Original Article

Relationship between Oxidative Stress and Essential Hypertension

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This study investigated the association of blood pressure with blood oxidative stress–related parameters in normotensive and hypertensive subjects. A cross-sectional design was applied to 31 hypertensive patients and 35 healthy normotensive subjects. All subjects were men between the ages of 35 and 60 years. Exclusion criteria were obesity, dyslipidemia, diabetes mellitus, smoking and current use of any medication. All patients underwent 24-h ambulatory blood pressure monitoring and sampling of blood and urine. Antioxidant enzymes activity, reduced/oxidized glutathione ratio (GSH/GSSG), and lipid peroxidation (malondialdehyde) were determined in erythrocytes. Parameters measured in the plasma of test subjects were plasma antioxidant status, lipid peroxidation (8-isoprostane), plasma vitamin C and E, and the blood pressure modulators renin, aldosterone, endothelin-1 and homocysteine. Daytime systolic and diastolic blood pressures of hypertensives were negatively correlated with plasma antioxidant capacity ($r=-0.46$, $p<0.009$ and $r=-0.48$, $p<0.007$), plasma vitamin C levels ($r=-0.53$, $p<0.003$ and $r=-0.44$, $p<0.02$), erythrocyte activity of antioxidant enzymes, and erythrocyte GSH/GSSG ratio, with hypertensives showing higher levels of oxidative stress. Blood pressures showed a positive correlation with both plasma and urine 8-isoprostane. Neither plasma vitamin E nor the assessed blood pressure modulator levels showed significant differences between the groups or correlation with blood pressures. These findings demonstrate a strong association between blood pressure and some oxidative stress–related parameters and suggest a possible role of oxidative stress in the pathophysiology of essential hypertension. (*Hypertens Res* 2007; 30: 1159–1167)

***Key Words*: antioxidants, essential hypertension, 8-isoprostane, oxidative stress, vitamin C**

Introduction

Increased vascular oxidative stress could be involved in the pathogenesis of hypertension (*1*, *2*), a major risk factor for cardiovascular disease mortality. Oxidative stress occurs when there is an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense systems so that the latter become overwhelmed (*3*, *4*). In human essential hypertension ROS may increase due to a diminution of the activity of antioxidant enzymes (*5*). The importance of ROS in vascular function and the development of hypertension has been recently reviewed (*6*, *7*). It is known that superoxide rapidly inactivates endothelium-derived nitric oxide (NO), the most important endogenous vasodilator, thereby promoting vasoconstriction (*8*, *9*). Thus oxidative stress may account for endothelial dysfunction, but it is unknown whether this abnormality is a primary event or a consequence of increased

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This study was supported by grants from FONDECYT (grant number 1040429, Chile Government), Procaps Laboratory (Colombia) and Gynopharm CFR Laboratory (Chile).

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Received March 12, 2007; Accepted in revised form July 3, 2007.

Table 1. Clinical Characteristics of Essential Hypertensive Patients ($n=31$) and Healthy Normotensive Subjects ($n=35$)

Characteristic	Normotensive subjects	Hypertensive patients	*p* value
Age (years)	44.4±1.3	45.9±1.6	0.48
Body mass index (kg/m^2)	25.5±0.4	26.2±0.3	0.17
Serum glucose (mmol/L)	4.94±0.07	5.11±0.10	0.16
Creatinine (μmol/L)	80.4±1.6	82.2±2.2	0.52
Total cholesterol (mmol/L)	4.54±0.16	4.86±0.13	0.13
HDL-cholesterol (mmol/L)	1.29±0.07	1.21±0.04	0.31
LDL-cholesterol (mmol/L)	2.69±0.16	2.90±0.11	0.26
Serum triglycerides (mmol/L)	1.33±0.08	1.53±0.10	0.10
Daytime SBP (mmHg)	119.5±0.8	137.5±0.2	<0.001*
Daytime DBP (mmHg)	78.2±0.8	91.9±1.3	<0.001*
Heart rate (beats/min)	71.6±1.3	73.5±1.1	0.28

Values are means±SEM. HDL, high density lipoprotein; LDL, low density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure. *Significant difference by unpaired *t*-test.

