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Behaviour of carbonyl groups in several clinical conditions: Analysis of our survey

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Abstract. Protein carbonylation is a marker of oxidative protein damage, that is likely involved in the pathogenesis of several diseases. The aim of this study was to evaluate the protein carbonyl (PC) groups in different clinical conditions. It included different groups of subjects: 81 trained subjects; 23 subjects with mild essential hypertension; 31 middle-aged subjects with metabolic syndrome (MS); 106 subjects with MS not selected for age (subdivided into two subgroups, with and without diabetes mellitus); 91 obese adults subdivided in two subgroups (BMI 30–35 Kg/m^2 and BMI > 35 kg/m^2); 48 subjects with obstructive sleep apnea syndrome (OSAS) subdivided in accordance with the apnea/hypopnea index (AHI); 27 subjects with chronic kidney disease (CKD) on conservative therapy; 31 subjects with CKD on haemodialysis treatment; and 50 subjects with juvenile myocardial infarction. PC groups were reduced in trained subjects in comparison with sedentary controls, while no variation was observed in mild essential hypertension. PC groups were increased in MS subjects and in adult obese subjects. In MS subjects the PC groups were not influenced by the presence of diabetes mellitus and in adult obese subjects were not influenced by the obesity degree. In OSAS subjects only those with AHI > 30 showed an increase of PC groups. PC groups increased in CKD subjects undergoing conservative treatment and haemodialysis therapy. In dialyzed subjects, after a standard dialysis session, there was a marked increase in PC groups. In juvenile myocardial infarction PC groups were higher than in controls; there was no difference between STEMI and NSTEMI and their concentration was unaffected by the number of cardiovascular risk factors or stenosed coronary vessels.

Keywords: Oxidative stress, arterial hypertension, metabolic syndrome, juvenile myocardial infarction, chronic kidney failure

1. Introduction

An imbalance between the synthesis of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the endogenous antioxidant mechanisms induces the oxidative stress. The oxidative stress is evident in a large variety of clinical disorders and it is believed to act as a pathogenetic factor [1, 2]. While ROS include free radicals such as superoxide, hydroxyl, peroxyl and hydroperoxyl, RNS include nitric oxide and nitrogen dioxide.

Proteins are the principal target of ROS and RNS because they are present in high concentration in biological systems and dismiss 50–75% of generated ROS [3]. The advanced oxidation protein products (AOPPs) are produced as a consequence of the action of chlorinated compounds, leading to the production of dityrosine residues and to protein cross-linking [3]. AOPPs may maintain and amplify oxidative stress and inflammation activating neutrophils, monocytes and T lymphocytes [4]. *In vitro*, AOPPs inhibit inducible NO synthesis by macrophages [5] and induce ROS synthe-

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sis in endothelial cells partially through NADPH oxidase activation [6]. ROS and RNS modify protein conformation directly or indirectly [7]. ROS- and RNS-induced lipid peroxidation generates certain relatively stable products, such as malondialdehyde (MDA), hydroxynonenal (HNE), oxynonenal (ONE) and isoprostanes [7]. Aminoacid modification induced by α/β unsaturated aldehydes often occurs at the nucleophilic residues of cysteine, histidine and lysine. The damaged proteins cause the cleavage of the primary structure, cross-linking or variation of a single aminoacid chain [7]. Oxidation of the thiol groups of cysteine residues by ROS and RNS may change the structure and function of proteins. Some of the ROS- and RNS-induced modifications of cysteine are reversible, while other reactions with ROS and RNS develop irreversible products [8, 9]. Some reversible protein changes may be protective against irreversible oxidation, but irreversible modifications of protein conformation may cause inhibition of enzymatic activity, increased susceptibility to aggregation and altered proteolysis [10]. Oxidized proteins are almost always catabolized by proteasomes and lysosomes, but some inactive proteins form aggregates inside the cells or in the extracellular matrix.

Protein carbonylation is a marker of oxidative protein damage. Its employment as an indicator of oxidative stress is useful because of the early synthesis and the stability of carbonylated proteins in comparison with other oxidation products. Carbonylation may occur through different mechanisms: direct oxidation of lysine, arginine, proline and threonine residues, interaction with reactive carbonyl species (RCS) produced from carbohydrate or lipid oxidation and non-oxidative reactions with dicarbonyl compounds [10].

We examined plasma concentrations of protein carbonyl (PC) groups in several groups of subjects: healthy sedentary or trained subjects, patients with mild essential hypertension, middle-aged subjects with metabolic syndrome (MS), diabetic and non-diabetic subjects with MS, obese subjects, patients with obstructive sleep apnea syndrome (OSAS), patients with chronic kidney disease (CKD) undergoing conservative treatment or haemodialysis, and a group of relatively young subjects with recent acute myocardial infarction (AMI).

