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CORRESPONDENCE

Inflammatory and oxidative responses to disturbed blood flow in hypertensive men

Amanda S. Storch[1] · Helena N. M. Rocha[1] · Vinicius P. Garcia[1] · Gabriel M. S. Batista[1] · João Dario Mattos[1] · Monique O. Campos[1] · Antonio Claudio L. Nóbrega[1,2] · Igor A. Fernandes[1] · Natalia G. Rocha[1,2]

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Atherosclerosis is a chronic inflammatory arterial disease that underlies many of the common causes of cardiovascular death/disability worldwide [1]. Atherosclerosis develops preferentially in irregular arterial regions that are exposed to disturbed blood flow (DBF), e.g., curvatures and bifurcations [2]. The close relationship between DBF and atherogenesis relies on endothelial dysfunction, which is associated with a proinflammatory phenotype with a higher level of reactive oxygen species production, elevated cell turnover, greater cell–cell permeability, and upregulated expression of adhesion molecules and chemokines [3]. These mechanisms are also common pathways in the pathophysiology of hypertension [4]. However, it is unclear whether similar DBF-induced inflammatory and oxidative responses occur in healthy and hypertensive subjects.

To clarify this issue, nine healthy men (CT group) and seven hypertensive men (HT group) underwent 75 mmHg-dominant forearm cuff occlusion for 30 min to disturb brachial artery blood flow and evoke impaired endothelial function [4, 5]. Blood flow was measured by Doppler ultrasound, while plasma samples were obtained at rest and during the last 30 s of cuff occlusion [4, 5]. The study protocol was approved by the local ethics committee (CAAE: 36681814.3.0000.5243), and the study was conducted in accordance with the principles of the latest revision of the Declaration of Helsinki (except for registration in a database).

The inclusion criteria were a sedentary lifestyle (<150 min/week of moderate intensity cardiorespiratory exercise), an absence of metabolic disorders/diagnosed diseases, and smoking habits. The HT group comprised subjects with grade I hypertension (systolic blood pressure between 140 and 159 mmHg and/or diastolic blood pressure between 90 and 99 mmHg) without regular pharmacological treatment [6].

Superoxide dismutase (SOD; Superoxide Dismutase Assay Kit, Cayman Chemical, Ann Arbor, MI, USA), catalase (CAT; Catalase Assay Kit; Cayman Chemical), adhesion molecules [intercellular (ICAM-1), vascular (VCAM-1) and P-selectin; Human Cardiovascular Disease Magnetic Bead Panel 2, Millipore, Sigma, Burlington, MA, USA] and inflammatory cytokines [interleukin (IL)-1b, IL-6, IL-8, IL-10, monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-α (TNF-α); Human Cytokine/Chemokine Magnetic Bead Panel, Millipore] were measured by commercial assays according to the manufacturer's instructions. The oxidative stress levels were determined by quantifying the levels of thiobarbituric acid reactive species (TBARS) [7].

Independent Student's *t*-test was used to compare clinical measurements between the CT and HT groups. Two-way analysis of variances (ANOVAs) were used to compare the groups in relation to the hemodynamic variables and plasma biomarkers before and during cuff occlusion. The ANOVAs were followed by Fisher's post hoc tests when appropriate. Data are presented as the means and standard deviations (SDs). Detected outliers (mean ± 2xSD) were removed from the analyses. A value of $P \leq 0.05$ was considered significant.

As expected, participants' anthropometric, clinical, and biochemical data were similar between groups, except for systolic and diastolic blood pressures, which were higher in the HT group ($P < 0.01$). Compared with the CT group, shear rate (SR) ($P < 0.01$), blood velocity ($P < 0.01$), blood

✉ Natalia G. Rocha
nataliagalito@id.uff.br

[1] Laboratory of Exercise Sciences (LACE), Department of Physiology and Pharmacology, Fluminense Federal University, Niteroi, Rio de Janeiro, Brazil

[2] National Institute of Science and Technology (INCT)— Physical (In)activity and Exercise, National Council for Scientific and Technological Development (CNPq), Fluminense Federal University, Niteroi, Rio de Janeiro, Brazil

Table 1 Diameter, blood velocity, blood flow, and shear patterns at baseline and during cuff occlusion in the cuffed and non-cuffed arms of healthy (CT, $n = 9$) and hypertensive subjects (HT, $n = 7$)

