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Early Increase in Autoantibodies Against Human Oxidized Low-Density Lipoprotein in Hypertensive Patients After Blood Pressure Control

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BACKGROUND

Oxidized lipoproteins and antioxidized low-density lipoprotein (anti-oxLDL) antibodies (Abs) have been detected in plasma in response to blood pressure (BP) elevation, suggesting the participation of the adaptive immune system. Therefore, treatment of hypertension may act on the immune response by decreasing oxidation stimuli. However, this issue has not been addressed. Thus, we have here analyzed anti-oxLDL Abs in untreated (naive) hypertensive patients shortly after initiation of antihypertensive therapeutic regimens.

METHODS

Titers of anti-oxLDL Abs were measured in subjects with recently diagnosed hypertension on stage 1 ($n = 94$), in primary prevention of coronary disease, with no other risk factors, and naive of antihypertensive medication at entry. Subjects were randomly assigned to receive perindopril, hydrochlorothiazide (HCTZ), or indapamide (INDA) for 12 weeks, with additional perindopril if necessary to achieve BP control. Abs against copper-oxidized LDL were measured by enzyme-linked immunosorbent assay.

RESULTS

Twelve-week antihypertensive treatment reduced both office-based and 24-h ambulatory BP measurements ($P < 0.0005$). The decrease in BP was accompanied by reduction in thiobarbituric acid-reactive substances (TBARS) ($P < 0.05$), increase in anti-oxLDL Ab titers ($P < 0.005$), and improvement in flow-mediated dilation (FMD) ($P < 0.0005$), independently of treatment. Although BP was reduced, we observed favorable changes in anti-oxLDL titers and FMD.

CONCLUSIONS

We observed that anti-oxLDL Ab titers increase after antihypertensive therapy in primary prevention when achieving BP targets. Our results are in agreement with the concept that propensity to oxidation is increased by essential hypertension and anti-oxLDL Abs may be protective and potential biomarkers for the follow-up of hypertension treatment.

Keywords: *angiotensin-converting enzyme inhibitor; antibodies; blood pressure; diuretic; hypertension; oxidized LDL*

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Hypertension affects ~50 million people in the United States, and up to 60% of the adult population is either prehypertensive or hypertensive.[1,2] Hypertension is considered an inflammatory disease and several studies suggested that the adaptive immune system contributes to blood pressure (BP) elevation.[3–5] Data from the Third National Health and Nutrition Examination Survey showed that C-reactive protein (CRP) and systolic BP (SBP) were positively associated since the prehypertensive stage.[6] In fact, low-grade inflammation in vascular tissue contributes to the pathophysiology of hypertension. Guzik *et al.*[4] reported that T cells modulate BP elevation caused by angiotensin II and in response to sodium and volume challenges, by activating perivascular fat, which releases cytokines that promote vasoconstriction.[5]

Hypertension is a known risk factor for atherosclerosis, and acts promoting, facilitating, or permitting the oxidation of low-density lipoprotein (LDL).[7] LDL from hypertensive patients is more susceptible to oxidation *in vitro*, is more promptly oxidized *in vivo*,[8] and antibodies (Abs) against modified LDL (antioxidized LDL (anti-oxLDL) Abs) could be a suitable index of *in vivo* LDL oxidation.[9] This increased susceptibility could be secondary to angiotensin II stimulation.[10] LDL particle can be modified by oxidation, aggregation, or glycosylation, followed by additional changes by enzyme activity leading to a complete disintegration of the particle, where residues can be identified in plasma.[11,12] Oxidized LDL expresses antigenic epitopes that elicit an immune response.[9] Oxidized lipoproteins and anti-oxLDL Abs have been detected in human plasma and atheromas of patients with coronary

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atherosclerosis,[13–15] in hypertensive individuals[9] and in apparently healthy controls.[16] It is not yet known whether these Abs are only an indication of the oxidative status of LDL or whether they contribute to atherogenesis. Thus, there is controversy regarding the protective or pathogenic role of these circulating autoantibodies.[17] Ketelhuth *et al.* investigated the Ab repertoire to oxLDL subfractions and observed higher titers in stable than in unstable patients.[16] We have previously shown that anti-oxLDL Ab titers were higher in untreated patients with hypertension, as compared to those after a recent acute coronary syndrome, and hypothesized that the mechanism could be related to consumption or diminished Ab production due to the acute inflammatory state.[18]

Here we evaluate the effects of BP lowering with different antihypertensive agents on anti-oxLDL Abs. Although there are several reports on the level of Abs related to hypertension, the effect of antihypertensive treatments on the immune response is not completely explored.[19–24] Therefore, based on the concept that there is a relationship between inflammation and hypertension and that immune mechanisms can be protective,[17,25,26] the immune response may be useful in preventing or treating this disease. In addition, it will further strengthen the causal link between immune mechanisms and coronary disease. We hypothesized that BP control dramatically changes the levels of anti-oxLDL Abs suggesting that it could be used both as a marker for follow-up and as a potential secondary treatment.

