REVIEW ARTICLE

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Protein Oxidation in Metabolic Syndrome

Abstract

Purpose: Oxidative stress plays a pivotal role in the pathogenesis of the metabolic syndrome and in the progression of its complications. Carbonylated proteins are a stable marker of severe oxidative stress because damage to the protein structure is irreversible and may cause an inhibition of their enzymatic activity or an increased susceptibility to proteolysis. There are few data regarding protein oxidation in metabolic syndrome, although elevated levels of carbonyl groups are often detected in subjects with obesity, diabetes mellitus, hypertension or dyslipidemia, well-known components of the metaboic syndrome. In particular, obesity, insulin resistance and diabetes mellitus are frequently associated with increased protein carbonylation. A relationship between insulin resistance, protein oxidative stress and inflammation has also been suggested as well as protein oxidation products are correlated with overexpression of resistin, TNF-α and IL-6.

Conclusion: Therapeutic interventions based on lifestyle modifications and pharmacological agents in order to correct all the main risk factors influence oxidative stress and protein carbonylation.

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Metabolic syndrome (MS) is characterized by a high risk of cardiovascular morbidity and mortality [1,2]. The components that define MS, such as visceral obesity, hypertension, diabetes mellitus and dyslipidemia, all show both the altered oxidant/antioxidant status and chronic subclinical inflammation that are responsible for accelerated atherosclerosis.

Previously, oxidative stress has been shown to play a role in MS development [3]. Several components of this syndrome, including hyperglycemia and inflammation, induce an increased production of reactive oxygen species (ROS) [4]. ROS and reactive nitrogen species (RNS) are also involved in aging, neurodegenerative diseases and other clinical conditions. The main ROS is the superoxide anion (O2-), which is rapidly neutralized by anti-oxidant enzymes in vivo, and, in the case of endothelial dysfunction, oxidizes nitric oxide (NO), producing peroxinitrite and starting a cascade of ROS generation that leads to the oxidation of carbohydrates, lipids and proteins [3].

Oxidative stress is often caused by hyperactivity of NADPH oxidase with a consequent synthesis of ROS and a reduced bioavailability of NO [5]. NO levels are decreased in subjects with metabolic diseases and are negatively correlated with body mass index (BMI), blood pressure and triglyceridemia [6]. Activated phagocytes are another source of ROS: neutrophils contain myeloperoxidase (MPO), which catalyzes the reaction between chloride ion and hydrogen peroxide to generate hypochlorous acid. Hypochlorous acid oxidizes proteins, especially albumin, producing advanced oxidation protein products (AOPPs) [7].

Proteins are the principal target of ROS and RNS because they are present in high concentrations in biological systems and remove 50-75% of the generated ROS [7]. AOPPs are formed mainly as a consequence of the action of chlorinated compounds, leading to the production of dityrosine residues and to protein crosslinking [7]. AOPP may play a part in the expansion and preservation of both oxidative stress and inflammation by the activation of neutrophils, monocytes and T lymphocytes [8]. *In vitro,* AOPPs are able to inhibit inducible NO production by macrophages [9] and to induce ROS production in endothelial cells via NADPH oxidase activation [10].

ROS and RNS may modify protein structure directly or indirectly [11]. ROS- and RNS-induced lipid peroxidation generates some relatively stable end products, such as malondialdehyde (MDA), hydroxynonenal (HNE), oxononenal (ONE) and isoprostanes [11]. Aminoacid modification by α/β-unsaturated aldehydes often occurs at the nucleophilic residues of cysteine, histidine and lysine. The damage of the protein may cause the cleavage of the primary structure, cross-linking or modifications of a single amino acid chain [11].

