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Impact of cardiovascular risk factors on oxidative stress and DNA damage in a high risk Mediterranean population

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Abstract
The impact of classic cardiovascular risk factors on oxidative stress status in a high-risk cardiovascular Mediterranean population of 527 subjects was estimated. Oxidative stress markers (malondialdehyde, 8-oxo-7′8′-dihydro-2′-deoxyguanosine, oxidized/reduced glutathione ratio) together with the activity of antioxidant enzyme triad (superoxide dismutase, catalase, glutathione peroxidase) were analysed in circulating mononuclear blood cells. Malondialdehyde, oxidized glutathione and the ratio of oxidized to reduced glutathione were significantly higher while catalase and glutathione peroxidase activities were significantly lower in high cardiovascular risk participants than in controls. Statistically significant differences were obtained after additional multivariate control for sex, age, obesity, diabetes, lipids and medications. Among the main cardiovascular risk factors, hypertension was the strongest determinant of oxidative stress in high risk subjects studied at a primary prevention stage.

Keywords: *Oxidative stress, 8-oxo-dG, cardiovascular risk factors, hypertension, primary prevention*

Introduction

The potential use of oxidative stress products as disease progression markers is the focus of much biomedical research. Among the well-known factors in the development of cardiovascular pathology, oxidative stress seems to play a particularly important role [1–3]. Increased oxidative stress by-products and/or a reduced antioxidant activity have been demonstrated in different animal and human models of atherosclerosis and endothelial dysfunctions [4–6]. Knowledge regarding the mechanisms

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underlying the pathogenesis of cardiovascular diseases (CVD) is extremely important, since these diseases are the leading cause of mortality and morbidity in the world [7].

Classic risk factors of CVD include age, a smoking habit and high levels of cholesterol (in the form of low density lipoproteins), hypertension, diabetes and obesity [8]. All these metabolic and haemodynamic alterations have in common an increased oxidative stress status, which is observed in both circulating and endothelial cells of affected patients [9–12]. In addition, different antioxidants such as glutathione peroxidase (GPx) activity and lipid peroxidation products have been proposed as independent cardiovascular risk factors [13,14]. Even though the pathophysiological implications of oxidative stress are well established, little research has been carried out concerning the contribution of individual cardiovascular risk factors. Recently, we have reported that the impact of the metabolic syndrome components on the oxidative stress burden generated by high blood pressure is minimal [15]. This is of great relevance as several epidemiological studies have attributed preventive cardiovascular effects to antioxidant-rich diets [16,17].

Growing evidence of the importance of oxidative stress in cardiovascular and other degenerative diseases [1] has prompted a need for reliable and reproducible markers, which can be used to monitor treatment-induced changes.

Increased oxidative stress of hypertensive subjects improves with anti-hypertensive treatment [18]. Methods for the quantification of different types of oxidative stress indicators have been developed to detect the oxidative modification of the most important cell molecules [1,18,19]. Malondialdehyde (MDA), protein carbonyl groups and DNA base oxidation products are examples of oxidative stress by-products with potentially promising clinical applications. In addition, antioxidants are employed as biomarkers of oxidative stress and tissue damage [20].

The DNA- damaged product 8-oxo-7'8'-dihydro-2'-deoxyguanosine (8-oxo-dG) is a reliable marker of oxidative stress changes in hypertension [16,17]. Upon oxidation, a hydroxyl group is added to position C8 of the guanine molecule, resulting in the oxidative and mutagenic by-product 8-oxo-dG [21,22], which is considered to be both an important biomarker of generalized cellular oxidative stress and a repair product [21,22].

The objectives of this study were: (1) To estimate oxidative stress by means of reduced glutathione (GSH) and oxidized glutathione (GSSG) levels and the GSSG/GSH ratio, 8-oxo-dG, MDA concentration and antioxidant enzyme activities in the nucleated blood cells of a large sample of high cardiovascular risk (HCR) subjects; and (2) To investigate the influence of each cardiovascular risk factor on the oxidative stress profile of the HCR subjects.