METHODS

Patients. Ninety-four middle-aged individuals of both genders, with recently diagnosed arterial hypertension on stage 1 (ref. 2) were included in the protocol. Hypertension was defined as the average of three measurements of sitting BP ≥140 mm Hg for SBP and/or ≥90 mm Hg for diastolic BP (DBP), obtained after a 5-min resting period and repeated at 5-min intervals. White coat hypertension was ruled out by 24-h ambulatory BP monitoring (ABPM) showing at least one measure (mean of 24-h SBP or DBP) above the normal range.[27]

Study design. This is a prospective intervention trial of hypertensive subjects, naive of antihypertensive treatment at entry, randomly assigned to receive perindopril 4 mg angiotensin-converting enzyme inhibitor (ACEI group), hydrochlorothiazide 25 mg (HCTZ group), or indapamide 1.5 mg (INDA group) for 12 weeks in a blinded-fashion. If BP goal at 6 weeks was not achieved, the patients of each therapeutic arm received addition of perindopril (4 mg) for the following 6 weeks. Drugs were coded and encapsulated before randomization and investigators were unaware of patients' treatment arm.

Presence of other risk factors, such as smoking or diabetes or, some treatment for them, hormone replacement therapy, renal or hepatic dysfunction, any active inflammatory or infectious disease was exclusion criteria.

This project was conducted at the Federal University of Sao Paulo, was approved by the local ethics committee and informed consent was obtained from all patients prior to protocol initiation.

Clinical parameters. Demographic and anthropometric data were collected at baseline and end of study. A 12-lead electrocardiogram was performed at entry. An intermediate visit at 6 weeks was performed to assess BP control and to add perindopril (4 mg) to those patients who were not at goal. Twenty-four-hour ABPM (Spacelabs 90207, Seattle, WA) was recorded at baseline and after 12 weeks.

Blood sample collection. Twelve-hour fasting samples were obtained for all patients at baseline and 12 weeks after treatment.

Biochemistry, serum lipids, and apolipoproteins. Electrolytes and creatinine were measured automatically, serum total cholesterol, high-density lipoprotein cholesterol and triglycerides were determined enzymatically (Opera Bayer, Leverkusen, Germany) with LDL-cholesterol estimated by the Friedewald equation when triglycerides were <400 mg/dl. Glucose was assessed by enzymatic method and glycated hemoglobin A_{1c} was measured using high-perfomance liquid chromatography. Concentrations of apolipoproteins were determined by nephelometry (Array 360 Beckmann, Fullerton, CA).

Markers of inflammation. High-sensitivity CRP was measured by nephelometry (R100 Analyser, Behringer, Mannheim, Germany), and plasma peroxidation was evaluated by the thiobarbituric acid-reactive substances (TBARS) assay, which measures malondialdehyde.[28]

Preparation of LDL and oxLDL. Blood was collected from one fasting normolipidemic blood-donor volunteer, and EDTA-plasma was obtained after centrifugation (1,000*g*, 4 °C, for 15 min). Benzamidine (2 mmol/l), gentamicin (0.5%), chloramphenicol (0.25%), phenyl-methyl-sulfonylfluoride (0.5 mmol/l), and aprotinin (0.1 unit/ml) were added to the plasma. LDL fraction (1.006 < d < 1.063 g/ml) was isolated by sequential ultracentrifugation (100,000*g*, 4 °C), using a 50Ti rotor (L-8 ultracentrifuge; Beckman Instruments, Palo Alto, CA), and thereafter dialyzed (4 °C) against phosphate-buffered saline (PBS; pH 7.4), containing EDTA (0.01%). LDL preparation was sterilized by filtration through a 0.22-mm filter (Milipore, Schwalbach, Germany).

