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Macrophage Superoxide Anion Production in Essential Hypertension: Associations With Biological and Psychological Cardiovascular Risk Factors

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ABSTRACT

Objective: Essential hypertension is an important risk factor for coronary artery disease and its underlying process atherosclerosis, but involved mechanisms are not fully understood. Both macrophages and superoxide anions have been proposed to play a major role in the pathogenesis of atherosclerosis. In the present study, we investigated whether macrophages of individuals with hypertension show higher nicotinamide adenine dinucleotide phosphate oxidase–derived superoxide anion production compared with normotensive individuals. Furthermore, we examined associations between macrophage superoxide anion production and the psychological factors depression and chronic stress independent from hypertension status.

Methods: We studied 30 hypertensive (mean [standard deviation] = 48.7[2.4]years) and 30 age-matched normotensive men (mean [standard deviation] = 48.6[2.4]years). We assessed macrophage superoxide anion production using the WST-1 assay. The assay is based on the chemical reduction of the cell-impermeative tetrazolium salt WST-1 by superoxide anions that are produced by activated human ex vivo isolated monocyte-derived macrophages. We further evaluated whether chronic stress or depressive symptom severity was associated with macrophage superoxide anion production. All analyses were adjusted for potential confounders.

Results: Individuals with hypertension showed higher superoxide anion production compared with normotensive individuals ($F(1,58) = 11.56$, $p = .001$). Complementary analyses using mean arterial blood pressure as a continuous measure revealed that higher mean arterial pressure correlated significantly with higher WST-1 reduction ($\beta = .38$, $p = .003$, $\Delta R^2 = .145$). These results remained significant when controlling for potential confounding influences. Chronic stress was related to higher WST-1 reduction scores, but this association was not statistically significant ($\beta = .24$, $p = .067$, $\Delta R^2 = .053$); depression levels were not significantly associated with WST-1 reduction scores ($p = .24$).

Conclusions: Our results indicate higher macrophage superoxide anion production in individuals with hypertension compared with normotensive individuals. This may suggest a mechanism underlying cardiovascular risk with hypertension.

Key words: atherosclerosis, essential hypertension, human macrophages, NADPH oxidase, superoxide anions.

INTRODUCTION

Essential hypertension is a major risk factor for coronary artery disease (CAD) and its underlying process atherosclerosis (1–4). However, the mechanisms that link hypertension with an increased risk of atherosclerosis are not fully understood (5,6).

The innate immune system plays a paramount role in initiation and progression of the inflammatory process in

ANOVA univariate analysis of variance, **BDI** Beck Depression Inventory, **BMI** body mass index, **BP** blood pressure, **CAD** coronary artery disease, **CSSS** Chronic Stress Screening Scale, **HDL** high density lipoprotein, **HMDM** human monocyte derived M1 macrophages, **LDL** low density lipoprotein, **MAP** mean arterial blood pressure, **NADPH** nicotinamide adenine dinucleotide phosphate, **PBMC** peripheral blood mononuclear cell, **PMA** phorbol myristate acetate, **ROS** reactive oxygen species, **WST 1** 2 (4 iodophenyl) 3 (4 nitro phenyl) 5 (2,4 disulfophenyl) 2*H* tetrazolium, monosodium salt

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atherosclerosis, whereby monocytes and macrophages are key cells in this process (7–10). One of the earliest events in atherosclerosis is the recruitment of monocytes into the intima as the inner layer of the arterial wall, where they mature into macrophages being important mediators of the innate immune response and of inflammation in atherosclerosis (7,11–13).

A key innate immune effector function of classically activated macrophages (or inflammatory M1 macrophages, respectively) is microbicidal activity, that is, the killing of microbes (14–16). Microbicidal effectiveness of human macrophages largely hinges on their production of reactive oxygen species (ROS) (17,18). ROS production in turn derives from the activated multisubunit enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase located in the phagolysosomal and plasma membrane of macrophages. Once activated, NADPH oxidase transfers electrons from NADPH in the cytosol to extracellular or intraphagolysosomal oxygen molecules. These oxygen molecules are then chemically reduced to highly reactive superoxide anions (O_2) and other ROS subtypes (17,19). Particularly, superoxide anions are of major importance for the microbicidal activity of macrophages in host defense (16,20,21).