Table 2. Plasma Blood Pressure Modulator Levels in the Study Participants

Modulator	Normotensive subjects ($n=35$)	Hypertensive patients ($n=31$)	*p* value
Renin activity (pmol/L/h)	26.07±4.00	20.86±2.64	0.29
Aldosterone (nmol/L)	0.24±0.02	0.26±0.02	0.47
Endothelin-1 (pmol/L)	2.72±0.25	2.45±0.33	0.51
Homocysteine (μmol/L)	8.85±0.31	9.91±0.51	0.07
Folic acid (nmol/L)	43.49±1.49	46.6±1.27	0.12
Vitamin B_{12} (pmol/L)	228.2±9.3	233.4±7.87	0.73

Values are means±SEM.

blood pressure (*10*). In keeping with the above results, acute pressure overload has been reported to induce self-limited superoxide production in the vascular wall (*11*). Attempts to counteract the hypertensive effect of ROS have led to the use of exogenous administration of antioxidants thought to improve the vascular function and reduce the blood pressure in animal models (*12*, *13*) and in human hypertension (*14*, *15*). Nevertheless, the available data are not conclusive and the relationship between blood pressure and oxidative stress in humans remains to be elucidated. The purpose of the present study was to investigate the association of blood pressure with oxidative stress in volunteer male normotensive and essential hypertensive subjects.

Methods

Study Design

A cross-sectional design was applied to 31 hypertensive patients and 35 healthy normotensive subjects. The study protocol was approved by the Ethics Committee of the University of Chile Clinical Hospital, in accordance with the Helsinki Declaration (Edinburgh revision, 2000). All participants signed a written consent form and no complications were encountered during the study.

Patients

We recruited untreated essential hypertensive outpatients (stage 1) (*16*). All subjects were males between the ages of 35 and 60 years. Hypertension was defined as mean daytime blood pressure values ≥135 mmHg systolic or ≥85 mmHg diastolic, by ambulatory blood pressure monitoring (*17*). Exclusion criteria were smoking, obesity (body mass index [BMI] >30 kg/m^2), diabetes, hypercholesterolemia, chronic diseases, and current use of any medication, including dietary supplements. Normotensive volunteer subjects participated as controls. The potential participants were subjected to a selection protocol consisting of clinical history, physical examination and appropriate tests. Patients showing evidence of target-organ-damage were excluded from the study. Cardiovascular damage (myocardial hypertrophy and/or valve dysfunction) was detected by echocardiographic examination (*18*). In addition, plasma creatinine levels and urine analysis were used to detect renal damage.

Table 3. Plasma, Erythrocyte and Urine Oxidative Stress–Related Parameters of the Study Participants

Parameter	Normotensive subjects ($n=35$)	Hypertensive patients ($n=31$)	p value
Plasma			
FRAP (μmol/L)	427.4±13.7	303.4±10.5	<0.001*
Uric acid (μmol/L)	296.2±11.5	305.8±12.2	0.57
Vitamin C (μmol/L)	42.8±2.1	35.7±2.1	0.02*
Vitamin E (μmol/L)	15.7±1.1	15.5±1.2	0.89
8-Isoprostane (pmol/L)	76.7±3.6	104.1±3.6	<0.001*
Erythrocytes			
Catalase (*k*/g Hb)	267.2±2.6	216.3±2.1	<0.001*
Superoxide dismutase (U/g Hb)	1,355±8	1,186±7	<0.001*
Glutathione peroxidase (U/g Hb)	6.23±0.04	5.31±0.03	<0.001*
GSH/GSSG ratio	7.2±0.5	5.6±0.4	<0.02*
Malondialdehyde (nmol/g Hb)	311.2±3.1	361.8±2.7	<0.001*
Urine			
8-Isoprostane (nmol/μmol creatinine)	126.5±8.8	167.1±10.9	<0.006*

Values are means±SEM. FRAP, ferric reducing ability of plasma; U, units; *k*, catalase first order kinetic constant for breakdown of hydrogen peroxide (mol/L/s); GSH, reduced glutathione; GSSG, oxidized glutathione. *Significant difference by unpaired *t*-test.