Oxidation of the thiol groups of cysteine residues by ROS and RNS can significantly modify the structure and function of proteins. By reacting with ROS and RNS, thiol groups function as antioxidants. Some of the ROS- and RNS-induced modifications of cysteine residues are reversible, including glutathionylation, sulfenic acid formation, S-nitrosylation, S-acylation, sulfanilamide production and disulfide formation, while some reactions with ROS and RNS produce irreversible products such as sulfinic or sulfonic acids and thioethers [12,13]. These protein changes may either be biomarkers of oxidative damage or may defend against oxidative stress.

Carbonylation is an irreversible ROS-induced protein modification. There are many ways to introduce carbonyl groups into a protein structure: ROS may directly oxidize lysine, arginine, proline or threonine residues, or may react with carbohydrates and lipids generating reactive carbonyl species (RCS), such as ketoamine, ketoaldehydes, MDA, HNE and ONE, which subsequently interact with proteins [14]. HNE and ONE may react with nucleophilic amino acids producing Michael and Schiff adducts that are then modified by tautomerization, oxidation, dehydration and sometimes condensation with another aldehyde to generate stable advanced lipoxidation end products (ALEs) [15]. The damage to the protein structure is generally irreversible and may cause inhibition of enzymatic activity, increased susceptibility to aggregation and proteolysis and altered cellular uptake [11].

Oxidized proteins ares usually catabolized by proteasomes and lysosomes, but some inactive proteins may be poorly degraded, generating protein aggregates that accumulate inside the cells or in the extracellular matrix [11].

In the literature, there are two papers regarding protein oxidation or carbonylation in MS subjects. In the first, [16] the behavior of protein oxidation, expressed as AOPPs/albumin ratio, has been examined in relation to a number of risk factors in MS subjects subdivided in omnivores and vegetarians. This AOPPs/albumin ratio, which is higher in the vegetarian group, was correlated with the following risk factors: BMI, blood pressure, glucose metabolism and lipid pattern. In the second paper, [17] protein oxidation, expressed as AOPPs and carbonyl groups, was investigated in 60 subjects with MS subdivided according to the presence or absence of metformin treatment; this study showed a significant decrease in AOPPs (49%) and in carbonyl groups (51%) only in the group treated with metformin (850 mg once daily for 1 year).

Even if there are only limited data on protein oxidation in subjects with MS, there is more evidence of these protein

changes in subjects with visceral obesity, diabetes mellitus, arterial hypertension and dyslipidemia; principal components of MS.

Obesity

In obese subjects, elevated AOPP levels were correlated with central obesity, triglyceridemia, lipid peroxidation and insulinemia, and are reduced by weight loss [18]. In a small group of obese subjects, Frohnert and coworkers [19] demonstrated higher concentrations of carbonylated proteins in subcutaneous adipose tissue, which positively correlated with adiposity and plasma levels of free fatty acids. In another study, [20] obese women showed increased plasma AOPP in comparison with lean controls. These AOPP levels were more elevated in those with diabetes mellitus and were related to higher concentrations of resistin, TNF-α and IL-6. Zhou *et al*. [21] demonstrated that the exposure of the adipocytes to levels of AOPPs, comparable to serum concentrations found in subjects with obesity and diabetes mellitus, induced a cellular alteration and an overexpression of TNF-α and IL-6 in a dose- and time-dependent manner, probably via NF-kB activation. This study underlines a correlation between insulin resistance, oxidative stress and inflammation. Atabek and coworkers [22] observed higher values of AOPP in obese adolescents with insulin resistance relative to lean controls, although the difference was not statistically significant.

Other authors have demonstrated a positive correlation between carbonylated proteins, HOMA index and BMI in obese subjects and also a decrease of these proteins after gastric banding [23]. Obese subjects who underwent bariatric surgery show a weight loss accompanied by the improvement of metabolic parameters and a decrease of carbonyl groups [24]. Even weight loss obtained with caloric restriction and physical activity was associated with a decrease in some parameters of oxidative stress, such as thiobarbituric acid-reactive substances (TBARS), nitrotyrosines and carbonylated proteins [25]. In obese children, following low-calorie diet, the consumption of mandarin juice, rich in antioxidants, contributed to a decrease in carbonyl groups and MDA [26]. In obese subjects with MS, metformin administration (for one year) reduced body weight, abdominal circumference and oxidative/nitrosative stress parameters and improved NO concentration and endothelial function [16].