To obtain oxLDL, LDL was dialyzed overnight against EDTA-free PBS, followed by incubation with $CuSO_4$ (2.5 mol/l per mg of LDL protein; 18 h; 37 °C). The oxidation process was stopped by the addition of 1 mmol/l EDTA.[24] This procedure is standardized in our laboratory and results in a completely oxidized LDL, as defined by both a plateau phase in the TBARS assay and an increase in net negative charge.[28]

Determination of anti-oxLDL Abs. To determine the Abs to copper-oxidized LDL, we used our own established assay as previously described.[16,25] Ninety-six-well microtiter plates were coated with oxLDL (7.5 µg/ml; 50 µl per well) suspended in carbonate/bicarbonate buffer (0.1 mol/l; pH 9.6) and left for sensitization (4 °C; overnight). After washing with PBS, the plate was blocked

with gelatin (3%; room temperature; 24 h). Patients' serum samples (50 µl) were diluted (1:400) before they were added to the wells. After incubation (2 h), the plate was washed with PBS containing Tween (0.05%) and horseradish peroxidase-conjugated goat antihuman immunoglobulin G (IgG) solution (diluted 1:1,000 in PBS; Kirkegaard & Perry Laboratories, Gaithersburg, MD) was added. After washing, 3,3′,5,5′-tetramethylbenzidine (6.5% in dimethyl sulfoxide; Sigma, St Louis, MO), and H_2O_2 (Sigma) diluted in citrate/phosphate buffer (0.1 mol/l; 250 µl; pH 5.5) were added (room temperature) as enzyme substrate. The reaction was stopped by addition of H_2SO_4 (2 mol/l) and measured in terms of optical density (λ = 450 nm).

Because some imprecision in quantifying anti-oxLDL Abs by the enzyme-linked immunosorbent assay method may occur, IgG (10 mg/ml; purified human IgG, Pierce Protein Research Products; Thermo Scientific, Rockford, IL) and a buffer blank (PBS) were used as controls to compensate intraplate variation. Interplate imprecision in the enzyme-linked immunosorbent assay was minimized by processing all the samples in the same period, at the end of the clinical protocol. To minimize false positive results due to cross-reactivity with antigen naive epitopes, Ab titers were expressed as the index of reactivity, calculated as index of reactivity = $(OD_{sample} - OD_{sample\ blank})/(OD_{IgG} - OD_{IgG\ blank})$, where IgG was used as a control. Samples were run in triplicate and the variation within the triplicates did not exceed 5% of the mean.

Endothelium-dependent and -independent vasorelaxation. Vasoreactivity tests were performed in the morning after an overnight fast, by an experienced ultrasonographer in accordance with the Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated dilation (FMD) of the brachial artery.[29] An ultrasound system (Sonos 5500; Hewlett-Packard-Phillips, Palo Alto, CA), equipped with vascular software for two-dimensional imaging, color and spectral Doppler ultrasound modes, internal electrocardiogram monitor, and linear-array transducer (with a frequency range from 7.5 to 12.0 MHz), was used. Image acquisition, endothelial-dependent FMD, and endothelium-independent dilation with isosorbide dinitrate (5 mg; sublingual) were assessed.[29] The percent change in vessel diameter from the baseline value was calculated to determine FMD or endothelium-independent dilation. The intra- and intersonographer variability values were <1 and 2%, respectively.

Statistical analyses. All analyses were performed using the Statistical Package for the Social Sciences software (17.0) for

Table 1 | Clinical characteristics of the study population at baseline and 12 weeks after antihypertensive treatment