Material and methods

We studied 527 subjects (346 women and 181 men) who participated in the multi-centre PREDIMED (Prevención con Dieta Mediterránea) study [16] and 25 healthy subjects (10 men and 15 women) without cardiovascular risk factors. The aim of the trial, which is currently under way, is to assess the effect of the Mediterranean diet on the primary prevention of cardiovascular diseases. Participants were consecutively recruited in primary care centres in the Valencia Region, Spain. The criterion for inclusion in the study was 50–80 years for men and 60–80 years for women. In addition, subjects needed fulfil at least one or two criteria: type 2 diabetes or three or more cardiac heart disease (CHD) risk factors, including current smoking habit, hypertension, (BP $>$ 140/90 mmHg) or treatment with anti-hypertensive drugs, elevated low density cholesterol (LDL) level ($>$4.13 nmol/L; $>$160mg/dl) or treatment with hypolipemic drugs, decreased high density (HDL) cholesterol level ($<$1.04 nmol/L; $<$40 mg/dl), body mass index (BMI) $>$25 kg/m^2 or a family history of premature CHD [16].

Exclusion criteria were a history of cardiovascular disease, severe chronic illness, addiction to drugs or alcohol, a history of allergy or intolerance to olive oil or nuts and low predictive likelihood of a change in dietary habits according to the stage-of-change [23]. Primary-care physicians based participant selection on a review of patient's clinical records and a screening visit. A list of candidates was obtained from computer-based records of patients regularly attending the participating centres. Potentially-eligible candidates were contacted by telephone and invited to attend a screening visit. The visit included an interview consisting of a 26-item questionnaire about the medical condition and risk-factor status of the subject. Of the candidates who fulfilled the entry requirements, 95% agreed to participate.

Data were recorded at baseline. The baseline examination included assessment of standard cardiovascular risk factors, use of medication, socio-demographic factors and lifestyle variables, as previously detailed [13]. BMI was calculated as weight (in kg) divided by height (in m^2). BP was measured by trained personnel using a validated semi-automatic oscillometre (Omron HEM-70CP; Hoofddrop, the Netherlands). Blood samples were obtained after overnight fast. Serum, EDTA plasma and buffy-coat were collected, coded, shipped to central laboratories and frozen at $-80°$ C until assay. All laboratory parameters were analysed in duplicate for each participant. Serum glucose, cholesterol and triglyceride levels were measured using a standard automated enzymatic method (Trinder, Bayer Diagnostics, Tarrytown, New York). High-density

lipo-protein (HDL) cholesterol was quantified after precipitation with phosphotungstic acid and magnesium chloride. LDL-C was calculated with the Friedewald formula. The approval of the Institutional Ethics Committee was obtained and all participants gave written informed consent.

Analysis of oxidative stress markers

Whole blood (14 ml) was diluted with saline medium and mononuclear cells were isolated by Ficoll-Hypaque centrifugation [24]. The antioxidant enzymes were analysed following the methodology described in Greenwald [25] with the modifications specified in Redón et al. [6], and included GPx, CAT, superoxide dismutase (SOD) and GSH. For the analysis of GSSG, samples were treated with N-ethylmaleimide and bathophenanthroline disul-phonic acid and were derivatized and analysed by high performance liquid columns (HPLC) [26,27]. MDA was analysed by the method of Wong et al. [28]. Protein content was measured using the Bradford method.

DNA isolation and assay of 8-oxo-7,8-dihydro-2′-deoxyguanosine

Nuclear DNA (nDNA) was isolated following the Gupta method with the modification described by Muñiz et al [26]. Isolated DNA was washed twice with 70% ethanol, dried and then dissolved in 200 µl of 10 mM Tris/HCl, 0.1 mM EDTA, 100 mM NaCl, (pH 7.0) for its enzymatic digestion, as previously described [29].