Elevated levels of protein oxidation have been demonstrated in response to obesity, and are correlated with the presence of adipose tissue, insulin resistance and inflammation. Furthermore, protein oxidation may be reduced by lifestyle modifications and pharmacological therapy directed towards weight loss.

Diabetes mellitus

Hyperglycemia may contribute to the plasma and cellular protein damage mediated by oxidation and glycation. Protein glycation produces advanced glycation end products (AGEs) and is often mediated by alpha-dicarbonyls (glyoxal and methylglyoxal), produced by protein glycation (non-oxidative reaction) or by lipid peroxidation (markers of oxidative stress).

In diabetes, an increment in the polyol or sorbital pathways may cause an altered NADH/NAD+ ratio and NADPH/NADP+ ratio, respectively, affecting the detoxification process [27,28]. These alterations, together with an elevated concentration of carbohydrate and lipid compounds, may produce higher levels of RCS [27,28]. This condition can be considered to be determined by carbonyl stress and not by oxidative processes, so some authors retain that it is possible to distinguish oxidative stress from carbonyl stress, especially in diabetes mellitus.

In diabetes, elevated concentrations of asymmetric dimethyl arginine (ADMA) and low levels of tetrahydrobiopterin (BH4) may lead to an impaired NOS regulation [29,30]. BH4 binds the oxidase domain of NO synthase (NOS) and it is an essential co-factor for the synthesis of NO while as it is known ADMA is an inhibitor of NOS [31].

Methionine sulfoxide and N'-formylkynurenine, which are derived from oxidation of methionine and tryptophan, are markers of protein oxidative damage [32]. Some authors [32] found a decrease in plasma levels of glycation products and of carboxyl-metil-lysine derived from glyoxal in patients treated with insulin to decrease post-prandial hyperglycemia.

Telci and coworkers reported an increase in plasma carbonyl levels in type 2 diabetic subjects without chronic complications [33], while others found an increase in protein carbonyl concentration in diabetic subjects with and without microangiopathy as compared with normal subjects [34]. In contrast, Odetti *et al.* demonstrated that the plasma protein carbonyl content in type 2 diabetic subjects was slightly but not significantly higher in comparison with normal subjects [35]. In the same group of type 2 diabetic subjects was also observed a correlation between HbA1c and protein carbonyl groups [35]; insulin resistance, expressed as HOMA index, was also correlated with higher carbonyl group levels [36].

In first-degree relatives of type 2 diabetic subjects, increased plasma protein carbonyl content has been described [37]. AOPP, carbonylated proteins and lipoperoxides levels were increased in subjects with poorly controlled diabe-

tes mellitus [38]. Their levels were elevated in most type 2 diabetic subjects, positively correlated with triglyceridemia [39] and higher in those with cardiovascular complications [40,41]. AOPP, carbonyl groups and thiol-proteins levels were increased in plasma and urine of type 2 diabetic subjects with nephropathy and the AOPP values were associated with albuminuria and kidney disease progression [42]. In women with gestational diabetes, an increased concentration of protein oxidation markers, such as AOPP, protein carbonyls, protein hydroperoxides and nitrotyrosine, was found [43]. This finding was associated with a reduced activity of paraoxonase-1, a peroxidase-like enzyme that defends against lipoprotein oxidation [43]. AOPPs are also involved in diabetic retinopathy development [44].

Increased protein carbonylation has been demonstrated in erythrocyte membrane of diabetic subjects, especially in subjects with proliferative retinopathy [45,47]. The presence of diabetic foot ulcers is also associated with higher plasma MDA and carbonyl levels, and these parameters were correlated with worsened outcomes [48].

