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# Antihypertensive therapy increases natural immunity response in hypertensive patients

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ABSTRACT

*Aims:* The aim of this work was to evaluate the effects of treatment of hypertension on the autoantibodies to apolipoprotein B-derived peptides (anti-ApoB-D peptide Abs) response, inflammation markers and vascular function.
*Main methods:* Eighty-eight patients with hypertension (stage 1 or 2) were recruited and advised to receive perindopril (4 mg), hydrochlorothiazide (25 mg), or indapamide (1.5 mg) for 12 weeks in a blinded fashion. Office and 24-h ambulatory blood pressure monitoring (24 h ABPM), flow-mediated dilatation (FMD), nitrate-induced dilatation (NID), titers of IgG and IgM anti-ApoB-D peptide Abs, hsCRP, and interleukins (IL-8 and IL-10) were evaluated at baseline and 12 weeks after therapies.
*Key findings:* All treatments reduced office BP, and improved FMD ($P < 0.05$ *vs.* baseline). The NID was improved only in the perindopril arm ($P < 0.05$ *vs.* baseline). The 24 h-ABPM was reduced with perindopril and hydrochlorothiazide therapies ($P < 0.05$ *vs.* baseline), but not with indapamide, and this effect was followed by increase in titers of IgM Anti-ApoB-D peptide Abs ($P < 0.05$ *vs.* baseline), without modifications in titers IgG Anti-ApoB-D peptide Abs and interleukins. Multivariable regression analysis has shown that change in the titers of IgM anti-ApoB-D peptide was associated with the changes in FMD ($\beta -0.347$; $P < 0.05$).
*Significance:* These findings shed light to a possible modulator effect of the antihypertensive therapy on the natural immunity responses and vascular function.



## 1. Introduction

Hypertension is responsible for high rates of morbidity and mortality worldwide and is well established as a risk factor for atherosclerosis. Suboptimal blood pressure values (>115 mm Hg, for systolic blood pressure) are related to ischemic cerebrovascular and heart diseases [1,2]. Hypertension presents itself as a multifactorial disease and the product of a dynamic interaction between different genetic, environmental and physiological factors.

Recent data strengthen the notion that hypertension also has an additional component associated with innate and adaptive immune responses that contribute to the hypertension condition [3]. This association is similar to the consensus on the immune system components that contribute to atherosclerosis [4].

In experimental models, infusion of angiotensin II leads to accumulation of macrophages, dendritic cells and T and B-lymphocytes within the blood vessel wall [5]. In particular, the B cells are capable of secreting antibodies of different isotypes. These antibodies are also found in the blood or inside the plaque either free or in the form of immunocomplexes [6,7]. These immunocomplexes may be formed from the oxidation of LDL and apolipoprotein B (ApoB) derived peptides [8,9].

The IgG autoantibodies anti-ApoB-D, so named by our group (ApoB-D is a synthetic peptide with the same amino-acid sequence as that of ApoB fragment), have shown an association with markers of inflammation and blood pressure assessed by different methods [10,11]. Thus, the existence of a possible immunomodulatory component of BP on the humoral response has been postulated.

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However, no data on the titers of autoantibodies to peptides derived from ApoB in response to antihypertensive therapy are available. Therefore, the aim of this study was to verify the titers of different isotypes of autoantibodies to the ApoB-D peptide in response to three different antihypertensive therapies.

## 2. Materials and methods

### 2.1. Subjects

Eighty-eight middle-aged individuals paired by both gender and age (50% males), with recently diagnosed arterial hypertension on stages 1 and 2 [12] were included in the protocol. Hypertension was defined as the average of three consecutive measurements of sitting blood pressure (BP) ≥140 mm Hg for systolic BP (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 at least one day's average value over 130/85 mm Hg by 24-h ambulatory BP monitoring (24-h ABPM) [13].

This project was approved by the local ethics committee and written informed consent was obtained from all patients prior to protocol initiation.