Ambulatory Blood Pressure Monitoring

Blood pressure levels were determined through ambulatory monitoring on a regular workday (over a period of 24 h beginning at 8:30 AM) with an oscillometric monitor (SpaceLabs 90207; SpaceLabs Inc., Redmond, USA) previously checked for accuracy against simultaneous measurements with a mercury sphygmomanometer. This device fulfils the validation criteria of the British Hypertension Society protocol (*19*) and satisfies the criteria of the Association for the Advancement of Medical Instrumentation (AAMI) for studies under ambulatory conditions (*20*). The blood pressure analyzed was the mean daytime pressure value. The oscillometric accuracy, assessed by SpaceLabs–intra-arterial average differences, was −0.6±5.9 and 0.9±6.4 mmHg (means±SD), for systolic blood pressure (SBP) and diastolic blood pressure (DBP), respectively, which are within the AAMI accuracy standard. The estimated oscillometric reproducibility was −0.3±3.2 and 0.1±3.5 mmHg (means±SD) for SBP and DBP, respectively (*21*).

Assessment of Oxidative Stress–Related Parameters

Venous blood samples were collected in chilled vacutainers. Plasma, erythrocyte lysates and urine samples were stored at −70°C. Plastic tubes for 8-isoprostane samples were previously treated with butylated hydroxytoluene (final concentration 1 mmol/L). Plasma antioxidant status was assessed by ferric reducing ability of plasma (FRAP), with a detection limit of 10 μmol/L (*22*). The inter-assay and intra-assay coefficients of variation for FRAP were 3.0% and 1.0%, respectively. The plasma uric acid levels were also assayed. Vitamin C was analyzed by a fluorometric method (*23*), and the inter-assay and intra-assay coefficients of variation were 4.9% and 2.7%, respectively. Vitamin E was analyzed by high performance liquid chromatography (HPLC) (*24*), and the inter-assay and intra-assay coefficients of variation were 6.4% and 4.5%, respectively. Lipid peroxidation was estimated through plasma and urine 8-isoprostane concentrations, a reliable biomarker of oxidative stress *in vivo* (*25*), using an ELISA kit (Cayman, Ann Arbor, USA). The inter-assay and intra-assay coefficients of variation were 9.5% and 10.7%, respectively. Renin and aldosterone were determined by radioimmunoassay, and endothelin-1, homocysteine, folic acid and vitamin B_{12} by ELISA.

Assessment of Erythrocyte Antioxidant Status

Lysates of erythrocytes were homogenized for the fluorometric determination of reduced glutathione (GSH) and oxidized glutathione (GSSG) (*26*). The GSH/GSSG ratio was determined. In addition, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities were determined according to previously described methods (*27*).

Statistical Analysis

The necessary sample size was calculated using blood pressure as the primary endpoint to detect a difference of about 20% between the blood pressure levels and oxidative stress–related parameters of both groups at a power of 80% and a p value of 0.05. Descriptive statistics of variables used the mean and SEM. The source of variation was assessed by unpaired Student's *t*-test for normally distributed parameters. The association of variables was studied by Pearson correlation test.