	ACEI (n = 29)		HCTZ (n = 33)		INDA (n = 32)			
Variables	Baseline	12 weeks	Baseline	12 weeks	Baseline	12 weeks	*P* (bg)	*P* (wg)
Age (years)[a] IQR	57.0 (49.0–62.5)	—	56.0 (48.0–64.0)	—	59.5 (50.0–66.7)	—	0.370	—
Male gender[b] (%)	15 (51.7)	—	17 (51.5)	—	15 (46.9)	—	0.909	—
Weight (kg)[a] IQR	76.0 (59.7–87.5)	—	71.7 (62.5–80.7)	—	72.3 (61.2–83.5)	—	0.751	—
BMI (kg/m^2)[a] IQR	27.0 (24.9–32.1)	—	27.0 (25.6–31.6)	—	28.3 (24.2–32.2)	—	0.837	—
Waist circumference (cm)[a] IQR	95.0 (86.5–106.5)	—	94.0 (85.0–100.5)	—	96.0 (88.0–106.0)	—	0.666	—
HR (bpm)[c] IQR	80.0 (72.0–87.0)	78.0 (72.0–78.7)	78 (68–81)	74.0 (69.0–82.0)	78 (74–86)	74.0 (70.0–82.0)	0.586	0.134
SBP (mm Hg)[c] IQR	150.0 (142.5–164.2)	135.0 (120.2–144.3)	150.0 (140.0–160.0)	130.7 (123.6–145.0)	153.8 (141.8–160.0)	140.0 (130.0–150.0)	0.324	<0.0005
DBP (mm Hg)[c] IQR	90.0 (90.0–98.5)	80.0 (80.0–90.0)	90.0 (89.3–92.5)	79.3 (80.0–90.0)	91.3 (89.5–98.6)	86.5 (80.0–90.5)	0.712	<0.0005
24-h SBP (mm Hg)[c,d] IQR	127.0 (118.0–142.0)	123.5 (115.0–134.3)	131.0 (124.0–142.5)	118.0 (116.0–130.0)	131.0 (119.3–143.0)	125.5 (112.0–129.7)	0.153	<0.0005
24-h DBP (mm Hg)[c,d] IQR	82.0 (77.5–89.5)	80.5 (71.7–84.7)	83.0 (75.5–90.0)	75.0 (70.0–82.0)	79.0 (70.0–86.3)	73.0 (69.0–79.7)	0.327	<0.0005
FMD (%)[c] IQR	7.3 (4.3–10.3)	9.3 (6.3–11.2)	8.3 (5.8–10.1)	9.0 (7.2–11.7)	6.7 (5.0–10.2)	9.2 (6.7–12.3)	0.718	<0.0005
EID (%)[c] IQR	11.1 (9.0–14.7)	13.4 (9.8–17.0)	12.6 (10.1–15.9)	12.4 (10.2–17.2)	13.0 (9.5–15.3)	13.4 (9.8–16.8)	0.989	0.089

$P < 0.05$. ACEI: perindopril group; HCTZ: hydrochlorothiazide group; INDA: indapamide group.
ABPM, ambulatory blood pressure monitoring; ACEI, angiotensin-converting enzyme inhibitor; bg, between groups; BMI, body mass index; bpm, beats per minute; DBP, diastolic blood pressure (mean values); EID, endothelial independent dilation; FMD, flow-mediated dilation; HCTZ, hydrochlorothiazide; HR, heart rate; INDA, indapamide; IQR, interquartile range (median values); SBP, systolic blood pressure (mean values); wg, within group.
[a]Analysis of variance. [b]χ^2-test. [c]General linear model, repeated measures. [d]Log-transformed variables.

Table 2 | Laboratory parameters of the study population at baseline and 12 weeks after antihypertensive treatment