Increasing evidence suggests that NADPH oxidase and the resulting production of superoxide anions are likely to play a critical role in the pathogenesis of atherosclerosis. For instance, NADPH oxidase–derived superoxide anions can induce low-density lipoprotein oxidation (19,22–25), an important cause of endothelial dysfunction as an initial step in atherosclerosis (4,10). Indeed, NADPH oxidase–deficient mice developed significantly less atherosclerosis as assessed by quantifying atherosclerotic lesion sizes (26). Furthermore, in mice, the extent of atherosclerosis correlated with higher aortic superoxide anion production (25). Similarly, a study in human coronary arteries revealed that NADPH oxidase–mediated superoxide anion production was highest in the macrophage-rich shoulder regions of the plaque and higher superoxide anion production correlated with higher severity of atherosclerosis (27).

To date, NADPH oxidase–derived superoxide anion production by inflammatory macrophages as cells present in atherosclerotic lesions has not yet been investigated in essential hypertension. Also, a potential role of superoxide anion production by inflammatory macrophages in mediation of atherosclerotic risk in hypertension is unclear. So far, two studies investigated NADPH oxidase–derived superoxide anion production in macrophage precursor cells, namely, circulating peripheral blood mononuclear cells (PBMCs). So, Fortuño and colleagues (28) reported a superoxide anion overproduction in circulating PBMCs among partially treated hypertensive men and women compared with normotensives. Furthermore, Watanabe and colleagues (29) observed in individuals with hypertension a higher ROS formation by circulating mononuclear cells with increasing carotid intima-media thickness as a vague index for atherosclerosis severity.

Here, we investigate for the first time whether individuals with hypertension differ from normotensives in their NADPH oxidase–derived superoxide anion production by inflammatory M1 macrophages. To account for a confounding influence by age (30), participant groups were matched for age. Given the atherosclerotic risk of hypertension in combination with the supposed role of inflammatory macrophages and superoxide anions in atherosclerosis, we hypothesized individuals with hypertension to show a higher macrophage superoxide anion production as compared with normotensives.

MATERIALS AND METHODS

Study Participants

This study was part of a larger project assessing psychoneurobiological mechanisms in essential hypertension. The project was approved by the ethics committee of the State of Bern, Switzerland, and all participants provided written informed consent.

Between December 2012 and May 2014, we recruited by aid of the Swiss Red Cross of the State of Bern apparently healthy, nonsmoking, and medication-free hypertensive and age-matched normotensive men. In detail, members of our study team accompanied the mobile blood donation unit of the Swiss Red Cross that routinely records blood pressure (BP) before blood donation. Male blood donors with elevated BP and expressing interest in participating in the study were given the written study information asking for the following inclusion criteria: age of 18 to 80 years, systolic BP of 140 mm Hg or greater, and/or diastolic BP of 90 mm Hg or greater; nonsmoker; no acute or regular intake of medication; and no alcohol or illicit drug abuse. Next, we assessed whether interested blood donors were actually hypertensive or not (see later). Those identified as hypertensive were then screened by telephone interview using an extensive health questionnaire. Explicit exclusion criteria were the following: regular strenuous exercise, alcohol and illicit drug abuse, liver and renal diseases, chronic obstructive pulmonary disease, allergies and atopic diathesis, rheumatic diseases, human immunodeficiency virus, cancer, major psychiatric disorders, neurological diseases, and current infectious diseases. In addition, eligible hypertensive participants provided blood samples for the routine assessment of serum creatinine, calcium, sodium, and potassium to find potential people with secondary hypertension. Furthermore, we measured HbA1c and low density lipoprotein (LDL)/high density lipoprotein (HDL) ratio in all participants. All participants eligible for our study were assessed for the macrophage activation assay as described later. In 2 of 32 recruited hypertensive men, macrophage activation data could not be obtained because of assay problems. No eligible hypertensive participant was diagnosed with secondary hypertension post hoc, so all were defined to have essential hypertension. Notably, calcium, sodium, and potassium could not be analyzed in three hypertensive participants and HbA1c and LDL/HDL ratio could not be assessed in two normotensive participants because of technical problems. For each of the 30 essential hypertensive participants with macrophage data, we recruited an age-matched (±4 years) normotensive control on a case-by-case basis to balance with regard to potential confounding effects of age on superoxide anion production (31), yielding a final study sample of 60 participants. Apart from hypertension-related criteria, controls had to meet the same inclusion and exclusion criteria as individuals with hypertension.