2. Materials and methods

2.1. We examined 9 groups of subjects.

The **first group** included 81 trained subjects (62 men and 19 women; mean age 31.5 ± 8.7 years; BMI 23.8 ± 2.6 kg/m^2). All subjects were free from cardiovascular diseases or other medical diseases. None of them assumed nutritional supplements of antioxidant substances. In each subject thr maximal oxygen consumption (VO_2max) was determined by means of a cardiopulmonary incremental test with a cycloergometer. This group was compared with a control group of sedentary 27 sedentary subjects (20 men and 7 women; mean age 33.2 ± 5.6 years; BMI 24.7 ± 2.7 kg/m^2). This one, as the other control groups described in this study, was recruited amongst the hospital staff members and students. In the whole group of trained subjects (TS) the value of VO_2max was 32.7 ± 11.2 ml/kg/min, while in the group of sedentary controls it was 22.0 ± 5.6 ml/kg/min.

The **second group** included 23 subjects with mild essential hypertension (17 men and 6 women; mean age 45.05 ± 6.14 years; range 31–53 years). The diagnosis of hypertension was based on blood pressure (BP) measurements taken on two separate occasions with the patient in a seated position after 15 minutes of rest. The mean values of these measurements were 144/87 mmHg; only a minority of the patients showed a simultaneous increase of systolic and diastolic BP above 140/90 mmHg, the great majority having only either systolic or diastolic values within the range of hypertension. In this group, the hypertension duration was 13.4 ± 11.9 months and the BMI was 27.04 ± 3.38. This group

was compared with 26 healthy subjects (17 men and 9 women; mean age 43.54 ± 6.92 years; BMI 25.58 ± 4.45 kg/m^2).

The **third group** included 31 middle-aged non-diabetic subjects with MS (18 men and 13 women; mean age 42.9 ± 6.5 years; BMI 31.72 ± 3.60 kg/m^2; waist circumference 103 ± 8 cm; systolic BP 128.3 ± 11.3 mmHg and diastolic BP 80.8 ± 10.0 mmHg; fasting glucose level 90.0 ± 9.7 mg/dl; total cholesterol 228.1 ± 44.0 mg/dl; LDL-C 153.5 ± 42.4 mg/dl; HDL-C 37.6 ± 9.5 mg/dl; triglycerides 268.2 ± 172.6 mg/dl). This group was compared with 54 normal subjects matched by age (mean age 41.3 ± 7.4 years) and sex (35 men and 19 women).

The **fourth group** included 106 subjects with MS (mean age 53.5 ± 8.9 years; waist circumference 106.7 ± 11.2 cm, BMI 32.21 ± 4.53 kg/m^2; SBP 132.1 ± 16.3 mmHg; DBP 81.2 ± 9.9 mmHg; fasting glucose level 114.3 ± 44.3 mg/dl; total cholesterol 213.9 ± 53.0 mg/dl; LDL-C 133.2 ± 46.5 mg/dl; HDL-C 40.4 ± 10.8 mg/dl; tryglicerides 220.2 ± 147.8 mg/dl), subsequently subdivided into diabetics (29 men and 14 women) and non-diabetics (32 men and 31 women). The entire group and the two subgroups were compared with a control group including 54 healthy subjects (35 men and 19 women; mean age 41.3 ± 7.4 years).

The **fifth group** included 91 subjects with obesity (58 men and 33 women; mean age 52.2 ± 11.4 years; waist circumference 115.3 ± 11.6 cm; BMI 35.64 ± 5.06 kg/m^2; SBP 132.9 ± 14.6 mmHg; DBP 81.3 ± 10.4 mmHg; fasting blood glucose 115.2 ± 49.8 mg/dl; total cholesterol 208.4 ± 54.2 mg/dl; LDL-C 130.1 ± 49.0 mg/dl; HDL-C 40.5 ± 11.11 mg/dl; triglycerides 212.1 ± 154.1 mg/dl), subsequently subdivided into two subgroups according to BMI (51 with BMI 30–35 kg/m^2 and 40 with BMI > 35 kg/m^2 respectively). The group of obese subjects as well as the two subgroups were compared with a control group including 44 subjects with normal weight (26 men and 18 women, BMI < 25 kg/m^2).

The **sixth group** included 48 subjects (36 men and 12 women; mean age 49.7 ± 1.6 years) with OSAS. The anthropometric characteristics and the oxygen status parameters were: BMI 35.37 ± 7.31 kg/m^2; waist circumference 118.8 ± 16.1 cm; neck circumference 44.41 ± 4.53 cm; apnea/hypopnea index (AHI) 38.47 ± 25.66; mean nocturnal SO_2 91.1 ± 3.68%; oxygen desaturation index (ODI) 39.34 ± 29.03. This group of OSAS subjects was subdivided according to the AHI value in two subgroups: Low ($L = 21$ subjects with AHI < 30) and high ($H = 27$ subjects with AHI > 30). The entire group and the two subgroups were compared with a control group including 59 normal subjects (43 men and 16 women; mean age 36.24 ± 8.25 years).