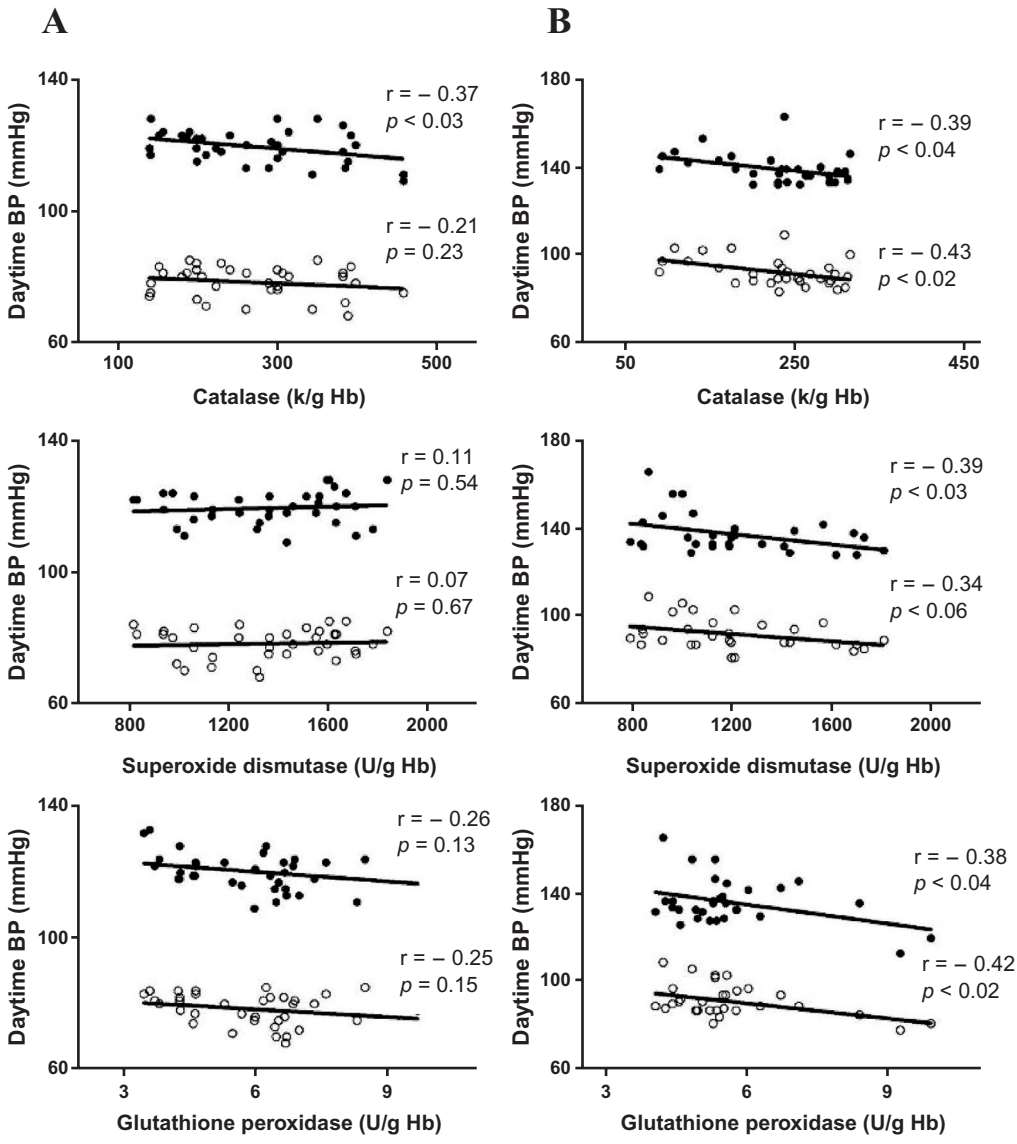

Fig. 1. *Pearson correlation between daytime SBP (solid circles and line) or DBP (open circles and line) and erythrocyte activities of catalase, superoxide dismutase, and glutathione peroxidase in normotensive (A) and hypertensive (B) subjects. SBP, systolic blood pressure; DBP, diastolic blood pressure; U, unit; k, catalase first order kinetic constant for breakdown of hydrogen peroxide (mol/L/s).*

Results

Clinical Characteristics

The clinical characteristics of the 66 study participants are shown in Table 1. Except for the significantly higher daytime SBP and DBP in the hypertensive group ($p < 0.001$), all parameters were within the normal ranges and showed no significant differences between the two groups.

Blood Pressure Modulators and Oxidative Stress–Related Parameters

Plasma renin, aldosterone, endothelin-1 and homocysteine levels (Table 2) were not significantly different between hypertensive and normotensive subjects.