Assessment of Essential Hypertension

Following written instructions, each participant was required to measure BP on 3 separate days at home using sphygmomanometry (Omron M6;

TABLE 1. Cutoff Values for the Classification of Hypertension and Normotension

Blood Pressure	Hypertension	Normotension
Home SBP, mm Hg	≥140	<135
Home DBP, mm Hg	≥90	<85
Study SBP, mm Hg	≥140	<140
Study DBP, mm Hg	≥90	<90

Home SBP systolic blood pressure from home measurements; home DBP diastolic blood pressure from home measurements; study SBP systolic blood pressure from study measurements; study DBP diastolic blood pressure from study measurements.

Omron Healthcare Europe B.V., Hoofdorp, the Netherlands). Home BP measurements were obtained in a seated position after a 15-minute rest twice per day (once in the morning and once in the evening, rendering a total of 6 measurements for each participant) and the average BP was computed. Deviating from instructions, two normotensive and seven hypertensive participants provided six BP measurements on 4 to 6 different days and one normotensive participant measured BP only once. Hypertension was conservatively defined (both for home and study measurements) by the World Health Organization/International Society of Hypertension definition, that is, systolic BP of 140 mm Hg or greater and/or diastolic BP 90 mm Hg or greater (32). Participants were conservatively classified as normotensive if home systolic BP was less than 135 mm Hg and home diastolic BP was less than 85 mm Hg according to recommendations for home BP measurements (33). Because of technical problems, home BP was missing in two hypertensive and two normotensive participants who were alternatively classified on the basis of their BP values recorded by the blood donation center. The classification of each participant as hypertensive or normotensive was verified by three additional seated study BP measurements after a 15-minute rest performed by trained personnel during the study session (see later). Six normotensive participants provided only two instead of three of those additional seated study BP measurements. Notably, for classification of normotension according to study BP measurements, we applied the regular World Health Organization/International Society of Hypertension definition; that is, systolic BP of less than 140 mm Hg and diastolic BP of less than 90 mm Hg. We calculated mean arterial blood pressure (MAP) from the study BP measurements by the formula (2/3 mean diastolic BP) + (1/3 mean systolic BP). Table 1 gives an overview of the BP measurement procedure and the different cutoff values (Fig. 1) for the classification of hypertension and normotension in our study.

Procedure

All participants abstained from caffeine and alcohol consumption for 24 hours and consumed a semistandardized breakfast following written instructions before arrival at the laboratory at 8:00 A.M. Then, questionnaires were administered. Blood was collected by short-term cannula insertion (see the study by Kuebler et al. (34)) for the assessment of superoxide anion production at 11:30 A.M., that is, after a fasting for 3.5 hours since arrival. Blood pressure was assessed by means of sphygmomanometry (Omron M6; Omron Healthcare Europe B.V., Hoofdorp, the Netherlands) 3 and 2.5 hours before and 10 minutes after blood sampling. In one participant, BP measurement was taken only once 10 minutes after blood sampling because of technical problems.

Macrophage Activation Assessment

Reagents and Chemicals

We used the following reagents: Ficoll-Paque PLUS (Ficoll; no. 17-1440-02; GE Healthcare, Uppsala, Sweden), 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)2*H*-tetrazolium (WST-1; no. 150849-52-8; Dojindo Laboratories, Kumamoto, Japan), interferon-gamma (IFN-γ; no. PHC4031; Invitrogen, Basel, Switzerland), tumor necrosis factor-α (no. PHC3016; Invitrogen, Basel, Switzerland), Hank's balanced salt solution without phenol red (no. 14025050; Invitrogen, Basel, Switzerland), fetal bovine serum (no. 10270-106; Invitrogen, Basel, Switzerland), lipopolysaccharide (no. L6529; Sigma-Aldrich, Buchs, Switzerland), phosphate-buffered saline (no. P5368; Sigma-Aldrich, Buchs, Switzerland), phorbol 12-myristate 13-acetate (PMA; no. P8139; Sigma-Aldrich, Buchs, Switzerland), RPMI 1640 medium with glutamax (RPMI 1640; no. W9925E; Fisher Scientific, Wohlen, Switzerland), and Diff-Quick Staining Set (Medical Solutions GmbH, Hünenberg, Switzerland).