The **seventh group** included 27 subjects (15 men and 12 women; mean age 58.25 ± 7.6 years) with clinically stable CKD in conservative therapy, at stages 2–5 according to the KDOQI classification. In this group the cause of CKD was diabetic nephropathy in 6 patients, nephroangiosclerosis in 5, chronic glomerulonephritis in 3, polycystic kidney disease in 2; the cause was unknown in 11 patients. In this group creatinine was 3.15 ± 1.70 mg/dl, creatinine clearance 26.63 ± 17.05 ml/min, leukocyte count 7721 ± 3092/ μl, haemoglobin 12.60 ± 2.15 g/dl. This group was compared with a control group of 26 subjects (17 men and 9 women; mean age 43.54 ± 6.92 years).

The **eighth group** included 31 subjects (16 men and 15 women; mean age 61.5 ± 12.8 years) with CKD on HD treatment. In this group, the cause of CKD was nephroangiosclerosis in 9 patients, diabetic nephropathy in 6, chronic glomerulonephritis in 5; the cause was unknown in 11 patients. Dialysis duration was 48.5 ± 35.7 months. This group was compared with a control group of 26 subjects (17 men and 9 women; mean age 43.54 ± 6.92 years).

The **ninth group** included 50 subjects (45 men and 5 women) aged < 46 years with recent AMI. In this group the mean age was 40.4 ± 4.8 years. The time interval between AMI onset and the examination was 13.0 ± 7.0 days. AMI subjects were subdivided according to the number of risk factors (family history of coronary artery disease, smoking, hypercholesterolemia, diabetes mellitus, arterial hypertension) into 3 subgroups: 14 of them had 0/1 risk factor, 21 had 2 risk factors and 15 had

3 to 5 risk factors. The 44 subjects in which coronary angiography was performed were subdivided into 3 subgroups on the basis of the extent of coronary lesions: 9 subjects did not show any significant coronary stenosis, 22 subjects had a single-vessel disease and 13 subjects had a multi-vessel disease. The control group included 42 subjects (35 men and 7 women; mean age 38.6 ± 5.3 years) without signs of acute or chronic vascular disease.

2.2. Protein oxidation

Plasma PC groups were examined using an enzyme-linked immunosorbent assay (ELISA) kit (BioCell PC test kit, Enzo Life Sciences AG, Switzerland) that uses the classic PC reagent 2,4-dinitrophenyl-hydrazyne (DNP), which reacts with the PC forming a stable hydrazone product. In brief, plasma samples were incubated with DNP, then plasma proteins were nonspecifically adsorbed to the wells of an ELISA plate. Unconjugated DNP and non-protein constituents were washed away. The absorbed proteins were probed with a biotinylated anti-DNP antibody, followed by streptavidin-linked horseradish peroxidase. A chromatin reagent was added, and the reaction was stopped by adding an acid solution. Absorbance for each well was measured at 450 nm and related to a standard curve prepared for serum albumin, containing increasing proportions of hypoclorous acid-oxidized protein, calibrated colorimetrically. Total protein concentration in plasma samples was evaluated by the method of Lowry et al [11].

2.3. Statistical analysis

Data were expressed as means $\pm$ SD; the difference between each group and the respective control group (N) was examined according to the Student's *t* test for unpaired data. The statistical difference between control subjects and TS subdivided according to the type of practiced sport (endurance, mixed or power) was estimated according to the 1-way analysis of variance (ANOVA) integrated with the Bonferroni test. The same statistical approach was used for obese subjects subdivided according to the BMI value, for OSAS subjects subdivided according to the AHI value and for AMI subjects subdivided according to the number of risk factors and the extension of coronary lesions. The evaluation of PC before and after dialysis was performed using the Student's *t* test for paired data. The correlations were effected using the linear regression test and the null hypothesis was rejected for p values > 0.05.

3. Results

3.1. Trained subjects

In the whole group of TS a significant decrease in PC groups was observed [12] in comparison with sedentary controls (SC) (TS = 0.351 ± 0.079 nmol/mg prot; SC = 0.405 ± 0.116 nmol/mg prot) (Fig. 1). Dividing the whole group of TS according to the type of sport (endurance, mixed and power), we noted a significant decrease in endurance (0.321 ± 0.045 nmol/mg prot; $p < 0.001$ vs SC) and in mixed (0.330 nmol/mg prot $p < 0.01$ vs SC) but not in power TS (0.416 ± 0.072 nmol/mg prot; not significant vs SC).