However, the erythrocyte GSH/GSSG ratio, the vitamin C levels, and the blood antioxidant activity as assessed by the plasma FRAP were 29%, 16% and 29% lower in hypertensives than normotensives, respectively (Table 3). In addition, the erythrocyte activities of CAT, SOD and GSH-Px of hypertensives were 19%, 12%, and 15% lower than the

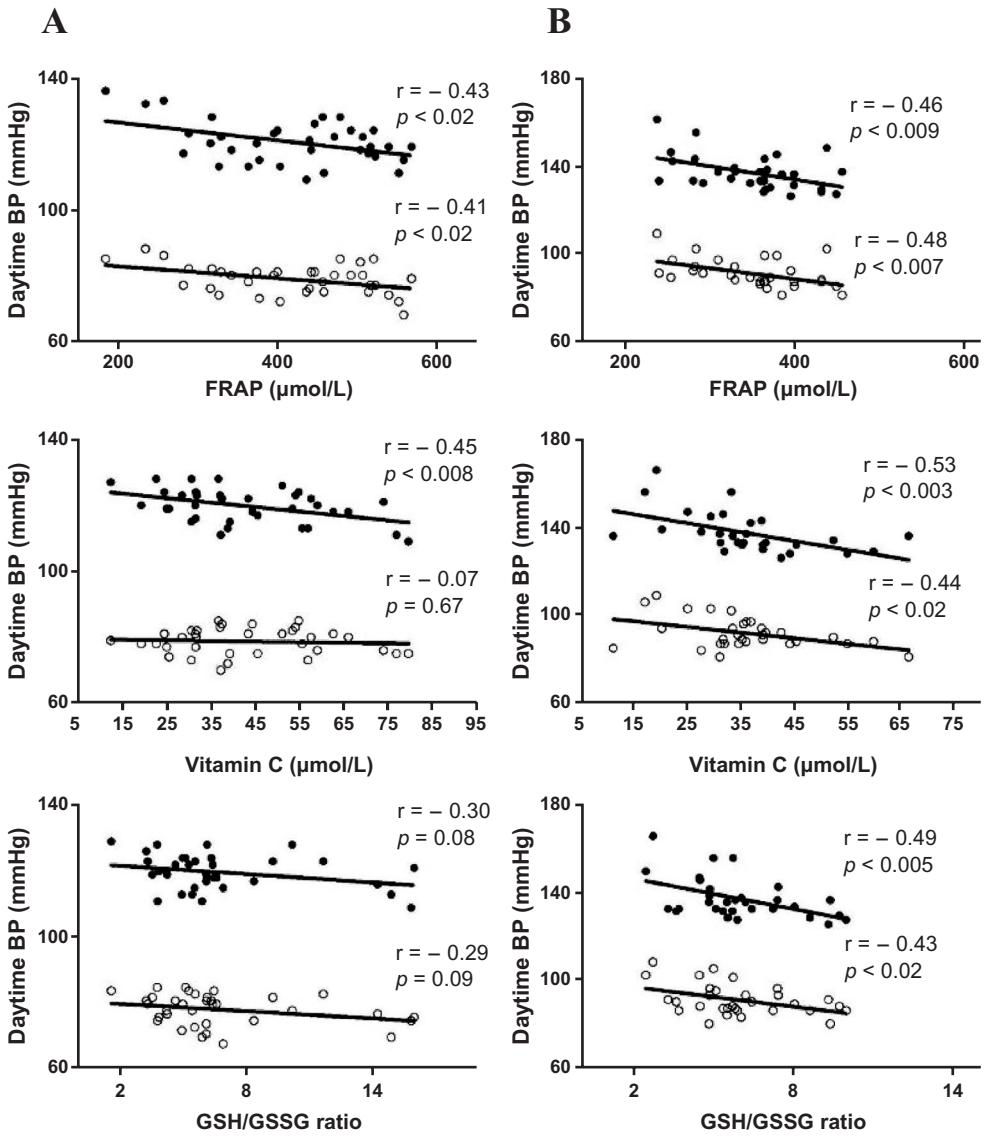

Fig. 2. *Pearson correlation between daytime SBP (solid circles and line) or DBP (open circles and line) and plasma vitamin C levels, erythrocyte GSH/GSSG ratio, and antioxidant status as assessed by FRAP in normotensive (A) and hypertensive (B) subjects. SBP, systolic blood pressure; DBP, diastolic blood pressure; FRAP, ferric reducing ability of plasma; GSH, reduced glutathione; GSSG, oxidized glutathione.*

respective normotensive values. Furthermore, the lipid peroxidation of hypertensives, as assessed by plasma and urine 8-isoprostane, and the erythrocyte malondialdehyde (MDA) concentrations were 36%, 32% and 16% higher, respectively, compared to normotensives.