DNA hydrolysates were dissolved in HPLC grade water and filtered through a 0.2 µm syringe filter before applying the samples to a Waters ODS HPLC column (2.5 × 0.46 i.d; 5 µm particle size). The amount of 8-oxo-dG and dG in the DNA digest was measured by electrochemical and UV absorbance detection, respectively [30]. To assess the optimization and accuracy of the HPLC-EC assay for the isolation and detection of 8-oxo-dG, appropriate chromatograms of both samples and standards were recorded at the beginning of each working day, as previously reported [19].

Statistical analysis

All continuous variables were examined for normality of distribution. Since triglyceride was shown to have a skewed distribution, the values were log-transformed prior to statistical analyses. Pearson χ^2 and Fisher tests were used to analyse differences in percentages. *T* and ANOVA tests were applied to compare crude multivariate adjustments of the association between cardiovascular risk factors and oxidative stress parameters, for which analysis of covariance was performed and adjusted means were estimated for age, sex, medication, smoking and other cardiovascular risk factors (hypertension, diabetes, obesity or dyslipidemia). Statistical analyses were performed with the SPSS package, version 14.0 (SPSS, Chicago, IL) for the whole sample and for men and women separately when indicated. All tests were two-tailed and *p*-values <0.05 were considered statistically significant.

Results

Table I shows demographic, anthropometric and biochemical characteristics of the 527 PREDIMED study participants (181 men and 346 women) at

Table I. Cardiovascular risk factors and oxidative stress parameters in patients and controls (means and standard deviations).

	High cardiovascular risk patients	Controls	p^*
Male/female	181/346	10/15	0.561
Age (y)	66.8 ± 6.2	66.1 ± 11.1	0.589
Weight (Kg)	78.3 ± 13.4	73.2 ± 11.6	0.065
BMI (Kg/m^2)	31.3 ± 5.30	26.70 ± 2.93	<0.001
SBP (mmHg)	146.8 ± 22.1	133.4 ± 9.2	0.003
DBP (mmHg)	81.21 ± 10.37	82.21 ± 11.27	0.555
Baseline glucose (mg/dL)	129.3 ± 44.4	100.2 ± 11.4	0.001
Total cholesterol (mg/dL)	212.4 ± 36.6	214.1 ± 29.6	0.824
HDL cholesterol (mg/dL)	52.0 ± 10.96	61.5 ± 13.2	<0.001
LDL cholesterol (mg/dL)	130.4 ± 32.4	136.2 ± 33.3	0.381
Triglycerides (mg/dL)	137.1 ± 86.6	106.4 ± 39.8	0.078
8-oxo-dG (8-oxo-dG/10^{6}dG)	5.61 ± 1.17	3.71 ± 0.65	<0.001
MDA (nmol/mg Prot)	0.52 ± 0.22	0.17 ± 0.06	<0.001
GSSG (nmol/mg Prot)	0.75 ± 0.40	0.20 ± 0.06	<0.001
GSH (nmol/mg Prot)	20.2 ± 4.9	20.3 ± 3.1	0.927
GSSG/GSH%	4.12 ± 2.88	0.99 ± 0.29	<0.001
CAT (U/g Prot)	107.8 ± 31.1	205.7 ± 28.6	<0.001
SOD (U/mg Prot)	4.54 ± 1.77	4.90 ± 0.57	0.321
GPx (U/g Prot)	35.2 ± 9.4	51.6 ± 6.9	<0.001

*p-value obtained in the test of comparison of means between high cardiovascular risk patients and healthy controls.

Table II. Prevalence of cardiovascular risk factors and medications by gender.