### 2.2. Study design

This study was conducted enrolling hypertensive subjects not receiving any previous antihypertensive treatment at the beginning of the study, randomly assigned to receive perindopril (4 mg) an angiotensin converting enzyme inhibitor, hydrochlorothiazide (25 mg), or indapamide (1.5 mg) for 12 weeks in a blinded-fashion. These drugs were chosen due to their different metabolic effects beyond blood pressure reduction that might possibly modulate immune responses. When BP goal was not achieved at 6 weeks, the patients of each therapeutic group received addition of perindopril (4 mg) for the following 6 weeks. Blood samples from baseline and follow-up were stored at −80 °C.

### 2.3. Exclusion criteria

Presence of other risk factors, such as smoking, diabetes or any treatment for this disease, hormone replacement therapy, renal or hepatic dysfunction, patients with left ventricular dysfunction, any active inflammatory or infectious disease were exclusion criteria.

### 2.4. 24 h ambulatory blood pressure monitoring

Twenty-four hour ABPM (Spacelabs 90,207, Seattle, WA) was performed at baseline and after 12 weeks.

### 2.5. Measurements of vascular reactivity

Vasoreactivity tests were performed in accordance with the Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated dilatation (FMD) of the brachial artery and endothelium-independent dilatation in response to isosorbide dinitrate (5 mg; sublingual route) (NID) [14]. Images of the FMD and NID were acquired using an ultrasound system (Sonos 5500; Hewlett-Packard-Phillips; Palo Alto, CA, USA). The percent change in vessel diameter from the baseline value was calculated to determine FMD or endothelium-independent dilatation. All assessments were performed in blinded fashion by an experienced ultrasonographer. The intra- and inter-sonographer variability values were <1 and 2%, respectively.

### 2.6. Blood sample collection

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

### 2.7. 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 autoanalyzer, Leverkusen, Germany). LDL-cholesterol was estimated by the Friedewald equation whenever triglycerides were <400 mg/dL. Glucose was assessed by enzymatic method and glycated hemoglobin (HbA1c) was measured using high-performance liquid chromatography. Concentrations of apolipoproteins and high sensitivity C-reactive protein (hsCRP) were determined by nephelometry (Array 360 Beckmann, Fullerton, CA).

### 2.8. Determination of plasma cytokine concentrations

Total plasma concentrations of IL-8 (pro-inflammatory cytokine) and IL-10 (anti-inflammatory cytokine) were determined by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's guidelines (R&D Systems, Minneapolis, MS, USA).

### 2.9. Determination of anti-ApoB-D peptide autoantibodies

Quantification of anti-ApoB-D autoantibodies (Abs) was assessed in total plasma by ELISA as previously described [10,15]. ApoB-D is a ApoB-peptide synthetic fragment with 22 amino acids derived from an ApoB conserved sequence, with amphipathic properties and not accessible to trypsin digestion. The immune response triggered by this peptide has been tested in animal model [16] and clinical studies [10,11]. Briefly, plates were coated overnight with 10 μg/mL of the synthetic ApoB-D peptide. After three washes, samples of voluntaries (1/1000 in phosphate buffer, PBS) were added to the plates. After three washes, purified goat anti-human IgG (0.1 μg/mL) or IgM (10 μg/mL) (KPL, Kirkegaard & Perry Laboratories, Gaithersburg, Maryland, USA) was added. After incubation for 2 h the plaques were washed and 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) (room temperature), were added, as enzyme substrate. The reaction was stopped by addition of $H_2SO_4$ (2 mol/l). The optical density (OD) of samples was measured at 450 nm. Autoantibodies (Abs) titers were expressed as the reactivity index (RI), calculated as $RI = (OD_{sample} - OD_{sample\ blank}) / (OD_{IgG\ or\ IgM} - OD_{IgG\ or\ IgM\ blank})$ where the IgG or IgM antibodies were used as controls. Samples were run in duplicate and the variation within the duplicates did not exceed 5% of the mean.

