.

[3] J.A. Batsis, T.A. Mackenzie, F. Lopez-Jimenez, S.J. Bartels, Sarcopenia, sarcopenic obesity, and functional impairments in older adults: National Health and Nutrition Examination Surveys 1999–2004, Nutr. Res. 35 (2015) 1031–1039.

[4] R.N. Baumgartner, S.J. Wayne, D.L. Waters, I. Janssen, D. Gallagher, J.E. Morley, Sarcopenic obesity predicts instrumental activities of daily living disability in the elderly, Obes. Res. 12 (2004) 1995–2004.

[5] T.N. Kim, K.M. Choi, The implications of sarcopenia and sarcopenic obesity on cardiometabolic disease, J. Cell. Biochem. 116 (2015) 1171–1178.

[6] M. Pahor, T. Manini, M. Cesari, Sarcopenia: clinical evaluation, biological markers and other evaluation tools, J. Nutr. Health Aging 13 (2009) 724–728.

[7] Obesity: preventing and managing the global epidemic. Report of a WHO consultation, World Health Organ. Tech. Rep. Ser. 894 (2000) i-253.

[8] S.W. Park, B.H. Goodpaster, E.S. Strotmeyer, L.H. Kuller, R. Broudeau, C. Kammerer, N. de Rekeneire, T.B. Harris, A.V. Schwartz, F.A. Tylavsky, Y.W. Cho, A.B. Newman, Accelerated loss of skeletal muscle strength in older adults with type 2 diabetes: the health, aging, and body composition study, Diabetes Care 30 (2007) 1507–1512.

[9] S.J. Meng, L.J. Yu, Oxidative stress, molecular inflammation and sarcopenia, Int. J.

Mol. Sci. 11 (2010) 1509–1526.
[10] G. Vendemiale, I. Grattagliano, E. Altomare, An update on the role of free radicals and antioxidant defense in human disease, Int. J. Clin. Lab. Res. 29 (1999) 49–55.
[11] H. Sies, Glutathione and its role in cellular functions, Free Radic. Biol. Med. 27 (1999) 916–921.
[12] I. Dalle-Donne, R. Rossi, R. Colombo, D. Giustarini, A. Milzani, Biomarkers of oxidative damage in human disease, Clin. Chem. 52 (2006) 601–623.
[13] J. Frijhoff, P.G. Winyard, N. Zarkovic, S.S. Davies, R. Stocker, D. Cheng, A.R. Knight, E.L. Taylor, J. Oettrich, T. Ruskovska, A.C. Gasparovic, A. Cuadrado, D. Weber, H.E. Poulsen, T. Grune, H.H. Schmidt, P. Ghezzi, Clinical relevance of biomarkers of oxidative stress, Antioxid. Redox Signal. 23 (2015) 1144–1170.
[14] S.B. Heymsfield, R. Smith, M. Aulet, B. Bensen, S. Lichtman, J. Wang, R.N. Pierson Jr., Appendicular skeletal muscle mass: measurement by dual-photon absorptiometry, Am. J. Clin. Nutr. 52 (1990) 214–218.
[15] G. Serviddio, A.D. Romano, A. Greco, T. Rollo, F. Bellanti, E. Altomare, G. Vendemiale, Frailty syndrome is associated with altered circulating redox balance and increased markers of oxidative stress, Int. J. Immunopathol. Pharmacol. 22 (2009) 819–827.
[16] M. Tsuchida, T. Miura, K. Mizutani, K. Aibara, Fluorescent substances in mouse and human sera as a parameter of in vivo lipid peroxidation, Biochim. Biophys. Acta 834 (1985) 196–204.
[17] S.D. Kanters, A. Algra, M.S. van Leeuwen, J.D. Banga, Reproducibility of in vivo carotid intima-media thickness measurements: a review, Stroke 28 (1997) 665–671.
[18] R.B. D'Agostino Sr., R.S. Vasan, M.J. Pencina, P.A. Wolf, M. Cobain, J.M. Massaro, W.B. Kannel, General cardiovascular risk profile for use in primary care: the Framingham Heart Study, Circulation 117 (2008) 743–753.
[19] S.G. Wannamethee, J.L. Atkins, Muscle loss and obesity: the health implications of sarcopenia and sarcopenic obesity, Proc. Nutr. Soc. 74 (2015) 405–412.
[20] T.E. Dorner, A. Rieder, Obesity paradox in elderly patients with cardiovascular diseases, Int. J. Cardiol. 155 (2012) 56–65.
[21] A.S. Cheung, C. de Rooy, R. Hoermann, E.J. Gianatti, E.J. Hamilton, G. Roff, J.D. Zajac, M. Grossmann, Correlation of visceral adipose tissue measured by Lunar Prodigy dual X-ray absorptiometry with MRI and CT in older men, Int. J. Obes. (Lond) 40 (2016) 1325–1328.
[22] M. Zamboni, G. Mazzali, F. Fantin, A. Rossi, V. Di Francesco, Sarcopenic obesity: a new category of obesity in the elderly, Nutr. Metab. Cardiovasc. Dis. 18 (2008) 388–395.
[23] M. Kozakowska, K. Pietraszek-Gremplewicz, A. Jozkowicz, J. Dulak, The role of oxidative stress in skeletal muscle injury and regeneration: focus on antioxidant enzymes, J. Muscle Res. Cell Motil. 36 (2015) 377–393.
[24] H. Oygarden, Carotid intima-media thickness and prediction of cardiovascular disease, J. Am. Heart Assoc. 6 (2017).
[25] J.L. Atkins, P.H. Whincup, R.W. Morris, L.T. Lennon, O. Papacosta, S.G. Wannamethee, Sarcopenic obesity and risk of cardiovascular disease and mortality: a population-based cohort study of older men, J. Am. Geriatr. Soc. 62 (2014) 253–260.
[26] C. Howard, L. Ferrucci, K. Sun, L.P. Fried, J. Walston, R. Varadhan, J.M. Guralnik, R.D. Semba, Oxidative protein damage is associated with poor grip strength among older women living in the community, J. Appl. Physiol. 103 (2007) 17–20.
[27] R. Lee, M. Margaritis, K.M. Channon, C. Antoniades, Evaluating oxidative stress in human cardiovascular disease: methodological aspects and considerations, Curr. Med. Chem. 19 (2012) 2504–2520.
[28] E. Pihl, K. Zilmer, T. Kullisaar, C. Kairane, A. Magi, M. Zilmer, Atherogenic inflammatory and oxidative stress markers in relation to overweight values in male former athletes, Int. J. Obes. (Lond) 30 (2006) 141–146.
[29] C. Chrysohoou, D.B. Panagiotakos, C. Pitsavos, I. Skoumas, L. Papademetriou, M. Economou, C. Stefanadis, The implication of obesity on total antioxidant capacity in apparently healthy men and women: the ATTICA study, Nutr. Metab. Cardiovasc. Dis. 17 (2007) 590–597.
[30] S.E. Espinoza, H. Guo, N. Fedarko, A. DeZern, L.P. Fried, Q.L. Xue, S. Leng, B. Beamer, J.D. Walston, Glutathione peroxidase enzyme activity in aging, J. Gerontol. A Biol. Sci. Med. Sci. 63 (2008) 505–509.