WST-1 Assay

We assessed superoxide anion production of ex vivo isolated human monocyte-derived M1 macrophages (HMDM) on the basis of our recently validated in vitro WST-1 assay (34). The assay principle is based on the chemical reduction of the cell-impermeative tetrazolium salt WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium

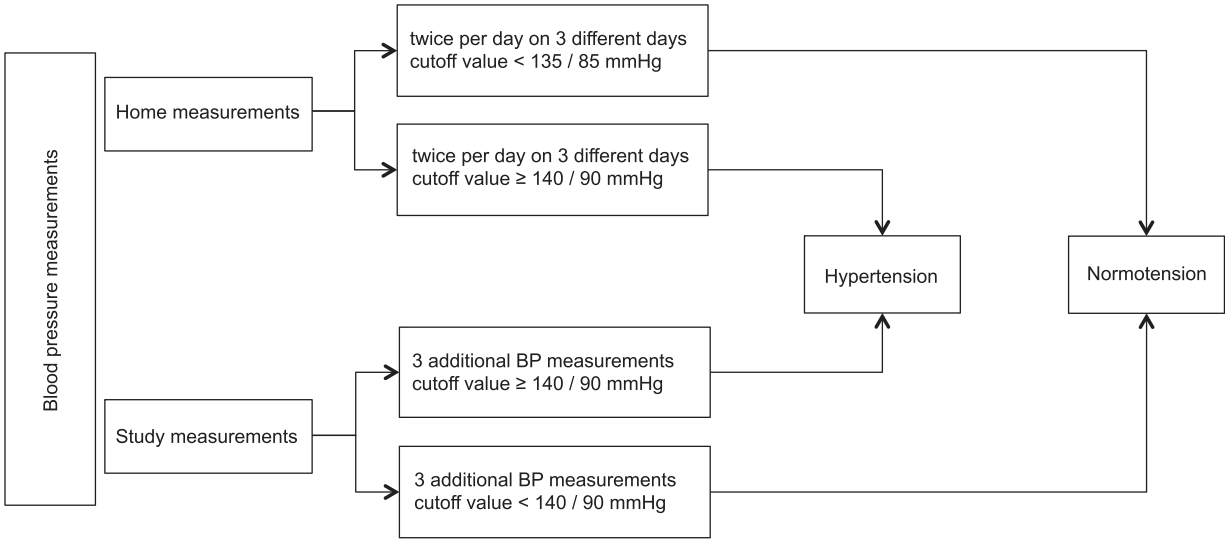

FIGURE 1. Procedure of BP measurements for the classification of hypertension and normotension. BP blood pressure.

monosodium salt) by superoxide anions that are produced by PMA-activated HMDM, which represent the classically activated M1 macrophages (15). The assay procedure is described in detail in the provided in Supplemental Digital Content 1 (http://links.lww.com/PSYMED/A277). Higher optical densities, as obtained in absorbance reading, are associated with higher amounts of WST-1 reduction and thus of superoxide anions generated by HMDM. In three hypertensive and three normotensive men, the assays were not performed in duplicates because of low PBMC numbers.

Cell Number Correction of WST-1 Reduction Scores

To rule out that possible differences in WST-reduction scores may relate to differences in the number of macrophages derived from 3.0×10^6/ml PBMCs, we followed previous methods (34) and determined the number of adherent cells per well as an indicator of the final macrophage number per well and calculated WST-1 reduction scores per 10,000 macrophages (corrected WST-1 reduction score). The procedure to correct for the number of cells is detailed in Supplemental Digital Content 1.

Psychological Assessment

The potential association of depressive symptom severity and chronic stress with superoxide anion production (34) was examined using regression analyses.