### 2.10. Statistical analyses

Categorical variables were expressed as number of subjects and percent values. Numerical data are presented as median values and interquartile range (IQR). Variables with non-Gaussian distribution were log-transformed for comparisons. At baseline, categorical variables were compared by the Chi-square test. Numerical data were tested by analyses of variance or Kruskal–Wallis test, when appropriate. The Wilcoxon test was utilized to compare effects of treatment within group and the Kruskal–Wallis test was used to evaluate percent changes between groups. To assess the effects of antihypertensive therapy in biochemical and vascular parameters, we used the difference between final − baseline levels (as indicated by delta, Δ). Correlation coefficients were tested by Pearson's or Spearman's correlation coefficient tests. A stepwise linear regression model was utilized to evaluate the impact of variables in Δ of titers of IgG autoantibodies anti-ApoB-D peptide (model 1) and Δ of titers of IgM anti-ApoB-D peptide (model 2). Values

of $P \leq 0.05$ were considered significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (17.0) for Windows.

## 3. Results

### 3.1. Subjects characteristics

Supplementary Table 1 shows the baseline characteristics of subjects. Most clinical and laboratory parameters were comparable in all therapeutic groups of the study.

Glucose levels were higher in the perindopril group (*vs.* indapamide group, ANOVA-Tukey; P = 0.004); hsCRP levels were higher in hydrochlorothiazide group (*vs.* perindopril group, Kruskal-Wallis; P = 0.006), but in the normal range and below 1.0 mg/L. Other vascular (Table 1) and biochemical parameters (Table S1) did not differ between therapeutic groups at baseline.

### 3.2. Effects of treatments

#### 3.2.1. 24-h ABPM, vascular reactivity and renal function

A significant reduction in the office systolic and diastolic BP was observed in the three arms of the treatment ($P < 0.05$). No difference was observed between drugs (SBP, $P = 0.109$; DBP, $P = 0.306$; Kruskal–Wallis) in the effectiveness of BP lowering (Table 1).

The mean values for 24-h ABPM showed a significant reduction in two treatment groups. In the perindopril group a reduction in the mean values for both systolic ($P = 0.018$) and diastolic ($P = 0.019$) BP were observed. The treatment with hydrochlorothiazide also showed reduction in the mean values for both systolic ($P = 0.001$) and diastolic ($P = 0.003$) BP. No reduction in the mean values of 24-h ABPM was observed in the indapamide group (Table 1).

The endothelial-dependent flow-mediated dilatation was improved in the perindopril ($P = 0.005$), hydrochlorothiazide ($P = 0.009$), and indapamide ($P = 0.003$) groups at 12 weeks, without difference between treatments. The endothelium-independent dilatation was modified only in the perindopril ($P = 0.010$) therapeutic group (Table 1).

#### 3.2.2. Cytokines and C-reactive protein

Levels of IL-8, IL-10, and C-reactive protein were not modified after treatment with any of the three antihypertensive therapies at 12 weeks. These results are shown in Table 2.

#### 3.2.3. Autoantibodies to ApoB-D peptide

The titers of IgG autoantibodies to ApoB-D peptide, at week 12 were not modified by any of the three antihypertensive regimens (Table 2).

**Table 1**
Vascular function, 24 h-ambulatory blood pressure monitoring, and estimated glomerular filtration rate in hypertensive patients.