3.2. Mild essential hypertension

In this small group of subjects with mild essential hypertension, we did not observe [13] any variation in PC groups in comparison with normal controls ($N = 0.440 \pm 0.134$ nmol/mg prot; Hypertensives

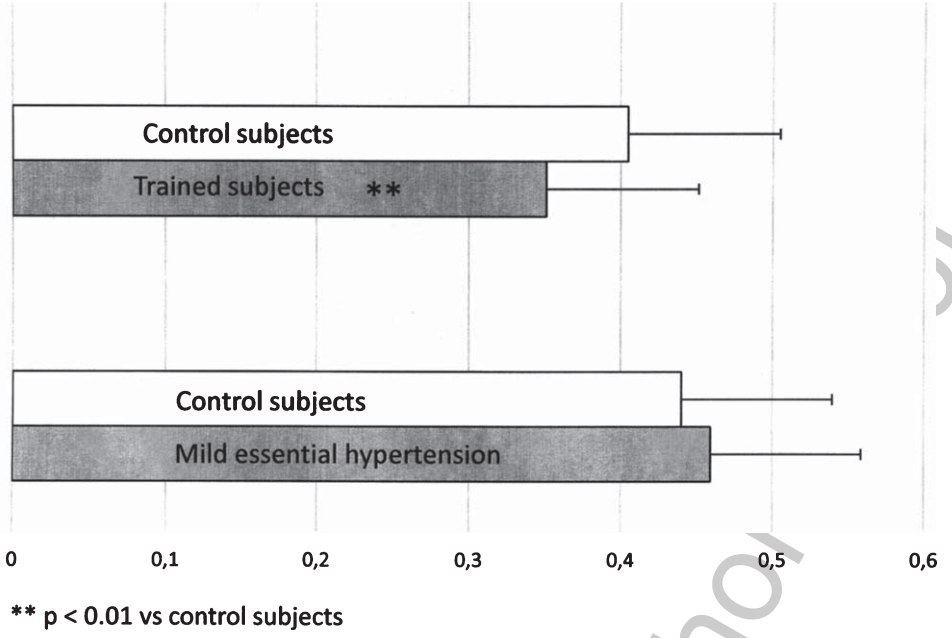

Fig. 1. Protein carbonyl groups in trained subjects and in patients with mild essential hypertension, compared with their respective control groups.

0.459 ± 0.136 nmol/mg prot) (Fig. 1). In hypertensives and in normal controls the PC were not related to age, BMI, metabolic parameters and blood pressure values.

3.3. Middle aged subjects with MS (MAMS)

In this group of middle-aged subjects with MS (MAMS), we observed [14] a significant increase in PC in comparison with normal subjects ($N = 0.424 \pm 0.139$ nmol/mg prot; MAMS = 0.856 ± 0.164 nmol/mg prot; $p < 0.001$) (Fig. 2). In this group, we only found a positive correlation between PC and total cholesterol level ($p < 0.05$).

3.4. Metabolic syndrome (MS)

In the entire group of MS subjects an increase in PC was present ($N = 0.424 \pm 0.139$ nmol/mg prot; MS = 0.874 ± 0.157 nmol/mg prot; $p < 0.001$) in comparison with normal subjects [15] (Fig. 2). The same datum was also observed in MS subjects with diabetes mellitus (DMMS) ($N = 0.424 \pm 0.139$ nmol/mg prot; DMMS = 0.879 ± 0.168 nmol/mg prot; $p < 0.001$) and in MS subjects without DM (NDMMS) ($N = 0.424 \pm 0.139$ nmol/mg prot; NDMMS = 0.871 ± 0.151 nmol/mg prot, $p < 0.001$) (Fig. 2). No difference was found, instead, between diabetic and nondiabetic subjects with MS. In the entire group of MS subjects, we observed a positive correlation between PC and HDL-cholesterol ($p < 0.05$); PC were not correlated with age, anthropometric profile, blood pressure values and metabolic pattern in DMMS, and only a positive correlation between PC and fasting blood glucose level ($p < 0.01$) in NDMMS subjects was observed.