	Male	Female	*p*
Diabetes %	57.4	51	0.17
Hypertension %	65.7	83.1	<0.001
Hypercholesterolemia %	64.7	68	0.465
Obesity %	45.1	64.8	<0.001
Smoking status %			
current smokers	26.7	3.0	<0.001
ex-smokers	46.1	5.6	<0.001
never smoker	27.2	91.4	<0.001
Medications %			
anti-hypertensive drugs	57.7	75.4	<0.001
hypolipidemic drugs	56.6	51.2	0.297
Anti-diabetic agents %			
insulin	9.4	10.7	0.737
oral anti-diabetics	32.6	26.9	0.201

p-value obtained in the comparison of prevalences between men and women.

baseline and the general characteristics of the 25 healthy subjects. The mean age of the PREDIMED study participants was 66.8 ± 6.2 years and no statistically significant differences were detected with respect to gender ($p = 0.277$). The mean age of healthy controls was 66.1 ± 11.1 years and, similarly, no significant differences were found in relation to sex ($p = 0.342$). The mean age of the control group did not differ from that of the PREDIMED participants (Table I). However, as expected, participants in the PREDIMED trial had significantly higher BMI, fasting glucose and systolic blood pressure and lower HDL-C concentrations than the healthy control group. Moreover, among PREDIMED participants, 69.1% were taking anti-hypertensive drugs and 53.1% were taking lipid lowering drugs, while none of the healthy controls were on medication (Table II). This explains the observation of no statistically significant differences between PREDIMED participants and healthy controls regarding plasma total cholesterol, LDL-C or diastolic blood pressure (Table I).

The prevalence of current smokers in the PREDIMED participants was low (11.1%) and no significant difference was detected in the healthy control group.

When oxidative stress parameters for PREDIMED cases and controls were compared (Table I), we found that the damaged DNA base 8-oxo-dG was statistically increased in the former group ($p < 0.001$). MDA, GSSG and the GSSG/GSH ratio were also statistically higher among PREDIMED participants than in the control population. No statistically significant differences were observed for GSH or SOD activity. On the other hand, CAT and GPx activity were statistically higher in controls than in the PREDIMED participants ($p < 0.001$).

When these analyses of oxidative stress parameters were stratified by gender (Table III), we observed homogeneity of damaged DNA base 8-oxo-dG, MDA and CAT in both cases and controls. However, in the PREDIMED participants, men exhibited higher GSH concentrations (20.77 ± 4.79 vs 19.82 ± 4.81; $p = 0.032$) and higher SOD ($p = 0.048$) and GPx ($p = 0.005$) activity than women. Conversely, in controls, SOD and GPx activity did not differ according to gender.

We investigated the contribution of different cardiovascular risk factors to oxidative stress in the PREDIMED participants. Table II shows the prevalence of diabetes, hypertension, hypercholesterolemia, obesity and smoking status with respect to gender. In addition, Table II shows the percentage of the population undergoing drug therapy for specific conditions. Considering diabetes, hypertension, hypercholesterolemia and obesity as the most important cardiovascular risk factors, we studied differences in oxidative stress parameters and anti-oxidant enzyme activity in relation to the presence or absence of these oxidative stress parameters (Table IV). Taking into account the statistically significant differences in gender and to control for other potential confounders, analyses were adjusted for age, gender, smoking, medication (lipid-lowering drugs, anti-hypertensive treatment, diabetic drugs) and the other conditions. We found that hypertension was the most important cardiovascular risk factor in determining significant differences in oxidative stress parameters and anti-oxidant enzyme activity.

Table III. Oxidative stress parameters in high cardiovascular risk patients and controls by gender (means and standard deviations).

	High cardiovascular risk patients			Controls		
	Male	Female	*p**	Male	Female	*p**
Age (y)	66.4 ± 6.6	67.0 ± 6.2	0.277	64.0 ± 8.5	68.3 ± 13.4	0.342
8-oxo-dG (8-oxo-dG/10^6dG)	5.5 ± 1.0	5.6 ± 1.2	0.171	3.6 ± 0.7	3.7 ± 0.5	0.693
MDA (nmol/mg Prot)	0.5 ± 0.2	0.5 ± 0.2	0.191	0.1 ± 0.0	0.2 ± 0.0	0.541
GSSG (nmol/mg Prot)	0.7 ± 0.4	0.7 ± 0.3	0.207	0.2 ± 0.0	0.2 ± 0.0	0.002
GSH (nmol/mg Prot)	20.7 ± 4.8	19.8 ± 4.8	0.032	20.9 ± 2.7	19.6 ± 3.5	0.302
GSSG/GSH %	3.8 ± 2.9	4.2 ± 2.8	0.115	1.2 ± 0.3	0.8 ± 0.2	0.012
CAT (U/g Prot)	109.8 ± 31.8	104.9 ± 31.5	0.092	199.7 ± 28.3	212.3 ± 28.5	0.281
SOD (U/mg Prot)	4.7 ± 1.7	4.4 ± 1.7	0.048	4.9 ± 0.6	4.8 ± 0.4	0.453
GPx (U/g Prot)	36.8 ± 10.2	34.3 ± 9.0	0.005	51.9 ± 3.9	51.2 ± 9.2	0.791