Variable	CT		HT	
	Baseline	Cuff occlusion	Baseline	Cuff occlusion
Cuffed arm				
Diameter (cm)	0.403 ± 0.054	0.391 ± 0.057*	0.402 ± 0.032	0.400 ± 0.033
Blood velocity (cm s^{-1})	3.56 ± 1.19	1.69 ± 1.36*	6.37 ± 1.83†	2.13 ± 1.57*
Blood flow (mL min^{-1})	26.95 ± 10.25	11.28 ± 8.42*	46.68 ± 10.88†	13.10 ± 5.41*
Antegrade SR (s^{-1})	50.63 ± 25.21	70.52 ± 30.27	72.60 ± 13.71	78.09 ± 18.14
Retrograde SR (s^{-1})	17.25 ± 17.66	61.74 ± 43.37*	9.30 ± 4.56	57.32 ± 14.56*
SR (s^{-1})	35.15 ± 11.73	17.14 ± 14.82*	65.10 ± 17.59†	20.04 ± 10.14*
OSI	0.20 ± 0.12	0.39 ± 0.18*	0.12 ± 0.05	0.43 ± 0.06*
Non-cuffed arm				
Diameter (cm)	0.393 ± 0.046	0.390 ± 0.046	0.429 ± 0.056	0.428 ± 0.050
Blood velocity (cm s^{-1})	4.56 ± 2.38	4.94 ± 1.99	6.30 ± 4.33	7.17 ± 4.75
Blood flow (mL min^{-1})	32.30 ± 15.34	33.21 ± 11.40	43.34 ± 32.61	50.63 ± 31.70
Antegrade SR (s^{-1})	64.76 ± 23.48	65.02 ± 23.68	65.49 ± 41.63	73.93 ± 45.82
Retrograde SR (s^{-1})	18.93 ± 13.60	16.05 ± 10.13	16.53 ± 11.36	16.05 ± 11.17
SR (s^{-1})	47.48 ± 26.73	49.62 ± 21.20	51.43 ± 46.88	61.80 ± 52.22
OSI	0.22 ± 0.11	0.19 ± 0.09	0.23 ± 0.13	0.20 ± 0.14

Mean ± SD

SR shear rate, *OSI* oscillatory shear index, *CT* healthy subjects, *HT* hypertensive subjects

*$P <$ 0.05 vs. baseline and $^{\dagger}P <$ 0.05 vs. CT group

flow ($P < 0.01$), IL-10 (CT: 1.30 ± 0.99 vs. HT: 3.07 ± 1.48 ng mL^{-1}, $P = 0.02$), ICAM-1 (CT: 185 ± 30 vs. HT: 233 ± 21 ng mL^{-1}, $P < 0.01$), VCAM-1 (CT: 636 ± 174 vs. HT: 886 ± 261 ng mL^{-1}, $P = 0.04$), and P-selectin (CT: 124 ± 43 vs. HT: 214 ± 101 ng mL^{-1}, $P = 0.04$) were higher at baseline in the HT group.

During cuff occlusion, retrograde SR ($P < 0.01$) and oscillatory shear index increased ($P < 0.01$), whereas mean SR ($P < 0.01$), blood velocity ($P < 0.01$), and blood flow ($P < 0.01$) decreased similarly in the cuffed arms in both groups (Table 1). The brachial artery diameter of the CT group declined compared to the baseline diameter ($P < 0.01$) (Table 1). The levels of IL-8 (CT, baseline: 2.02 ± 0.79 vs. occlusion: 3.06 ± 1.09 ng mL^{-1}, $P = 0.03$), IL-10 (CT, baseline: 1.30 ± 0.99 vs. occlusion: 3.06 ± 1.54 ng mL^{-1}, $P < 0.01$), MCP-1 (CT, baseline: 104.96 ± 23.15 vs. occlusion: 148.81 ± 30.02 ng mL^{-1}, $P = 0.03$), SOD (CT, baseline: 3.36 ± 0.69 vs. occlusion: 3.99 ± 0.95 IU mL^{-1}, $P < 0.01$), and CAT (CT, baseline: 74.80 ± 13.87 vs. occlusion: 89.88 ± 14.38 mmol min^{-1} mL^{-1}, $P = 0.02$) increased in the CT group, whereas the levels of MCP-1 (HT, baseline: 101.71 ± 26.54 vs. occlusion: 140.02 ± 59.09 ng mL^{-1}, $P = 0.05$), and TBARS (HT, baseline: 4.40 ± 0.79 vs. occlusion: 5.10 ± 1.31 mmol mL^{-1}, $P = 0.03$) increased in the HT group during cuff occlusion (Fig. 1). No differences were observed regarding inflammatory/oxidative stress markers between the groups/measurement times in the non-cuffed arm.

Briefly, the study's main findings were threefold: (1) DBF increased the proinflammatory chemokine levels in both groups; (2) DBF increased lipid peroxidation in hypertensive subjects; and (3) DBF increased anti-inflammatory cytokine and antioxidant enzyme levels in healthy subjects.

The increased MCP-1 concentration in both groups during cuff occlusion suggests that DBF stimulates chemoattractant cytokines for monocytes and neutrophils at injury sites, while the increased IL-8 level might be related to the formation of anti-inflammatory monocyte-platelet complexes in the DBF environment, which is preserved only in healthy subjects [5, 8, 9]. Additionally, cuff occlusion increased the TBARS concentration in hypertensive subjects, whereas it increased the levels of SOD and CAT in healthy subjects. It is believed that the combined effects of hypertension and DBF on oxidant production seem to overwhelm the available antioxidant system [10].