	ACEI ($n = 29$)		HCTZ ($n = 33$)		INDA ($n = 32$)			
Variables	**Baseline**	**12 weeks**	**Baseline**	**12 weeks**	**Baseline**	**12 weeks**	***P* (bg)**	***P* (wg)**
Total cholesterol (mg/dl) IQR	205.0 (185.5–228.0)	215.0 (172.5–242.5)	198.0 (169.5–209.5)	202.0 (175.5–228.5)	185.0 (161.0–209.7)	184.0 (162.0–211.0)	<0.05	0.196
HDL-C (mg/dl) IQR	47.0 (41.0–56.5)	47.0 (40.0–56.5)	44.0 (38.0–58.5)	45.5 (39.5–59.7)	47.5 (39.3–60.0)	46.0 (41.0–62.0)	0.879	0.273
LDL-C (mg/dl) IQR	126.5 (111.0–148.7)	117.0 (95.3–146.5)	110.0 (84.0–136.5)	125.0 (96.0–150.0)	115.0 (93.5–134.5)	118.0 (91.0–129.5)	0.634	0.367
Triglycerides[a] (mg/dl) IQR	110.0 (84.5–189.0)	121.0 (78.0–181.5)	121 (76.0–188.5)	126.0 (98.5–169.5)	108.0 (73.3–142.5)	111.0 (75.0–156.0)	0.395	0.233
Apolipoprotein AI (mg/dl) IQR	128.0 (110.0–146.0)	131.5 (122.3–151.0)	127.0 (113.0–142.5)	127.0 (111.5–145.0)	120.5 (104.0–146.3)	128.5 (110.3–148.0)	0.363	0.110
Apolipoprotein B100 (mg/dl) IQR	101.0 (89.5–123.5)	113.0 (91.1–128.5)	92.8 (77.2–111.5)	107.0 (94.8–119.0)	88.7 (68.9–117.0)	96.5 (76.9–111.0)	0.184	<0.05
Sodium ion[a] (mEq/l)	140.0 (138.0–142.0)	140.0 (139.0–141.5)	142.0 (140.5–143.0)	140.0 (138.0–143.0)	141.0 (139.3–143.0)	140.0 (138.0–141.0)	0.236	0.052
Potassium ion (mEq/l)	4.20 (3.85–4.50)	4.0 (3.7–4.5)	4.10 (3.75–4.40)	3.8 (3.5–4.2)	4.00 (3.80–4.30)	3.9 (3.5–4.1)	0.107	<0.005
Calcium ion (mEq/l)	1.16 (1.11–1.22)	1.18 (1.13–1.21)	1.15 (1.07–1.18)	1.15 (1.11–1.21)	1.17 (1.08–1.22)	1.15 (1.09–1.20)	0.535	0.220
Creatinine (mg/dl)	1.0 (0.8–1.1)	0.90 (0.80–1.05)	0.9 (0.9–1.1)	0.90 (0.80–1.10)	0.9 (1.0–1.2)	1.00 (0.85–1.10)	0.420	<0.05
Glucose (mg/dl)	93.0 (81.0–104.0)	88.0 (83.0–101.0)	87.0 (84.0–96.5)	92.0 (84.3–104.0)	87.5 (76.0–95.0)	89.0 (78.0–98.0)	0.293	0.918
Glycated hemoglobin (%)	5.4 (5.0–5.7)	5.5 (5.1–5.9)	5.5 (5.1–5.9)	5.5 (4.9–6.1)	5.4 (5.1–5.7)	5.5 (5.3–5.9)	0.980	0.330
TBARS[a] (µmol/ml)	1.54 (0.87–2.07)	1.33 (0.90–1.83)	1.44 (0.91–1.96)	1.19 (0.75–1.51)	1.58 (0.92–2.18)	1.35 (0.87–1.68)	0.599	<0.05
hsCRP (mg/l)	0.41 (0.27–0.56)	0.39 (0.27–0.59)	0.46 (0.28–0.99)	0.41 (0.31–0.86)	0.48 (0.27–0.99)	0.38 (0.23–0.47)	0.119	0.888
Anti-oxLDL Abs[a] (IR)	1.69 (1.21–2.15)	1.83 (1.28–3.25)	1.75 (1.26–2.45)	2.04 (1.53–2.59)	1.54 (1.29–1.99)	1.77 (1.30–2.30)	0.371	<0.005

$P < 0.05$. ACEI: perindopril group; HCTZ: hydrochlorothiazide group; INDA: indapamide group. General linear model, repeated measures.
ACEI, angiotensin-converting enzyme inhibitor; Anti-oxLDL Abs, Anti-oxLDL autoantibodies; bg, between groups; HCTZ, hydrochlorothiazide; hsCRP, high-sensitivity C-reactive protein; INDA, indapamide; IQR: interquartile range (median values); TBARS: thiobarbituric acid-reactive substances; wg, within group.
[a]Log-transformed variables.

Windows. Numerical data were expressed as median values and interquartile range, or means and s.e.m. are presented. Categorical variables were expressed as number of subjects and percent values. Variables with non-Gaussian distribution were log-transformed for comparisons. In descriptive statistics, analysis of variance-Tukey test was used for independent samples, and Pearson's χ^2-test was used for categorical variables. A general linear model with repeated measures was used to compare the effects of treatments within and between groups. Pearson's or Spearman's correlation coefficients were tested. A P value of <0.05 was considered significant.

RESULTS

Baseline characteristics of the study population

Demographic characteristics, clinical parameters and laboratory results are presented in Tables 1 and 2. Subjects in the ACEI, HCTZ, or INDA groups were comparable regarding body weight, body mass index (BMI), waist circumference, heart rate (HR), office-based, and 24-h ABPM measurements of SBP and DBP.

Endothelium-dependent and independent dilation of the brachial artery at baseline were not different between groups. However, FMD was marginally impaired. Laboratory variables were similar among groups of treatment, except for total cholesterol and sodium ion (Table 2). Titers of anti-oxLDL Abs and values for other inflammation markers (high-sensitivity CRP, TBARS) were also comparable in the three groups at baseline.