**p*-value obtained in the comparison of means for each parameter between men and women.

We further investigated the specific influence of anti-hypertensive treatment on oxidative stress parameters (Table V). Hypertensive patients undergoing pharmacological treatment presented lower 8-oxo-dG and GSSG/GSH ratio and higher SOD activity than participants that were not taking anti-hypertensive drugs. No statistically significant differences in oxidative stress parameters were observed among dyslipidemic subjects when those receiving lipid-lowering drugs were compared with those who were not (results not shown).

Discussion

In this study we have demonstrated that high cardiovascular risk patients from a Mediterranean population have a more negative oxidative stress profile than healthy subjects. This profile was characterized by more damaged DNA base 8-oxo-dG, higher MDA, GSSG and the GSSG/GSH ratio and lower CATand GPx activity. No statistically significant differences were observed for GSH or SOD activity. Moreover, hypertension was found to be the most important cardiovascular risk factor related to the abnormal oxidative stress profile. In recent years, a great deal of attention has been paid to the role of oxidative stress in the pathogenesis of atherosclerosis-derived processes, since it is well known that enhanced oxidation status can influence the inflammation and atherosclerosis of intima-media cells [31,32]. A special effort has also been made to identify reliable markers in different oxidative stress-associated diseases.

Although the beneficial effects of monounsaturated fatty acids and other Mediterranean-style nutrients on cardiovascular risk factors, obesity and diabetes [33,34] are well documented, there is very limited information about the role of oxidative stress byproducts in a primary prevention strategy phase. In fact, an integral oxidative stress study in such conditions has yet to be performed. In this sense the present work was designed in order to establish whether or not oxidative stress-induced by-products are correlated with and/or influenced by classic cardiovascular risk factors in a cohort of subjects without clinical events and prior to dietary interventions.

The most representative oxidative stress markers were simultaneously analysed in mononuclear cells of healthy volunteers in order to compare them with those of high risk subjects. The results demonstrate that a pro-oxidant state exists in the circulating blood cells of the high risk population. In these subjects, MDA and 8-oxo-dG levels were significantly higher than those of healthy volunteers. Increased oxidative stress products were accompanied by a reduction of the most important antiox-idant enzyme activity, such as that related to catalase and GPx, and was also

Table IV. Means and standard deviation of oxidative stress parameters and antioxidant enzymes depending on the presence or absencek of cardiovascular risks factors.