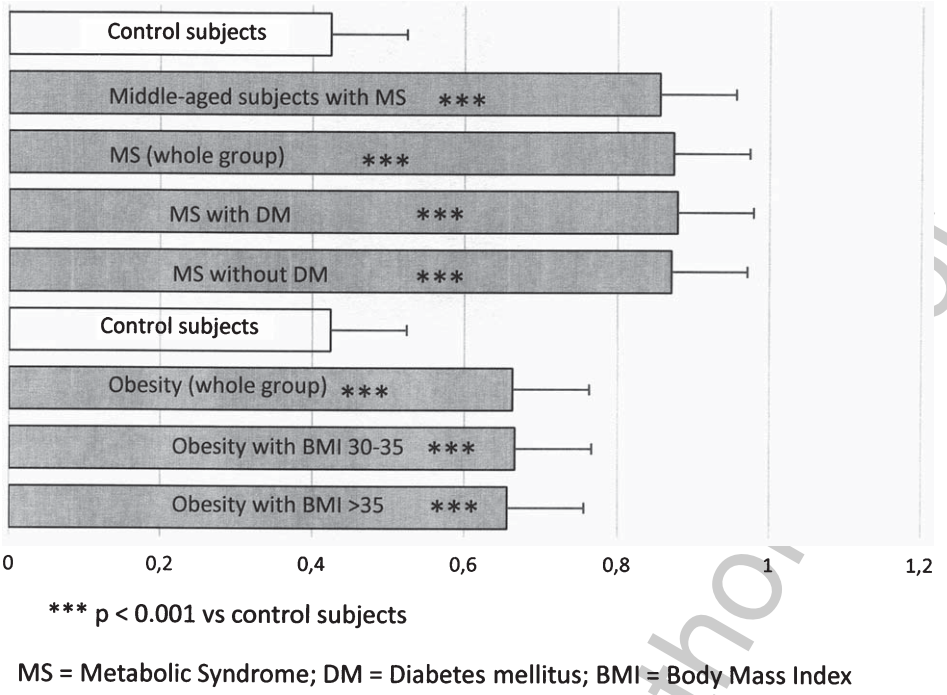

Fig. 2. Protein carbonyl groups in middle-aged subjects with metabolic syndrome, in the whole group of patients with metabolic syndrome and in obese subjects, compared with their respective control groups.

3.5. Obese subjects

In the group of obese subjects (O), we found [16] a significant increase in PC ($N = 0.424 \pm 0.139$ nmol/mg prot; $O = 0.663 \pm 0.314$ nmol/mg prot; $p < 0.001$) (Fig. 2). Splitting the entire group of obese subjects according to BMI (BMI 30–35 kg/m^2 and BMI > 35 kg/m^2) and using the 1-way ANOVA analysis integrated with the Bonferroni test, we observed that the values of PC groups differed significantly ($p < 0.0001$) among normal controls, obeses with BMI 30–35 kg/m^2 (0.666 ± 0.318 nmol/mg prot) and obeses with BMI > 35 kg/m^2 (0.656 ± 0.312 nmol/mg prot) although no statistical difference was noted between the two subgroups of obeses (Fig. 2).

3.6. Obstructive sleep apnea syndrome (OSAS)

In the whole group of OSAS subjects no variation in PC groups was observed in comparison with normal controls ($N = 0.347 \pm 0.094$ nmol/mg prot; OSAS $= 0.316 \pm 0.120$ nmol/mg prot) (Fig. 3). Subdividing the subjects with OSAS in accordance with the AHI values, we found that PC were significantly increased in the H subgroup (0.382 ± 0.099 nmol/mg prot) in comparison with the L subgroup (0.230 ± 0.088 nmol/mg prot) and the entire group of OSAS subjects (0.316 ± 0.120 nmol/mg prot) (Fig. 3). In the entire group of OSAS patients, we observed a positive correlation between PC and neck circumference ($r = 0.61$, $p < 0.0001$) and a positive correlation between PC and waist circumference ($r = 0.35$, $p < 0.02$). We also found a positive correlation between PC and AHI ($r = 0.68$, $p < 0.0001$), and between PC and ODI ($r = 0.63$, $p < 0.0001$), and a negative correlation between PC and mean oxygen saturation (($r = -0.46$, $p < 0.001$) [17].

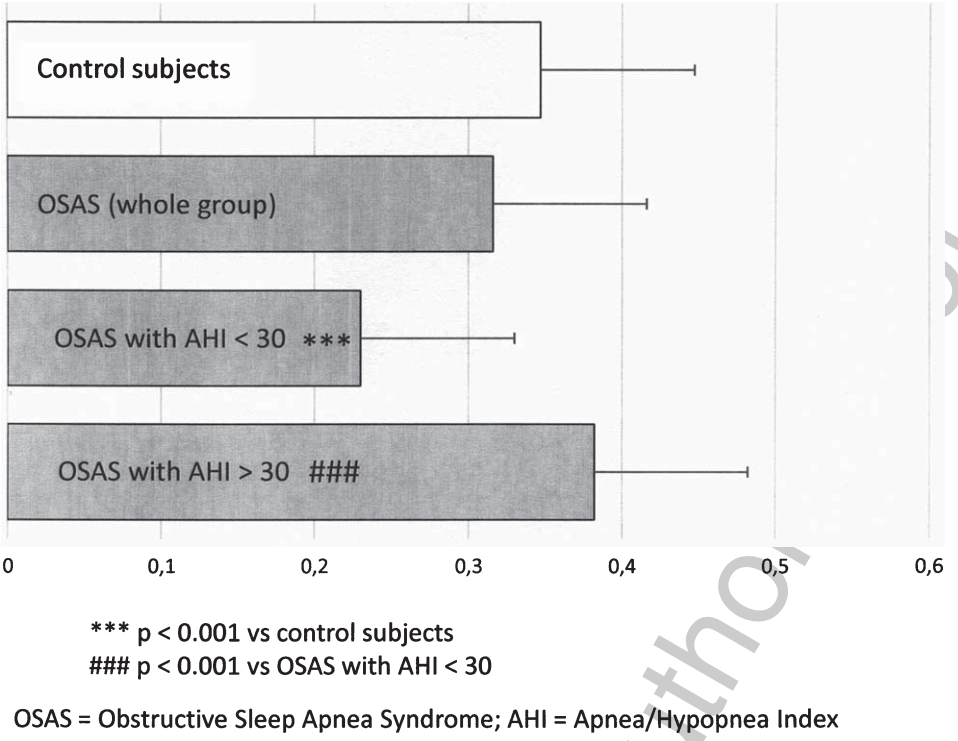

Fig. 3. Protein carbonyl groups in OSAS subjects, compared with the control group.