A three-fold higher concentration of residues modified by glycation, oxidation and nitrosylation in plasma proteins and a two-fold higher concentration in hemoglobin was noted in type 1 diabetic subjects in comparison with normal subjects [49].

These observation are comparable with those of Dominguez and coworkers [50]who found that the plasma carbonyl groups were significantly higher in diabetic children and adolescents in comparison with normal subjects.

In streptozotocin-induced diabetic rats, the administration of antioxidants, such as stobadine, reduced plasma levels of sulfhydrylic groups and thiol-proteins and liver concentrations of carbonylated proteins and AOPP, but not of N-tyrosine (51); as expected, stobadine did not improve glycometabolic control. In animal models, glomerular stores of AOPP cause podocyte apoptosis via activation of NADPH oxidase and caspases, thus promoting glomerulosclerosis (52).

In rats, after 8 weeks of the induction of diabetes, carbonylation of ventricular myosin heavy chains was observed, demonstrating that heart modifications induced by RCS may lead to diabetic cardiomyopathy (53).

Protein alterations in diabetes mellitus can be observed early in the course of disease and are correlated with glycemic control and vascular complications; administration of insulin reduces intermediates of oxidative and carbonyl stress involved in the generation of RCS.

Hypertension

NADPH oxidase activation, due to stimulation of AT1 receptors, may be responsible for an increase in the production of ROS and a consequent decrease in NO availability in response to arterial hypertension [3]. In addition, ADMA is increased in hypertension, causing a decrease in vascular compliance and elevated sodium retention [31].

A prospective study [54] showed that elevated RCS levels are associated with the development of pre-hypertension and hypertension, and that RCS and blood pressure values are significantly correlated. This study seems to suggest how protein oxidation is involved in hypertension progression. Some authors have proposed a possible role of RCS and oxidized thiol-proteins in the pathogenesis and in the maintenance of renovascular hypertension [55-57]. In contrast, others consider oxidative stress as a consequence of hypertension, due to the altered activity of enzymatic antioxidants [58]. These same authors found greater protein oxidative damage at all stages of hypertension and a positive correlation between blood pressure values and oxidative stress parameters. In subjects in the initial stage of essential hypertension, an increase in plasma carbonylated protein, associated with a decrease of antioxidant defences, in particular the reduced glutathione, was noted[59]. Even in subjects with sustained hypertension and white coat hypertension, increased levels of carbonylated proteins were observed, and no significant differences were observed between the two groups of hypertensives [60]. Caimi and coworkers did not find any difference in carbonyl groups between untreated subjects with mild essential hypertension and normal subjects [61]. Anti-hypertensive treatment with perindopril (an ACE inhibitor) and hydroclorothiazide for 45 days reduced carbonylated proteins and increased the activity of superoxide dismutase and catalase and the NO concentrations in elderly hypertensive subjects [62].

In spontaneously hypertensive rats, an increase in carbonylated proteins has been described in myocardial tissue [63], where it is associated with higher caspase activity, as well as in the kidney and aorta [64].

Pharmacological control of arterial hypertension may, therefore, attenuate the oxidative damage and delay the progression of cardiovascular complications.

Dyslipidemia

Hyperlipidemia is associated with oxidative stress and inflammation. In hypercholesterolemic subjects, a correlation between total cholesterol and protein carbonyl levels was found, as well as a difference in carbonyl groups between subjects with

total cholesterol varyng from 200 to 240 mg/dl and those with total cholesterol above 240 mg/dl [65]. Hypercholesterolemic subjects showed an increase in TBARS and carbonylated proteins, especially if subjects had a superoxide dismutase genetic polymorphism (Ala16Val) [66]. The subjects with homozygous familial hypercholesterolemia have higher levels of MDA and carbonylated proteins than subjects with heterozygous familial hypercholesterolemia, which correlated with LDL-C and Lpa levels, intima-media thickness and with increased cardiovascular risk [67]. AOPP concentration was positively correlated with triglyceridemia in a heterogeneous group of hospitalised patients [68]. In diabetic dyslipidemic subjects, the treatment with statins decreased carbonyl groups and MDA levels [69] and thiol groups were correlated inversely with apolipoprotein B and positively with apolipoprotein A-I.