Depressive Symptom Severity

Depressive symptom severity was measured with the validated German version (35) of the 21-item Beck Depression Inventory Second Edition (BDI-II) (36), where scores of 11 or higher indicate possible clinical depression. The BDI-II measures a somatic and a cognitive-affective dimension of depression and assesses the frequency and/or severity of symptoms related to sadness, feelings of guilt, perceptions of self-worth, suicidal ideation, and changes in appetite and body weight, among other characteristics. Items are rated on a 4-point Likert scale ranging from 0 (symptom not present) to 3 (symptom very much present) that add to a total BDI score ranging from 0 to 63. Higher scores indicate higher depressive symptom severity. Cronbach α of the BDI total score was .89 in our sample. In analyses controlling for a potential influence of BDI, missing BDI data of one hypertensive and one normotensive participant were estimated using the expectation-maximization algorithm (37,38).

Chronic Stress

To assess chronic stress, we used the 12-item Chronic Stress Screening Scale (CSSS) (39). The CSSS includes questions about frequency of experiencing work overload (four items), worries (four items), lack of social recognition (two items), excessive demands at work (1 item), and social overload (1 item). Items have a 5-point rating format reflecting stress frequency (1 "never" to 5 "very often"). Possible scores range from 12 to 60 with higher scores meaning greater levels of chronic stress. Cronbach α of the CSSS total score was .91 in our sample. Missing CSSS data of one normotensive and three hypertensive participants were estimated using the expectation-maximization algorithm (37,38), and results were used in analyses controlling for a potential influence of chronic stress.

Statistical Analysis

Data were analyzed using SPSS (Version 20) statistical software package for Macintosh (IBM SPSS Statistics, NY). All analyses were two-tailed, with the level of significance set at a p value of less than .05. Results are shown as mean and standard deviation (M [SD]).

Before statistical analyses, data were tested for normal distribution and homogeneity of variance using Kolmogorov-Smirnov and Levene tests, respectively. All data were normally distributed within the two groups, but homogeneity of variance of WST-1 reduction scores was not verified in the group comparisons. To calculate univariate analyses of variance (ANOVAs) testing for group differences, we therefore logarithmically transformed WST-1 reduction scores and homogeneity of variance was verified. For reasons of clarity, we show original WST-1 reduction scores in all figures. Body mass index (BMI) was calculated as the ratio of weight in kilograms to height in square meters. WST-1 scores are presented with and without correction for macrophage numbers. "Corrected WST-1 reduction" refers to WST-1 reduction scores per 10,000 macrophages.

We used ANOVAs to test for differences in the characteristics of the two groups and to test whether individuals with hypertension exhibited higher WST-1 reduction scores or higher corrected WST-1 reduction scores as compared with normotensives. To investigate linear associations and to take into account that our age-matching procedure may compromise independence of the two study groups, we calculated complementary analyses using multivariate linear regression models (enter method) with the continuous measure study MAP instead of the dichotomous (age-matched) group variable. We tested whether WST-1 reduction scores or corrected WST-1 reduction scores (dependent variables) were associated with study MAP, CSSS, or BDI (independent variables). In all WST-1 reduction analyses of the study (i.e., ANOVAs and regression analyses), we adjusted for traditional cardiovascular risk factors (i.e., BMI, LDL/HDL ratio, creatinine), for psychological factors (i.e., BDI and CSSS), and for the full set of these potential confounders. In linear regression models with MAP, instead of the age-matched group variable, we in addition adjusted for age. Control variables were selected a priori on the basis of previous literature showing associations with immune activation or superoxide anion production of HMDM, respectively (34,40,41).

RESULTS

Group Characteristics

Hypertensive participants, as expected, presented with a higher systolic BP, a higher diastolic BP, and a higher MAP as compared with normotensive participants. In addition, individuals with hypertension had a higher BMI and LDL/HDL ratio than normotensives. The two groups did not significantly differ in terms of age, BDI, CSSS, and HbA1c (Table 2). Hypertensive participants had serum levels of creatinine, calcium, sodium, and potassium in the normal reference range, thus supporting a diagnosis of essential hypertension. WST-1 reduction scores related to all BP measures but to none of the other group characteristic parameters. The unadjusted correlations for the full group are provided in Supplementary Table S1, Supplemental Digital Content 2, http://links.lww.com/PSYMED/A278.