Effect of BP-lowering treatment

Twelve weeks after the antihypertensive treatments with ACEI, HCTZ, or INDA, there was a decrease in SBP and DBP office ($P < 0.0005$ vs. baseline) and also in BP measurements obtained by 24-h ABPM ($P < 0.0005$), with no differences regarding treatment (Table 1). Forty-nine patients (52%) had addition of perindopril (4 mg) at week 6 in order to achieve BP targets.

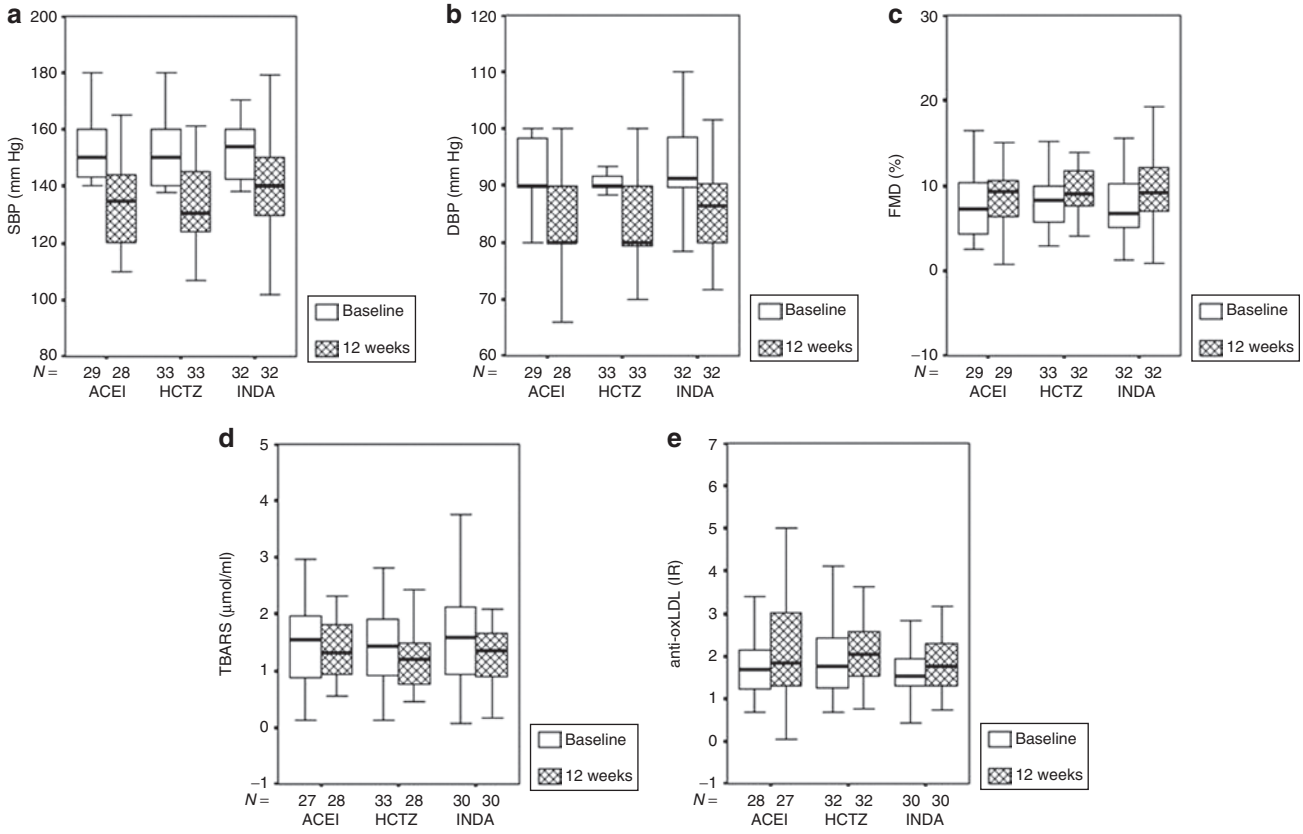

Figure 1 | Box-plots showing medians, interquartiles and 95% confidence interval for (**a**) systolic blood pressure (SBP), (**b**) diastolic blood pressure (DBP), (**c**) flow-mediated dilation (FMD), (**d**) thiobarbituric acid-reactive substances (TBARS), and (**e**) anti-oxLDL Abs at baseline and 12 weeks after antihypertensive treatment with angiotensin-converting enzyme inhibitor (ACEI), hydrochlorothiazide (HCTZ), or indapamide (INDA). ACEI: perindopril (4 mg); HCTZ: hydrochlorothiazide (25 mg); INDA: indapamide (1.5 mg) $P < 0.05$, baseline vs. 12 weeks; general linear model (GLM): repeated measures. ACEI = HCTZ = INDA.