The relation between daytime SBP or DBP and the activity of antioxidant enzymes is shown in Fig. 1 for both normotensives (Fig. 1A) and hypertensives (Fig. 1B). Daytime SBP and DBP were both negatively correlated with the activity of CAT in hypertensives, but in normotensives only the correlation between daytime SBP and CAT activity was statistically significant. SOD activity in hypertensives was negatively correlated with SBP, but not with DBP, whereas in normotensives neither relation was significant. GSH-Px activity in hypertensives was negatively correlated with both SBP and DBP, whereas in normotensives neither correlation was significant. The relations between daytime SBP or DBP and the plasma FRAP and vitamin C levels and the erythrocyte GSH/GSSG ratio are shown in Fig. 2 both for normotensives (Fig. 2A) and hypertensives (Fig. 2B). Both groups showed strong negative correlations between SBP and FRAP and also between DBP and FRAP. Plasma vitamin C levels were negatively correlated with SBP and DBP in hypertensives, whereas in normotensives only the correlation between

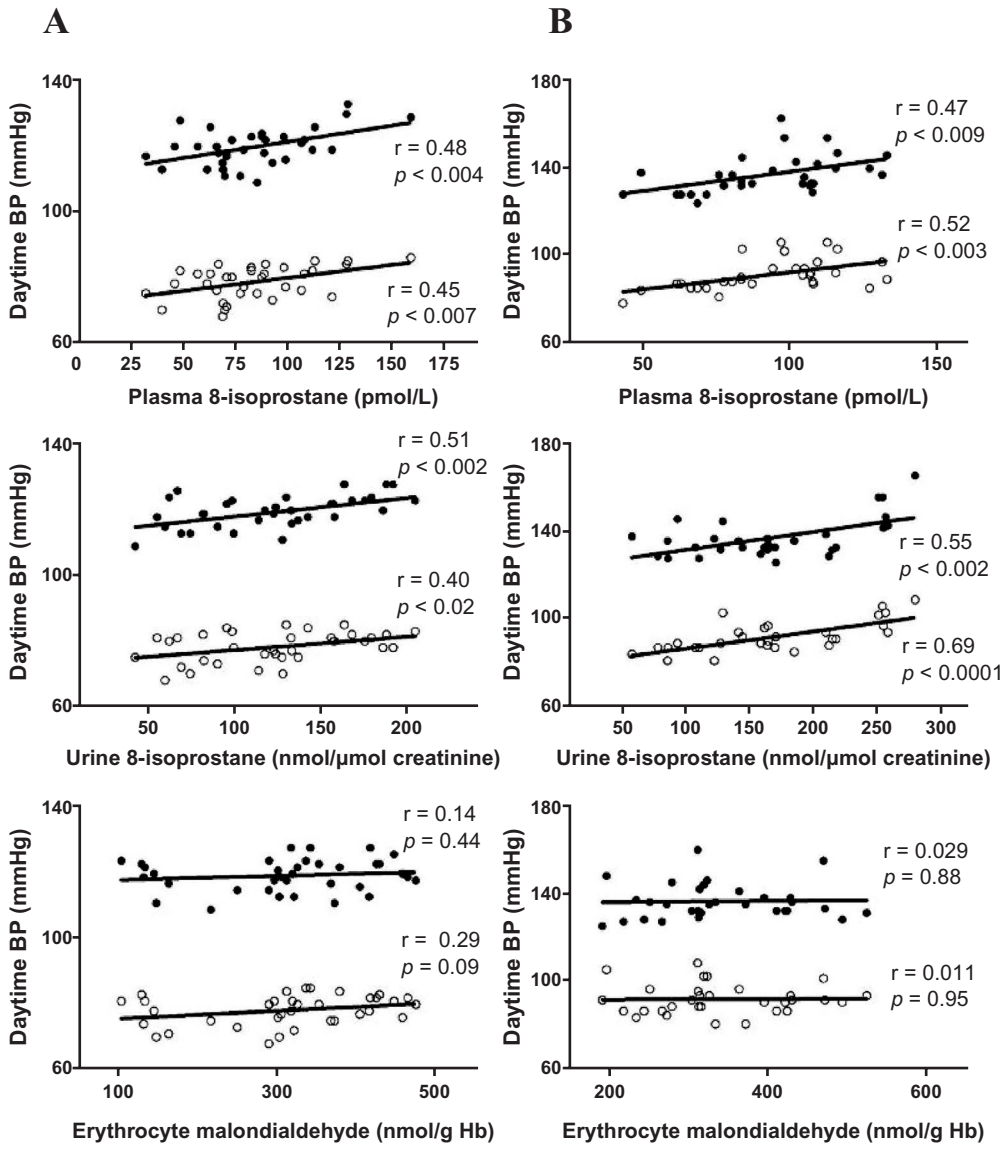

Fig. 3. *Pearson correlation between daytime SBP (solid circles and line) or DBP (open circles and line) and lipid peroxidation assessed by plasma and urine 8-isoprostane, and erythrocyte MDA concentrations of normotensive (A) and hypertensive (B) participants. SBP, systolic blood pressure; DBP, diastolic blood pressure; MDA, malondialdehyde.*

plasma vitamin C and SBP was significant. The erythrocyte GSH/GSSG ratio was negatively correlated with SBP and DBP in hypertensives, but was not significantly related to either SBP or DBP in normotensives. The relation between SBP or DBP and the plasma and urine 8-isoprostane levels and erythrocyte MDA levels is shown in Fig. 3 for normotensives (Fig. 3A) and hypertensives (Fig. 3B). Notably, there was a strong positive correlation between plasma and urine 8-isoprostane concentrations and both SBP and DBP in both groups. No correlation was found between SBP or DBP and the erythrocyte MDA concentrations or any of the blood-pressure modulators analyzed.

Discussion

The present findings demonstrate a strong association between blood pressure and some oxidative stress–related parameters. The increased oxidative stress levels that we observed in essential hypertensive patients are consistent with the findings of several previous studies (*28–30*). In other studies, however, no significant increase in plasma 8-isoprostane levels was observed, although these studies were performed in the early stages of the disease (*31*) or with patients receiving statin medication (*32*), a confounding factor of oxidative stress (*33*). The correlation of blood pressure levels with oxi-