	Hypertension			Obesity			Diabetes			Hypercholesterolemia		
	Yes	No	p^*	Yes	No	p^*	Yes	No	p^*	Yes	No	p^*
8-oxo-dG (8-oxo-dG/10^6dG)	5.6 ± 0.2	5.3 ± 1.1	0.33	5.7 ± 1.1	5.5 ± 1.2	0.119	5.6 ± 1.9	5.6 ± 1.2	0.876	5.6 ± 1.2	5.5 ± 1.2	0.388
MDA (nmol/mg Prot)	0.5 ± 0.2	0.4 ± 0.1	0.051	0.5 ± 0.23	0.5 ± 0.2	0.421	0.5 ± 0.2	0.5 ± 0.2	0.303	0.5 ± 0.2	0.5 ± 0.2	0.216
GSSG (nmol/mg Prot)	0.7 ± 0.4	0.6 ± 0.3	0.003	0.7 ± 0.4	0.7 ± 0.4	0.783	0.7 ± 0.4	0.7 ± 0.3	0.354	0.7 ± 0.4	0.7 ± 0.4	0.928
GSH (nmol/mg Prot)	19.8 ± 4.8	20.9 ± 4.5	0.038	20.1 ± 4.8	20.1 ± 4.8	0.971	20.1 ± 4.9	20.1 ± 4.6	0.911	20.3 ± 4.7	19.8 ± 4.9	0.295
GSSG/GSH (%)	4.3 ± 2.9	3.3 ± 2.4	0.002	4.2 ± 2.8	4.2 ± 3.1	0.988	4.2 ± 3.1	4.0 ± 2.6	0.25	4.0 ± 2.8	4.2 ± 2.9	0.622
CAT (U/g Prot)	107.3 ± 30.7	104.2 ± 36.1	0.339	108.5 ± 29.5	105.2 ± 34.4	0.246	108.7 ± 26.6	104.3 ± 37.2	0.121	107.4 ± 31.2	105.2 ± 33.8	0.457
SOD (U/mg Prot)	4.4 ± 1.7	4.9 ± 1.7	0.005	4.4 ± 0.7	4.5 ± 1.6	0.497	4.6 ± 1.8	4.4 ± 1.5	0.245	4.5 ± 1.7	4.4 ± 1.7	0.634
GPx (U/g Prot)	34.6 ± 9.2	36.9 ± 10.7	0.021	35.4 ± 9.5	35.1 ± 9.7	0.731	34.8 ± 9.1	35.6 ± 10.	0.338	34.8 ± 9.7	35.7 ± 9.2	0.362

*p-value were obtained in the corresponding multivariate model adjusted for gender, age, tobacco smoking, medications and the other cardiovascular risk factors.

Table V. Oxidative stress parameters and antioxidant enzyme activities depending on the medication in patients having hypertension (adjusted means and standard errors).

	No medication		Medication		
	Mean	SE	Mean	SE	p^*
8-oxo-dG (8-oxo-dG/10^6dG)	5.7	0.1	5.4	0.1	0.023
MDA (nmol/mg Prot)	0.5	0.0	0.5	0.0	0.203
GSSG (nmol/mg Prot)	0.7	0.0	0.6	0.0	0.011
GSH (nmol/mg Prot)	19.9	0.3	20.8	0.4	0.122
GSSG/GSH %	4.3	0.2	3.4	0.3	0.007
CAT (U/g Prot)	108.8	1.2	102.4	3.2	0.096
SOD (U/mg Prot)	4.3	0.1	4.8	0.2	0.011
GPx (U/g Prot)	35.1	0.5	37.1	0.9	0.058

SE: Standard error.

*p-value and means were adjusted for gender, age, smoking, obesity, diabetes and dislipemia.

manifested by the intracel-lular thiol oxidation status increasing the GSSG/ GSH ratio. It must be emphasized that, although the control group (subjects of the same age and without associated cardiovascular risk factor) was smaller than the PREDIMED group, differences between the groups in terms of oxidative stress parameters were statistically significant. Studies performed with peripheral blood of hypertensive subjects have shown increased rates of lipid peroxidation and DNA damage and an inadequate response of cytoplasmic antioxidant enzymes in these patients [35,36].

Reduced GSH and increased GSSG have also been related with early atherosclerosis in healthy adults, which supports a role for oxidative stress in the pathogenic process and, subsequently, as a useful parameter for identifying asymptomatic subjects at risk of cardiovascular disease at an early stage [37]. Levels of blood GSH and GSSG have been considered essential as indexes of whole-body GSH status and useful indicators of oxidative stress in humans.