Table 3 | Percent changes in blood pressure, anti-oxLDL Abs, and FMD after treatment compared to baseline

Variable	ACEI	HCTZ	INDA	*P*
SBP	−12.0 (1.5)	−12.1 (1.2)	−8.0 (1.9)	0.099
DBP	−8.3 (1.7)	−9.1 (1.1)	−5.5 (1.8)	0.229
Anti-oxLDL Abs	15.8 (7.6)	17.2 (6.6)	13.8 (6.5)	0.939
FMD	34.9 (10.6)	32.8 (10.9)	51.9 (24.3)	0.673

Data are means and s.e.m. $P > 0.05$; Analysis of variance. ACEI: perindopril group; HCTZ: hydrochlorothiazide group; INDA: indapamide group.
ACEI, angiotensin-converting enzyme inhibitor; anti-oxLDL Abs, antioxidized low-density lipoprotein antibodies; DBP, diastolic blood pressure; FMD, flow-mediated dilation; HCTZ, Hydrochlorothiazide; INDA, Indapamide; SBP, systolic blood pressure.

Box-plots showing medians, interquartiles and 95% confidence interval for SBP and DBP at baseline and 12-week are presented in Figure 1a,b.

FMD was improved after BP reduction ($P < 0.0005$ vs. baseline), without differences between treatment arms (Table 1 and Figure 1c). Conversely, the effect of nitrate on vasorelaxation was not affected by BP control (Table 1).

Decrease in creatinine and potassium ion levels and increase in apolipoproteins B were observed. The type of treatment did not affect other lipid parameters or glucose levels (Table 2).

There was a decrease in lipid peroxidation as measured by the TBARS assay after BP lowering ($P < 0.05$). High-sensitivity CRP remained unchanged, with values in the normal range since baseline (Table 2). Figure 1d shows baseline and 12-week distribution of TBARS.

Interestingly, BP control following a 12-week period of antihypertensive treatment was accompanied by increase in anti-oxLDL Ab titers ($P < 0.005$ vs. baseline), with no difference between groups regarding therapeutic regimen (Table 2 and Figure 1e).

When assessing percent change in SBP and DBP, anti-oxLDL Ab titers, and FMD from baseline values, it was observed that endothelial function was improved and Ab titers increased as BP and TBARS levels decreased. Similar results were exhibited by all treatment groups (Table 3). However, there were no significant correlations between anti-oxLDL Ab titers (or change in this marker after treatment) and the other variables (at baseline, end of study, and percent changes) (data not shown).

DISCUSSION

Our results clearly show that anti-oxLDL Ab titers increased shortly after BP lowering with ACEI, HCTZ, or INDA in a sample population with high BP, who was previously naive of antihypertensive treatment and without other known risk factors. Moreover, this study strengthens the possibility that increase in the anti-oxLDL Ab titers is protective against atherosclerosis. A recent study from our group has pointed out to a difference

between the anti-oxLDL Ab titers in two different clinical scenarios: patients with metabolic syndrome after a recent acute coronary syndrome and stable individuals with hypertension.[18] Other studies,[16,30] suggested that anti-oxLDL Ab titers in unstable patients were lower than in stable ones. These lower levels could be related to increased consumption of Abs due to either an inflammatory response occurring in the circulation or a decreased production as a result of deficient immune capacity.[18] Holvoet *et al.*[31] showed that high plasma levels of malondialdehyde-modified LDL are present in individuals with stable coronary artery disease and in those with unstable angina. However, he could not differentiate these two subgroups. If we assume that there should be stronger stimuli for LDL oxidation in plaque instability, the decrease in anti-oxLDL Ab titers could be related to formation of immune complexes, their presence in the arterial wall, or even their elimination. Part of the difference resides in the complex nature of the antigen (i.e., oxLDL) that will induce a polyclonal Ab response. Part of these Abs could be protective and another part could aggravate the disease.[32] However, the close correlation observed between the *in vivo* levels of circulating or tissue-fixed oxLDL or degree of lesion strongly supports the hypothesis that elevated titers of anti-oxLDL Abs may be protective against atherosclerosis.