dative stress–related parameters in both normotensives and hypertensives suggests that these parameters have an additional blood pressure–modulating effect distinct from those previously observed. Essential hypertensive subjects showed an impairment of the antioxidant defense system as assessed by a diminution of plasma and erythrocyte antioxidant status, in agreement with previous data (*34*–*36*). Furthermore, the negative correlation between SBP and DBP and both the antioxidant enzyme activity (Fig. 1) and the erythrocyte GSH/GSSG ratio (Fig. 2) points out the importance of the blood antioxidant status in blood pressure modulation. Previous studies found a low antioxidant enzyme activity (*37*) and a negative correlation of catalase activity with both daytime SBP and DBP (*28*) in essential hypertensives, in agreement with our results. The fact that normotensives did not show an association between blood pressure and the activity of most of the erythrocyte antioxidant enzymes (Fig. 1A), whereas hypertensives did (Fig. 1B), deserves special analysis. It is well documented that exposure to ROS increases the expression of antioxidant enzymes (*38*, *39*). Thus, genes encoding these enzymes are coordinately regulated by the antioxidant responsive elements (ARE) in their regulatory regions (*40*), a process occurring through the activation of the transcription factor NF-E2–related factor 2 (Nrf2). The binding of Nrf2 to these ARE sites leads to up-regulation of downstream genes that regulate the activity of antioxidant enzymes to compensate against ROS toxicity. It may be that this mechanism is triggered in most hypertensives in response to their ROS levels, which were here shown to be elevated compared to normotensives based on the plasma 8-isoprostane levels of both groups (Table 3). The negative correlation between blood pressure and the erythrocyte GSH/GSSG ratio observed in hypertensives (Fig. 2B) may indicate that GSH oxidation by increased ROS is not followed by a compensatory response of glutathione metabolism–related enzymes. In turn, this correlation was not significant in normotensives (Fig. 2A), likely due to a lack of ROS increase that would be responsible to change the erythrocyte GSH/GSSG ratio.