3.7. Chronic kidney disease (CKD) on conservative treatment

In this group of CKD undergoing conservative treatment (CT-CKD), we found an increase in PC in comparison with normal controls ($N = 0.440 \pm 0.134$ nmol/mg prot; CT-CKD=0.709 ± 0.107 nmol/mg prot; $p < 0.01$) [18] (Fig. 4). In CT-CKD subjects there was a negative correlation between PC and creatinine clearance ($r = -0.46$, $p < 0.02$). PC were not correlated with creatinine levels, leukocyte count and haemoglobin level.

3.8. Chronic kidney disease (CKD) on haemodialysis treatment

In this group of CKD on haemodialysis treatment (HD-CKD), we observed a marked increase in PC in comparison with normal controls ($N = 0.440 \pm 0.134$ nmol/mg prot; HD-CKD=1.230 ± 0.192 nmol/mg prot; $p < 0.001$) (Fig. 4). The standard haemodialysis session was followed by a further increase in PC (HD-CKD before dialysis = 1.230 ± 0.129 nmol/mg prot; HD-CKD after dialysis = 1.394 ± 0.352 nmol/mg prot; $p < 0.01$) [18] (Fig. 4).

3.9. Juvenile myocardial infarction (JMI)

In JMI, PC groups were significantly increased in comparison with normal controls ($N = 0.422 \pm 0.129$ nmol/mg prot; JMI = 0.605 ± 0.179 nmol/mg prot; $p < 0.001$) (Fig. 4). No significant difference in PC was observed between STEMI (0.593 ± 0.189 nmol/mg prot) and NSTEMI (0.594 ± 0.134 nmol/mg prot). Subdividing JMI subjects according to the number of risk factors, no significant difference was observed between the 3 subgroups; similarly, no difference was observed

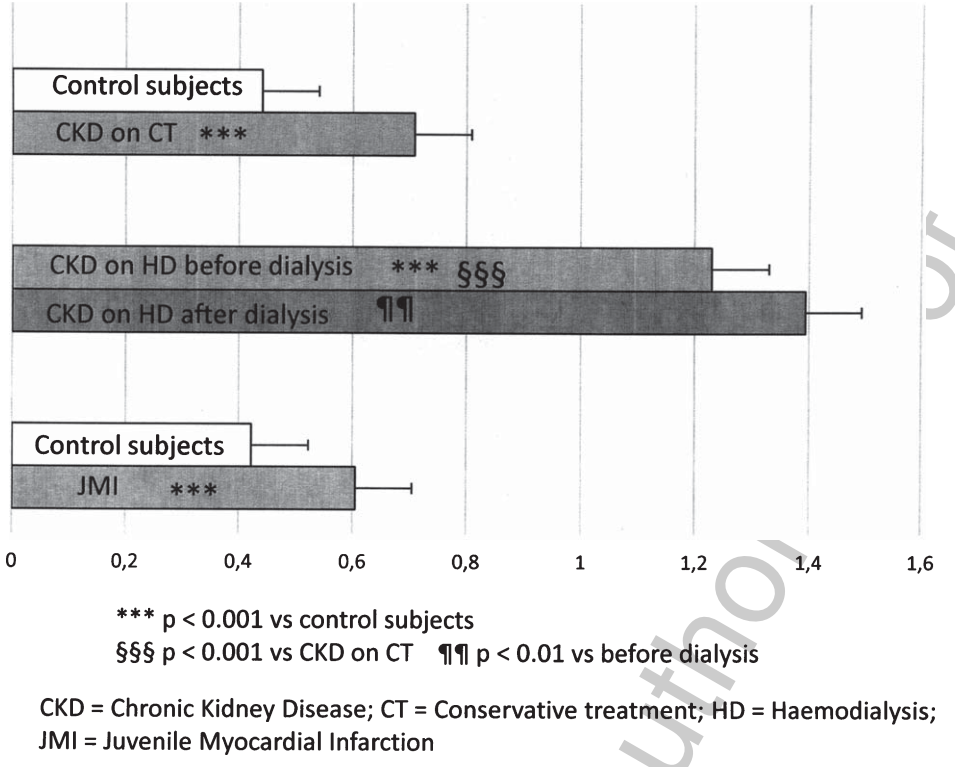

Fig. 4. Protein carbonyl groups in patients with chronic kidney disease on conservative or hemodialysis treatment and in patients with juvenile myocardial infarction, compared with their respective control groups.

subdividing the subjects according to the number of stenosed coronary vessels. No correlation was found between ejection fraction (evaluated by echocardiography) and PC in all JMI subjects nor in the subgroups of STEMI and NSTEMI subjects [19].