The nature of the hypertensive disease seems to be much more complex than a simple elevation in BP measurements, involving a milieu of vascular, cell, humoral, and inflammatory alterations that ultimately lead to the disease itself, promoting activation of the inflammatory cascade, generation of reactive oxygen species, which end up enhancing LDL oxidation,[5–8] and leading to macrophage uptake of oxLDL.[10] The degraded particle is immunostimulatory,[33] immunogenic, and stimulates production of autoantibodies to LDL and a series of cytokines. These Abs circulate and form complexes with oxLDL and CRP,[9] and titers of remaining-free Abs can be measured in the plasma. There is a decrease in reactive oxygen species after BP is lowered and therefore fewer stimuli for LDL oxidation. In the present study, these known phenomena can be inferred from both the observed decrease in TBARS and increase in FMD. Thus, it is reasonable to assume that the consumption of anti-oxLDL Abs would decrease, enabling uncomplexed Abs to increase in the plasma. Shoenfeld *et al.* have indeed demonstrated that these circulating complexes are not harmful, and their effects only appear when deposited in tissues.[17]

Endothelial function was mildly impaired in our naive hypertensive subjects. Oxidized LDL may contribute to atherosclerosis progression by enhancing endothelial injury and by inducing foam cell generation and smooth muscle proliferation.[34] We observed similar reductions in BP with ACEI, HCTZ, or INDA. These changes elicited short-term improvement in endothelial function and decreased plasma peroxidation, as measured by reduction in the TBARS, thus showing that BP control improves other biological parameters related to the risk of atherosclerosis. In previous studies from our group, we have shown attenuation of experimental atherosclerosis in the cholesterol-fed rabbit model[35] and in alloxan-induced type 1 diabetes[36] by using ACEI, quinapril. Other studies have shown reduced LDL peroxidation in hypertensive patients as well as in the apolipoproteins E-deficient mice, and a decrease in experimental atherosclerosis[37] following inhibition of angiotensin II formation by ACEIs, blocking of the angiotensin II type 1 receptor, and using the latter in combination with statins.[38] This confirms the importance of renin–angiotensin system activation on atherogenesis and the possibility of its reversal with renin–angiotensin system blockade.

Because risk factors were excluded, our population was relatively homogeneous and other biases related to LDL modification and immune-response activation were avoided. In addition, because our patients had stage 1 hypertension and were naive of antihypertensive therapy at baseline, we were able to individually evaluate the effect of BP control on anti-oxLDL Ab titers. Recent studies have evaluated the potential therapeutic role of anti-oxLDL autoantibodies either through active or passive immunization,[15] shedding light into immune modulation as novel approaches to treat patients with atherosclerosis and cardiovascular disease.

Limitations and future directions

Our study has limitations for the TBARS assay is relatively insensitive and does not give a conclusive picture of the oxidative state of LDL based on the presence of malondialdehyde adducts. Another issue is the quantification of immune complexes and free Abs. OxLDL does not only constitute one antigen, but a large amount where some react with haptens (e.g., malondialdehyde), some with lipid or lipid-associated antigens, some with proteic antigens either linear or conformational. These questions need to be answered when more defined epitopes are available. However, these circulating immune complexes are not in themselves harmful, causing damage only if they are deposited in tissues.[17]

The role of the immunoglobulin M anti-oxLDL Abs has been completely assessed.[39] Natural pre-existing immunoglobulin M Abs have been suggested to play a role. These are mainly directed against carbohydrates and glycolipids. However, because the anti-oxLDL response is likely to be a secondary response as Abs always are present reacting against protein epitopes, and that protective Abs can be generated through vaccination,[17,26] we speculate that the most important subclass is IgG. Therefore, the further delineation of IgG specific Ab epitopes may be future directions of investigation in this fascinating field.

In conclusion, our results are novel and show that human IgG type anti-oxLDL Abs increase shortly after BP control. This effect on the immune response, including anti-oxLDL Ab elevation, seems to be protective since we observed decreased lipid peroxidation and improved endothelial function, known to favorably affect atherosclerosis. These results support the concept that hypertension increases the propensity to LDL oxidation and indicate anti-oxLDL Abs as potential markers in the follow-up assessment of hypertension treatment.

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