In animal models of hyperlipidemia, increased ROS generation and over-expression of the NADPH oxidase gp91phox subunit in granulocytes have been observed [70]. Hypercholesterolemic rabbits had higher NADPH oxidase activity in their aorta, with stores of nitrotyrosine in the endothelium and intima and reduced eNOS and SOD activity [71]. In dyslipidemic rats following a hypercaloric diet, an increase in MDA, nitrotyrosine and carbonylated proteins was noted and these parameters decreased with the administration of eicosapentaenoate-lipoate (EPA-LA) derivative [72]. In dyslipidemic rabbits, administration of atorvastatin reduced protein oxidation [73].

An increase in protein oxidation is associated with dyslipidemia; thus, the use of drugs that decrease lipid levels may reduce oxidative stress and attenuate the development of vascular lesions.

Conclusions

Protein carbonylation is an indicator of oxidative protein damage. Its use as a biomarker of oxidative stress has some advantages because of the early synthesis and the stability of carbonylated proteins in comparison with other oxidation products. Protein structure is irreparably compromised by carbonylation, which may occur via different ways: direct oxidation of lysine, arginine, proline and threonine residues, or interaction with RCS produced from carbohydrate and lipid oxidation and by non-oxidative reactions with dicarbonyl compounds. Increased levels of protein oxidation products may be observed in obesity, diabetes, hypertension and dyslipidemia, principal components of MS, suggesting that the presence of carbonyl groups in proteins may be considered as important markers of oxidative stress in this syndrome (Table 1). Diet and physical activity, besides inducing weight loss and exerting the well-known effects on metabolic control, blood pressure and lipid profile, reduce the parameters of lipid and protein oxidation, so lifestyle modifications are useful to normalize oxidative status too. Interestingly, some drugs commonly employed in the treatment of MS, such as metformin and insulin for the treatment of diabetes, perindopril and hydrochlorothiazide for hypertension and statins for dyslipidemia, have antioxidant effects. Regarding the effects of antioxidant supplementation on cardiovascular outcomes, data in the literature are conflicting:, while *in vitro* and *in vivo* studies have demonstrated promising antiatherogenic and cardioprotective effects of vitamin C and E, metallothionein, resveratrol and glutathione peroxidase analogues [74,75], several clinical trials, in which patients were given antioxidant vitamins, showed no benefit [76-80]. One explanation for those negative findings may be the concomitant use of other drugs, such as statins or antiplatelets, commonly prescribed to patients with high cardiovascular risk. Statins could influence oxidative status and then abolish the potential beneficial effect of vitamins, while antiplatelet drugs, such as aspirin and clopidogrel, may interfere with the activity of antioxidants [81]. Some authors instead suggest that a complex supplementation with vitamins B, C, D, E, K, N-acetylcysteine, allopurinol, co-enzyme Q and alpha-lipoic acid associated with a diet rich in flavonoids and carotenoids may be necessary in order to obtain effective antioxidant prevention and protection against cardiovascular disease [82].

TABLE 1. Trend of plasma levels of advanced oxidation protein products (AOPP) and carbonylated proteins in metabolic syndrome and its components.

	AOPP	Carbonylated Proteins
Metabolic Syndrome	↑[16,17]	↑[17]
Obesity	↑[18,20,22]	↑[19,23-25]
Diabetes Mellitus	↑[37-41,43]	↑[33-41,43,48,50]
Hypertension	Unavailable Data	↑[54-60] =[61]
Dyslipidemia	↑[70]	↑[67-68]

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