4. Discussion

In trained subjects the data of our research show a significantly lower concentration of PC in comparison with sedentary controls, even though the difference was significant only in those who practiced endurance and mixed sports while no variation was found in those practicing power sports. Other authors [20, 21] found a similar trend of PC groups in TS. However, none of these studies considered a comparison between the different types of training. To date, several studies have examined the variation of PC groups produced by an acute bout of exercise [22–25]; only a few have tested PC groups at rest in trained versus untrained subjects. In TS at rest the protein oxidation markers are reduced, whereas the indicators of lipid peroxidation are often increased [21, 26–28]. This datum is apparently a paradox, since epidemiological studies clearly indicate that a regular physical activity is associated with a lower incidence of oxidative-related disease, such as atherosclerosis, cancer and neurodegenerative diseases [29–31]. The simultaneous evidence of increased lipid oxidation and decreased protein oxidation, observed in TS at rest by us [27] and by other authors [21], have no univocal explanation. The cause may be a greater protection of protein molecules from oxidative damage, as well as a more effective repair and/or degradation of damaged proteins.

Our finding in mild essential hypertension shows no variation of PC groups in comparison with normal controls. This datum is different from those observed by other authors who observed an increase in PC groups in hypertensives, although it must be noted that in such research subjects with hypertension were much older [32]; in other studies, the patients had higher blood pressure values [33–35]. However, in the papers by Simic [35] and Nandeesha [34], an increase in PC groups was found in subjects with grade I hypertension. Recently, other authors [36] described a significant increase of PC groups in sustained and white coat hypertension. Previously [37], we had found an increase in lipid peroxidation in a group of hypertensive subjects, including all the subjects evaluated for plasma PC groups. This contrasting datum might suggest that protein oxidation may develop at later stages during the clinical course of essential hypertension; its evaluation at the early stage of the disease might underestimate the impact of oxidative stress in hypertensive subjects.

Very interesting are the data regarding the increase of PC groups in middle-aged subjects with MS. We had previously examined the role of oxidative stress in MS and the relative contribution of all the MS components [38]. To date, there is a lack of significant data regarding the profile of oxidative stress in middle-aged subjects with MS. None of the subjects belonging to this group had dietary restrictions before testing for oxidative stress parameters and none had been taking antioxidant supplements; only 8 subjects out of this group were taking statins. It must be underlined that in this small group of middle-aged MS subjects we also found an increase in lipid peroxidation, an increase in nitric oxide metabolites and a significant decrease in total antioxidant status [38].

In MS patients not selected for age, there was an increase of the PC groups, unaffected by diabetes mellitus. To date, the behaviour of protein oxidation has been examined mainly in the principal components of MS (obesity, diabetes mellitus, hypertension and dyslipidemia); only Sebekova [39] and Meaney [40] evaluated protein oxidation in MS. Previously [41], the authors of the current research had undertaken the same research approach.

In our group of 106 MS subjects, the trend of PC groups was evaluated for the entire group, and for the two subgroups with or without diabetes mellitus. PC groups were correlated with age, anthropometric profile, blood pressure values and glycometabolic profile. In agreement with other authors [42, 43], we found no correlation between PC groups and age, but others [44, 45] demonstrated such association. Differently from other authors [33, 35, 46], we observed no correlation between PC groups and blood pressure values. In agreement with other authors [47], PC groups were not statistically correlated with BMI and waistline. However, others [42] found a positive correlation between protein oxidation, waist circumference and waist-to-hip ratio in juvenile overweight and obesity. In the whole group of MS subjects, we found a correlation between PC groups and HDL-cholesterol, not easily explainable, that may be fortuitous. We observed a correlation between PC groups and fasting blood glucose levels only in nondiabetic subjects with MS. Also this datum might be occasional, since no relation has been observed between protein oxidation and fasting blood glucose levels in both types of diabetes [48] and in severely obese children [47].

In our group of adult obese subjects the behaviour of protein oxidation is similar to that of lipid peroxidation [16]. The alteration of the oxidative stress observed by us in obesity confirms previous research papers [49–58]. This trend was observed not only in the entire group but also in the two subgroups (BMI 30–35 kg/m^2 or BMI > 35 kg/m^2). In accordance with other authors [42], a positive correlation between PC groups and TBARS was found, both in the entire group and in the two subgroups of adult obese subjects. The study of correlations with the anthropometric variables only showed a negative correlation of uncertain meaning between PC groups and waist circumference. Some authors [59] examined PC groups in a small group of severely obese patients (BMI = 48 ± 9 kg/m^2) before and six months after bariatric surgery, noting a marked decrease of their concentration ($p < 0.01$). In clinical practice alterations in the oxidative status may be corrected by weight loss, even though other therapeutic strategies deserve consideration.