The results from this study should be interpreted considering the small sample size. However, the number of subjects recruited was appropriate with respect to the calculation of the minimum sample size required for an analysis that considered the TBARS concentration as the main outcome, that is, a statistical power of 0.8 and an alpha error of 0.05.

In conclusion, these preliminary results show that acute exposure to DBF seems to induce inflammatory and

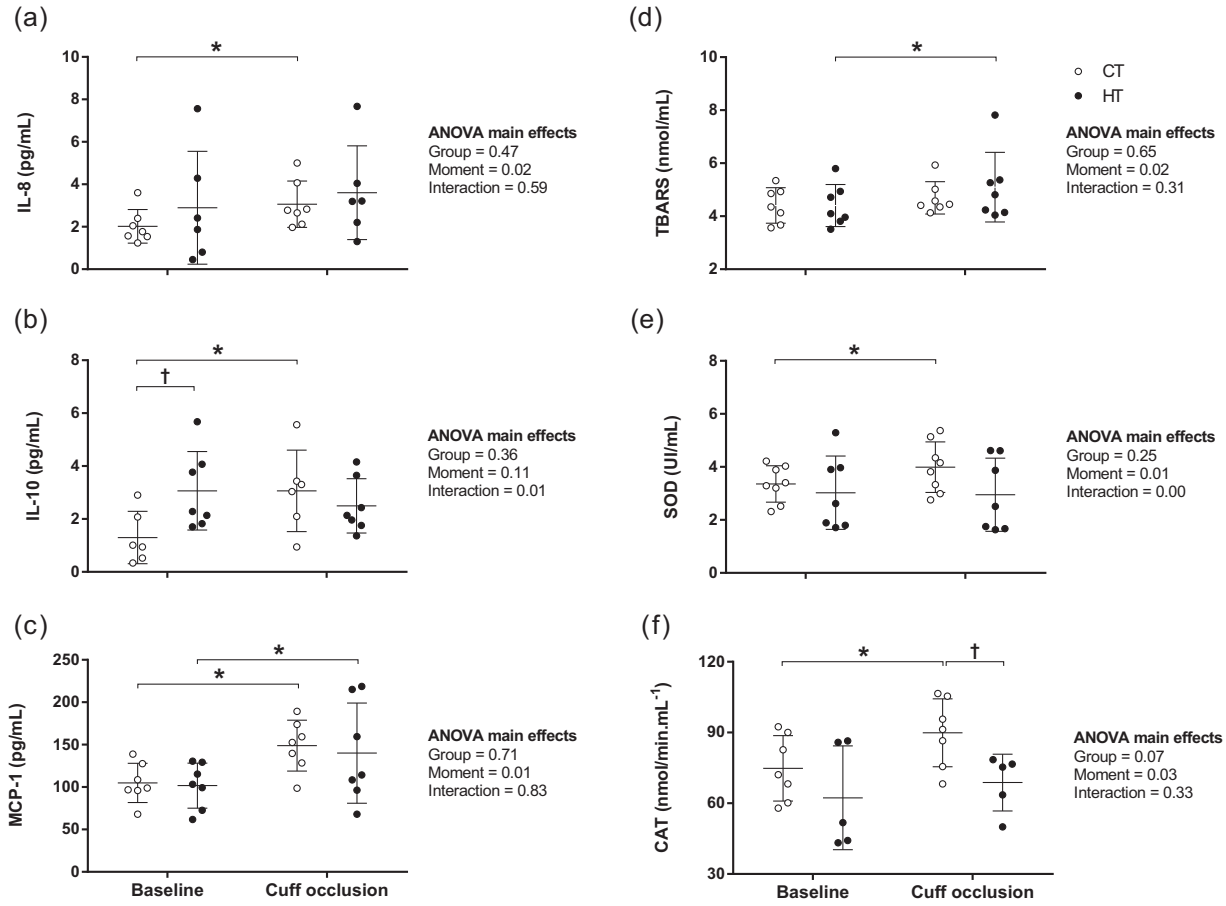

Fig. 1 Cytokine levels (**a**, **b**, **c**) and oxidative stress markers (**d**, **e**, **f**) at baseline and during cuff occlusion in the healthy (CT, $n = 9$) and hypertensive subjects (HT, $n = 7$). Data are expressed as individual values; means and standard deviations are also plotted. TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; CAT, catalase; IL, interleukin; MCP-1, monocyte chemoattractant protein-1. $^{*}P < 0.05$ vs. baseline and $^{\dagger}P < 0.05$ vs. CT group

oxidative responses in the vasculature of hypertensive subjects without stimulating anti-inflammatory or anti-oxidant regulatory mechanisms, which seem to be preserved in healthy men.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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