An interesting observation derived from our study is the apparently greater DNA instability of high cardiovascular risk subjects, which implies an increase in the damaged base 8-oxo-dG detected in their mononuclear cells. We have previously reported higher values of 8-oxo-dG in both the nuclear and mitochondrial DNA of hypertensive subjects [6]. In recent years, a considerable amount of research has focused on the role of DNA damage in the pathology of atherosclerosis and related cardiovascular alterations [38,39]. Recently the yield of 8-oxo-dG in urine has been proposed as a reliable marker of oxidative stress in human hypertension [19]. Guanine oxidation by ROS can be the result of generation of hydroxyl radicals (·OH) through a Fenton-type reaction, which requires the interaction of hydrogen peroxide (H_2O_2) with transition metal ions. Both reaction precursors are known to occur in mono-nuclear cells and macrophages of hypertensive patients with atherosclerosis [40].

Circulating mononuclear cells with an elevated oxidative state may act as a mechanism by which cell damage is amplified, thereby affecting macro-phages and vascular smooth muscle cells (SMC). In the inflammatory response to injury hypothesis, SMC hyperplasia is considered to be merely a reactive process that occurs as a result of injury to the endothelium. In addition, ROS and 8-oxo-dG may trigger different signal transduction pathways implicated in DNA repair and apoptosis, including the expression of different transcription factors under the control of the p53 protein. The p53 gene plays a major role in genomic surveillance by stimulating base scission repair and coordinating the cell's response to damage [41]. Therefore, the study of DNA damage may provide new insights into the pathogenesis of cardiovascular alterations and alternative perspectives for therapeutic approaches.

We have not observed differences in terms of oxidative stress status between males and females. It must be emphasized that the age range as the inclusive criteria was 60–80 years. All women were postmenopausal and only a 5.4% were taking hormonal replacement therapy. Different studies have demonstrated an effect of oestrogen withdrawal and administration on oxidative stress and ageing. Some experimental evidence argues against a cardioprotective role of oestrogen [4]. Ginestein, an isoflavone with similar structure to estradiol, has been shown to improve endothelial function and up-regulate antioxidant genes through ERK1/2 and NFκB, which results in beneficial effects on the cardiovascular system [42]. Therefore the role of hormone therapy in cardiovascular disease is complex and further research is required to arrive at a more solid conclusion.

Regarding the influence of cardiovascular risk factors on the degree of oxidative stress in our study population, results show that hypertension was the most important. This finding is in accordance with previous observations concerning the impact of components of the metabolic syndrome (MS) on

oxidative stress and enzymatic antioxidant activity in essential hypertension [15]. High BP values, abdominal obesity, dyslipidemia and a subtle increase in baseline glucose are common diagnostic criteria of the MS. High BP values are one of the main MS components and MS has been found in ~ 30–40% of hypertensive patients. However, there are doubts concerning whether MS itself confers more risk than the sum of the effects of each of the components [43]. Determination of oxidative stress by-products in HCR patients may help to throw light on which of these components plays a pivotal role in the development of atherosclerosis and cardiovascular events. Hypertension is considered a state of oxidative stress that can contribute to different types of hypertension-induced organ damage [44]. In a previous study, the oxidative stress caused by an increase of GSSG/GSH and ROS-derived by-products in both blood and peripheral mononuclear cells observed in hypertensive subjects was not enhanced by additional components of the so-called MS. Likewise, the reduction in the activity of antioxidant enzymes was not affected. Neither the sum of the components nor each of them separately (low HDL, triglycerides, abdominal obesity or fasting glucose) produced further impact on the oxidative stress abnormalities observed in patients with only hypertension. In that study, diabetes patients were excluded. In the present study, diabetes itself did not significantly enhance the production of oxidative stress by-products or the impairment of antioxidant enzymes. Although oxidative stress may be associated with increased BP values, its potential impact on ROS production in the vascular wall and other mechanisms of lesion development can not be excluded.

The present study reveals that oxidative stress is elevated in a cohort of asymptomatic HCR subjects of a Mediterranean region, and that hypertension was the most important factor influencing oxidative stress in these subjects. Our findings may be of potential value in the prediction of cardiovascular events and as reference data for diet and/or drug intervention research.

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