The occurrence of oxidative stress may arise from a primary decrease in the antioxidant defense system activity or from an elevation of ROS concentration. This derangement leads to oxidative damage to the structure of biomolecules, likely involving the antioxidant enzymes, thus contributing to the oxidative stress found in hypertensives, but not in normotensives (Table 3). As a consequence of increased ROS, a reduction in the endothelium-dependent vasodilation of the vascular smooth muscle cells of hypertensives could be expected (*6*, *7*). In turn, elevations of blood pressure could also contribute to the increase of ROS, thereby enhancing the mechanism of ROS-mediated hypertension through a complex interdependency.

Alternatively, the elevation of plasma 8-isoprostane levels in hypertensives has been suggested to be a contributory factor in the elevation of vascular peripheral resistance (*41*), although this finding needs confirmation. Regardless of whether the 8-isoprostane increase arises from a primary metabolic derangement or from the elevation of blood pressure itself, the strong positive association of both SBP and DBP with the plasma and urine 8-isoprostane concentration (Fig. 3) suggests that lipid peroxidation should be considered as a risk factor for blood pressure elevation. Additionally, the low plasma FRAP levels in hypertensives and their strong negative correlation with SBP and DBP levels in both groups (Fig. 2) suggests a role of the plasma antioxidant status in the modulation of blood pressure. The latter result in hypertensives is consistent with their low plasma levels of vitamin C, a contributor to FRAP (Table 3). The association of vitamin C and blood pressure is in agreement with previous studies that were performed on an epidemiologic scale (*42*, *43*) but not with the rigorous selection criteria applied in the present investigation in order to preclude the potential confounders. Although our data were obtained from a smaller sample, our approach allowed us to investigate the role of oxidative stress in the modulation of blood pressure. It is noteworthy that the effects of antioxidants and vitamin C in human hypertension remain controversial (*7*, *44*), although they may exert their antihypertensive effect through their ability to scavenge ROS, regulate NO synthases, and through cooperative action with enzymes that generate (NADPH oxidase) or depurate ROS (superoxide dismutase) (*45*). In addition, it has also been reported that the antioxidan adrenomedullin could exert antihypertensive effects derived from its antioxidant properties (*46*). The idea that oxidative stress contributes to the etiology of essential hypertension is further supported by our present finding that the plasma levels of the various modulators of blood pressure were not significantly different between hypertensives and normotensives (Table 2).

It was notable that 8-isoprostane and FRAP had the highest correlations with blood pressure among the oxidative stress–related parameters studied. Because of the relationship between oxidative stress and hypertension, it is worth noting that drugs with antioxidant effects can also be expected to lower blood pressure. Accordingly, the antihypertensive effects of statins could arise from their antioxidant properties, *via* their ability to decrease the expression of NAD(P)H oxidase subunits and upregulate catalase expression *in vivo* (*47*). In addition, the administration of candesartan and valsartan has been shown to cause a decrease in oxidative stress in essential hypertensives (*2*, *48*). Along these lines, antioxidant vitamins have been shown to exert antihypertensive effects in spontaneously hypertensive rats, although the extensibility of these results to human beings remain controversial (*49*), and awaits the completion of large scale clinical trials that are currently underway.

The usefulness of the results of the present study may be limited by our use of only male subjects. For example, it is well known that endogenous and exogenous estrogens and progestogens, which could be subjected to time-dependent variations in women, are able to modulate renal sodium metabolism as well as the activity of the renin-angiotensin-

aldosterone axis (*50*). However, future studies should be devoted to the analysis of these subjects.

In conclusion, these data provide evidence of blood pressure modulation by measurable oxidative stress–related parameters and contribute for the first time to the characterization of a functional dependence between these so far seemingly unrelated parameters. Accordingly, oxidative stress might one day be considered as a novel therapeutic target for the therapy of essential hypertension.

Acknowledgements

The authors wish to thank to FONDECYT, Procaps Laboratory (Colombia) and Gynopharm CFR Laboratory (Chile) for their financial support of this study. Dr. Myriam Orellana, Professor Irene Schiattino, and Mr. Diego Soto are also acknowledged.

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