Group Differences in WST-1 Reduction Scores

We observed that individuals with hypertension had significantly higher WST-1 reduction scores compared with normotensives, either without ($F(1,58) = 11.56$, $p = .001$, $\text{Eta}^2 = 0.17$, $f = 0.45$, Fig. 2) or with correction for macrophage numbers ($F(1,58) = 6.98$, $p = .011$, $\text{Eta}^2 = 0.11$, $f = 0.35$). Controlling for BMI, LDL/HDL ratio, and creatinine (WST-1 reduction score: $F(1,53) = 13.40$, $p = .001$, $\text{Eta}^2 = 0.13$, $f = 0.38$; corrected WST-1 reduction score: $F(1,53) = 8.34$, $p = .006$, $\text{Eta}^2 = 0.10$, $f = 0.34$) or CSSS and BDI (WST-1 reduction score: $F(1,56) = 13.14$, $p = .001$, $\text{Eta}^2 = 0.19$, $f = 0.48$; corrected WST-1 reduction

TABLE 2. Group Characteristics of Hypertensive and Normotensive Participants

	Individuals With Hypertension (n = 30)	Normotensives (n = 30)	p
Age, y	48.7 (13.0, 21 74)	48.6 (13.8, 22 74)	.98
BMI, kg/m^2	28.4 (3.5, 21.6 34.6)	24.9 (2.9, 19.8 30.9)	<.001
Blood pressure, mm Hg			
Home SBP	143.6 (7.8, 115.7 158.5)	120.3 (6.6, 105.2 129.3)	<.001
Home DBP	86.7 (10.0, 66.0 117.5)	70.8 (5.5, 60.0 80.8)	<.001
Study SBP	149.2 (9.5, 129.3 173.3)	122.4 (6.3, 108.7 137.3)	<.001
Study DBP	93.2 (9.5, 73.7 113.7)	76.8 (6.4, 58.3 85.0)	<.001
MAP	111.9 (8.9, 95.8 133.4)	92.0 (6.0, 75.3 99.5)	<.001
LDL/HDL ratio, µmol/l	2.8 (0.8, 1.4 4.3)	2.2 (0.7, 1.1 4.4), n = 28	.007
Creatinine, µmol/l	79.9 (9.5, 66 100)		
Sodium, mmol/l	140.4 (1.8, 137 144), n = 27		
Calcium, mmol/l	2.4 (0.1, 2.1 2.6), n = 27		
Potassium, mmol/l	4.2 (0.2, 3.9 4.7), n = 27		
HbA1c, mmol/mol	36.6 (3.2, 29 43)	36.1 (2.9, 30 41), n = 28	.60
Chronic stress (CSSS)	11.9 (8.2, 1 dhyph; 35), n = 27	13.0 (7.7, 0 26), n = 29	.62
Depressive symptom severity (BDI)	3.52 (4.7, 0 18), n = 29	4.59 (4.9, 0 19), n = 29	.41

BMI body mass index; SBP systolic blood pressure; DBP diastolic blood pressure; MAP mean arterial blood pressure, LDL/HDL low density lipoprotein/high density lipoprotein; CSSS Chronic Stress Screening Scale; BDI Beck Depression Inventory; n number of participants in case of missing data.

Values are expressed as mean (SD, range).

score: $F(1,56) = 8.31$, $p = .006$, Eta$^2 = 0.13$, $f = 0.38$) did not change results. The effect persisted when controlling for the full set of confounding variables (WST-1 reduction score: $p < .001$; corrected WST-1 reduction score: $p = .003$).

Association Between WST-1 Reduction Scores and Mean Arterial Blood Pressure

Complementary analyses using study MAP as a continuous measure revealed that higher MAP correlated significantly with higher WST-1 reduction scores, either without ($\beta = .38$, $p = .003$, $\Delta R^2 = .145$, Fig. 3) or with macrophage number correction ($\beta = .35$, $p = .006$, $\Delta R^2 = .124$). Results remained significant after controlling for age, BMI, LDL/HDL ratio, and creatinine (WST-1 reduction score: $\beta = .43$, $p = .007$, $\Delta R^2 = .124$; corrected WST-1 reduction score: $\beta = .43$, $p = .004$, $\Delta R^2 = .131$) or BDI and CSSS (WST-1 reduction score: $\beta = .40$, $p = .002$, $\Delta R^2 = .155$; corrected WST-1 reduction score: $\beta = .37$, $p = .004$, $\Delta R^2 = .133$). Controlling for age, BMI, LDL/HDL ratio, and creatinine in addition to BDI (WST-1 reduction score: $p = .006$; corrected WST-1 reduction score: $p = .004$) or CSSS (WST-1 reduction score: $p = .006$; corrected WST-1 reduction score: $p = .005$) did not significantly change results.