In OSAS subjects, the behaviour of PC groups was influenced by the severity of the condition. In fact, protein oxidation markedly differentiated subjects with mild to moderate OSAS from those with severe OSAS. Previous research has underlined the correlation between cardiovascular complications and the severity of OSAS [60–63]. In OSAS subjects we found a positive correlation between PC groups and both neck and waist circumference. These findings agree with those of other authors [64] and contrast with others [65]. Another aspect of our research involved the interrelationship between the PC groups and the polysomnographic parameters. In contrast with some authors [66], but in agreement with others [64], we found a positive correlation between PC groups and AHI values, a positive correlation between PC groups and ODI and a negative correlation between PC groups and mean oxygen saturation. It is known that protein oxidation is especially dependent on the hypoxia-reoxygenation episodes that characterize OSAS [67, 68]. The treatment of OSAS using continuous positive airway pressure (cPAP) allows the modification this parameter. An antioxidant therapy, for example consisting of vitamin C and N-acetylcysteine, could be recommended in OSAS as a complementary treatment, in particular in subjects with low adherence to cPAP [69].

For CKD in conservative treatment the increase in PC groups observed by us is in agreement with the data observed by other authors [70–73]. In our small group of CT-CKD subjects, we found a negative correlation between PC groups and creatinine clearance [18]. In the study by Matsuyama et al. [72], a group of CT-CKD subjects was subdivided into 4 groups according to the value of creatinine clearance, and a positive correlation between carbonyl stress and the degree of renal dysfunction was demonstrated. Other authors [73] correlated the levels of PC groups with the estimated glomerular filtration rate without finding any significant result. Other researchers [74] did not observe any variation of PC groups in CT-CKD subjects compared with normal controls. Another study [75] included children with CKD ($n = 65$, age range 1.4–18.6), who were subdivided according to CKD stage; only few subjects had an increase of PC group concentration.

CKD subjects undergoing haemodialysis (HD) are at risk of high oxidative stress and systemic inflammation, which aggravate the cardiovascular disease. We examined the PC groups in 31 HD-CKD subjects before and after a HD session. The levels of PC groups were increased in comparison with normal controls before, and especially after the HD session. The data obtained by us [18] agree with those of some authors [71, 74, 76, 77] although others [70] did not find any difference in PC groups before and after a standard HD session [70]. We did not note any difference in PC groups after subdividing HD-CKD subjects according to their dialysis vintage or the type of filter employed for HD.

To date, literature data are controversial about the influence of dialysis vintage on protein oxidation. Some authors [78] observed increased PC groups in subject on HD longer than 12 months, while others [77] did not find any variation. In the same study [77] HD was ineffective in controlling oxidative stress in uremia. With regard to the filters, some authors [76] reported a decrease in PC groups after using a high flux membrane, although no difference between polysulfone and cellulase membranes was observed. More biocompatible and antioxidant filters, such as vitamin E-coated polysulfone membrane [79, 80] have been proposed. The use of peritoneal dialysis may impact protein oxidation positively [71, 81]. PC groups decreased significantly after kidney transplantation [82]. Considering the behavior of protein oxidation in CKD on conservative and on HD treatment, it is useful to pursue therapeutic strategies able to mitigate or to oppose such unfavourable event. In fact, it involves the activation of granulocytes, monocytes and vascular endothelial cells [83] and accelerates the atherosclerotic process by promoting further oxidative stress and inflammation [84].

In JMI there was an evident increase of PC groups, both in STEMI and NSTEMI subjects. The subdivision according to the number of risk factors and the extent of coronary disease did not show significant differences, although an opposite trend emerged: PC groups tended to be higher in patients with more risk factors and in those with less coronary lesions. An increased protein oxidation has been

observed by several authors [85–89] in AMI subjects not selected for age. Our data do not confirm the correlation between protein oxidation and the severity of coronary disease, which was previously described by other authors [88]. There are many studies on the carbonyl scavenger activity played by some molecules [90–93]; in particular, L-arginine seems to reduce the level of protein oxidation in ischemic heart disease [94, 95].

In *conclusion*, we evaluated protein oxidation in different conditions by means of the same method. PC groups were reduced in trained subjects and did not show any variation in mild essential hypertension. However, they were significantly higher in middle-aged MS subjects, in MS subjects non selected for age and in adult obese subjects. In MS subjects the behaviour of PC groups was not influenced by diabetes mellitus; similarly, in obese subjects they were unaffected by the obesity degree. In OSAS subjects the PC groups were significantly increased only in the subgroup with AHI > 30. They were increased in CKD subjects on conservative treatment and negatively correlated with creatinine clearance. In CKD subjects on HD treatment, the PC groups increased significantly after the HD session. In this respect, useful could be the employment of more biocompatible and antioxidant filters, such as vitamin E-coated polysulfone membranes. The marked increase of PC groups observed in young AMI patients is consistent with our previous findings about lipid peroxidation, total antioxidant status and nitric oxide metabolites in the same clinical condition [96].

Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this paper.

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