Associations Between WST-1 Reduction Scores, Chronic Stress, and Depressive Symptom Severity

We further correlated WST-1 reduction scores with CSSS and BDI for the full group. CSSS (WST-1 reduction score: $\beta = .22$, $p = .098$, $\Delta R^2 = .047$; corrected WST-1 reduction score: $\beta = .23$, $p = .074$, $\Delta R^2 = .054$) but not BDI (WST-1 reduction score: $\beta = .16$, $p = .236$, $\Delta R^2 = .024$; corrected WST-1 reduction score: $\beta = .19$, $p = .148$, $\Delta R^2 = .036$) was marginally positively associated with WST-1 reduction scores. After controlling for age, BMI, MAP, LDL/HDL ratio, and creatinine both higher CSSS and higher BDI related to (marginally) higher corrected WST-1 reduction scores (CSSS: WST-1 reduction score: $\beta = .24$, $p = .067$, $\Delta R^2 = .053$; corrected WST-1 reduction score: $\beta = .28$, $p = .031$, $\Delta R^2 = .074$; BDI: WST-1 reduction

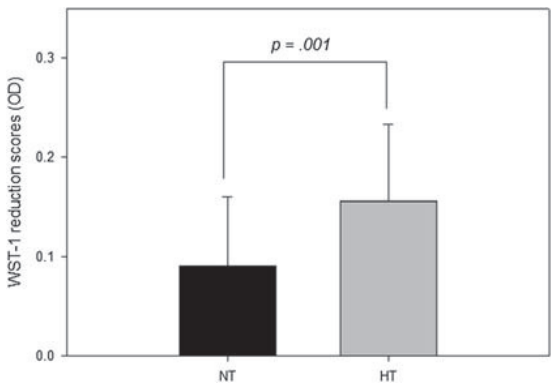

FIGURE 2. WST 1 reduction scores in hypertensive and normotensive men (M[SD]). Hypertensive men had significantly higher WST 1 reduction scores than normotensive men (p .001). OD optical densities; NT normotensive; HT hypertensive.

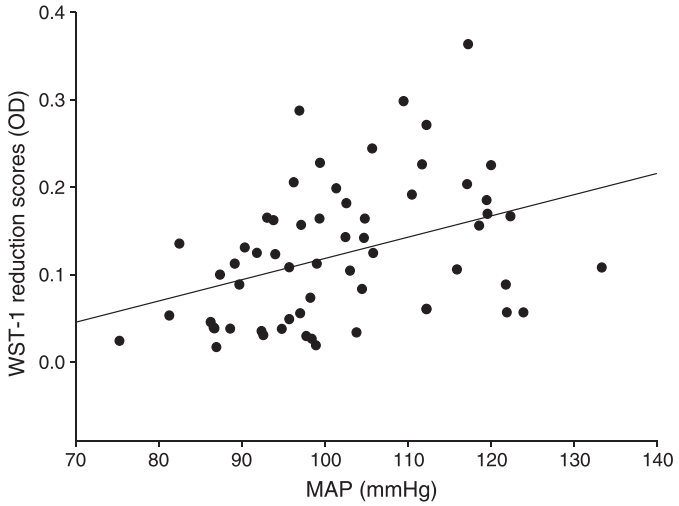

FIGURE 3. WST 1 reduction scores in relation to mean arterial blood pressure. The scatter plot shows a linear positive relationship between mean arterial blood pressure and WST 1 reduction scores (β .38, p .003). OD optical densities; MAP mean arterial blood pressure.

score: $\beta = .15$, $p = .238$; corrected WST-1 reduction score: $\beta = .23$, $p = .078$, $\Delta R^2 = .051$).

DISCUSSION

In this study, we investigated for the first time whether hypertensive men differ from age-matched normotensive men in terms of superoxide anion production by ex vivo isolated monocyte-derived macrophages. In complementary analyses, we in addition examined the association between MAP as a continuous measure and superoxide anion production.

We found that our hypertensive participants showed higher WST-1 reduction scores of PMA-activated ex vivo isolated monocyte-derived macrophages than normotensive controls. In addition, higher MAP was independently associated with higher superoxide anion production. Moreover, chronic stress but not depressive symptom severity was marginally associated with higher macrophage superoxide anion production. Given the importance of superoxide anions for microbicidal activity (16,20,21), our results suggest that macrophages of individuals with hypertension are characterized by an increased preparedness to kill microbes in reaction to stimulating agents. Importantly, we found this association to be linear, that is, the preparedness to kill microbes rose with increasing MAP.

Our observation is in line with previous studies that also found increased NADPH oxidase–derived ROS production by circulating macrophage precursor cells in human hypertension (28,29). Our study extends the previous findings by pointing to tissue-based monocyte-derived inflammatory macrophages and thus cells known to be present in atherosclerotic lesions as the source of higher superoxide anion production in human hypertension. In individuals with hypertension, higher ROS formation by circulating PBMCs including monocytes has been found to correlate with increasing intima-media thickness as a proxy measure of atherosclerosis severity (29). If this correlation also applies to monocyte-derived tissue-based inflammatory macrophages as key cells in atherosclerosis development and progression, our findings may suggest a mechanism underlying the cardiovascular risk with hypertension.

At present, we can only speculate about mechanisms underlying the observed higher phagocytic NADPH oxidase–derived superoxide anion production in hypertension. Several prospective studies in humans reported that inflammatory processes can be predictive for the development of hypertension (42–44). Experimental animal studies confirmed this causal relationship (7,45) and suggested that inflammatory mechanisms may prime macrophages to increased microbicidal preparedness (46,47). Moreover, it is conceivable that increased levels of oxidized LDL in individuals with hypertension (48) may trigger macrophages to produce greater amounts of superoxide anions to ingest oxidized LDL. This may promote vascular wall injuries with subsequent chronic inflammation that ultimately result in atherosclerotic events.

Therapeutic implications of our findings may relate to modulation of NADPH oxidase–derived superoxide anion production in the treatment of hypertension and prevention of atherosclerotic risk. A potential treatment option may include antioxidants, for example, vitamin C, that inhibit NADPH oxidase–derived generation of ROS (49–51). Furthermore, antihypertensive agents such as AT-II receptor antagonists are known to inhibit NADPH oxidase activity and thus decreasing the generation of ROS production (51).

Notably, our study was cross-sectional and hence we cannot draw a conclusion on the direction of the hypertension–microbicidal activity–atherosclerosis link. Prospective studies or randomized controlled trials are needed to address this important issue. The present study focused on apparently healthy, nonsmoking, and medication-free men and may therefore not be generalized to women or patients with other forms of cardiovascular disease. Also, although unlikely, we cannot completely rule out that some of the hypertensive participants may suffer from secondary hypertension. Moreover, we did not examine the association of superoxide anion production with macrophage microbicidal activity, for example, in atherosclerotic lesions. Nevertheless, this is the first study that investigated superoxide anion production by ex vivo isolated human monocyte-derived macrophages in human hypertension using the reliable, and valid WST-1 assay. Our observation of increased superoxide anion production may provide new insights into mechanisms that underlie the increased risk of atherosclerosis and CAD in hypertension. Furthermore, the inclusion of apparently healthy, nonsmoking, and medication-free hypertensive men and age-matched normotensives reduced potential confounding factors, while controlling for selected other potential confounders. Nevertheless, we cannot rule out that potential confounders that we did not assess may have an impact on macrophage superoxide anion production and thus on the results of this study. Of note, despite missing data, we consider the collected BP measurements to be reliable because missing data points resulted from problems other than participants' instruction adherence.

In sum, our data show that macrophage superoxide anion production is higher in hypertensive men compared with normotensives suggesting a mechanistic role in mediation of cardiovascular risk in hypertension with potential implications for intervention strategies. Future studies are needed to confirm our findings and to further investigate the precise mechanisms underlying atherosclerotic risk in human hypertension.

This research was previously presented in a poster at the American Psychosomatic Society meeting March 18–21, 2015, in Savannah, Georgia.

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