| Variable | Perindopril N = 27 | Hydrochlorothiazide N = 32 | Indapamide N = 29 | *P*-value |
|---|---|---|---|---|
| Office and 24-h ambulatory blood pressure | | | | |
| Systolic blood pressure (mm Hg) | | | | |
| Baseline | 150 (141–168) | 149 (140–160) | 151 (140–160) | 0.996 |
| 12 weeks | 130 (120–140) | 130 (123–145) | 140 (129–149) | |
| *P*: baseline *vs* 12 weeks | <**0.001** | <**0.001** | **0.002** | |
| Mean change (%) | −13.5 (−17.5 to −7.0) | −12.0 (−15.0 to −8.0) | −7.0 (−16.5 to −0.5) | 0.109 |
| Diastolic blood pressure (mm Hg) | | | | |
| Baseline | 90 (90–98) | 90 (89–92) | 91 (88–98) | 0.881 |
| 12 weeks | 80 (80–90) | 80 (79–90) | 85 (80–90) | |
| *P*: baseline *vs* 12 weeks | **0.002** | <**0.001** | **0.004** | |
| Mean change (%) | −11.0 (−16.5 to −4.0) | −11.0 (−12.5 to −4.5) | −9.0 (−12.0 to 0.5) | 0.306 |
| 24 h ambulatory SBP (mm Hg) | | | | |
| Baseline | 127 (115–141) | 121 (113–127) | 126 (110–133) | 0.299 |
| 12 weeks | 124 (112–134) | 109 (105–120) | 119 (112–132) | |
| *P*: baseline *vs* 12 weeks | **0.018** | **0.001** | 0.554 | |
| Mean change (%) | 3.5 (−3.0 to 7.5) | 10.0 (3.0 to 15.0) | 1.0 (−8.5 to 11.5) | 0.079 |
| 24 h ambulatory DBP (mm Hg) | | | | |
| Baseline | 79 (72–88) | 78 (71–86) | 74 (64–81) | 0.235 |
| 12 weeks | 76 (68–86) | 69 (63–78) | 70 (66–75) | |
| *P*: baseline *vs* 12 weeks | **0.019** | **0.003** | 0.432 | |
| Mean change (%) | −1.5 (−7.5 to 3.0) | −6.0 (−14.0 to 1.0) | 0 (−10.5 to 7.0) | 0.179 |
| Endothelial function | | | | |
| FMD (%) | | | | |
| Baseline | 7.2 (4.2–10.3) | 8.3 (5.8–10.1) | 6.5 (4.7–10.1) | 0.824 |
| 12 weeks | 9.3 (6.4–11.9) | 9.0 (7.5–11.7) | 9.2 (6.3–12.1) | |
| *P*: baseline *vs* 12 weeks | **0.005** | **0.009** | **0.003** | |
| Mean change (%) | 3.0 (−20.0 to −28.0) | −1.0 (−12.5 to 25.5) | −5.5 (−27.5 to 20.0) | 0.709 |
| NID (%) | | | | |
| Baseline | 9.4 (8.2–12.8) | 11.1 (9.6–14.3) | 11.3 (7.9–14.5) | 0.442 |
| 12 weeks | 11.4 (8.6–14.2) | 11.2 (8.8–13.9) | 11.8 (8.6–14.3) | |
| *P*: baseline *vs* 12 weeks | **0.010** | 0.708 | 0.991 | |
| Mean change (%) | 12.0 (−6.0 to 22.5) | −4.5 (−18.0 to 17.5) | −2.0 (−13.5 to 13.0) | 0.103 |
| Glomerular filtration rate | | | | |
| eGFR (mL/min) | | | | |
| Baseline | 81 (67–106) | 78 (65–98) | 67 (59–92) | 0.460 |
| 12 weeks | 85 (72–114) | 86 (65–101) | 75 (60–106) | |
| *P*: baseline *vs* 12 weeks | 0.109 | 0.670 | 0.058 | |
| Mean change (%) | 0 (−8.0 to 15.0) | 0 (−11.0 to 12.5) | 0 (8.5 to 13.5) | 0.691 |

Abbreviations: SBP: systolic blood pressure; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate (calculated using the Cockcroft–Gault equation); FMD: flow-mediated dilatation; IQR: interquartile range; NID: nitrate-induced dilatation. Variables are expressed as median values and IQR. Mean changes from baseline were tested using the ANOVA-Tukey test. Differences between time-points were analyzed using the Wilcoxon test. *$P < 0.05$; baseline *vs.* 12 weeks.
$P < 0.05$ represented in bold.

**Table 2**
Effects of antihypertensive drugs on immune markers and autoantibodies anti-ApoB-D peptide.

| Variables | Perindopril (N = 27) | Hydrochlorothiazide (N = 32) | Indapamide (N = 29) | *P*-values |
|---|---|---|---|---|
| IL-8, pg/dL | | | | |
| Baseline | 42.3 (22.6–81.3) | 46.4 (27.5–59.1) | 43.1 (14.2–66.7) | 0.217 |
| 12 weeks | 34.5 (22.1–61.9) | 49.6 (38.2–74.6) | 38.3 (27.0–68.1) | |
| *P*: baseline *vs* 12 weeks | 0.182 | 0.581 | 0.275 | |
| Mean change (%) | 12.0 (−39.0–15.0) | 5.5 (−38.5–106.5) | 11.5 (−21.5–464.5) | 0.262 |
| IL-10, pg/dL | | | | |
| Baseline | 5.1 (2.29–6.86) | 5.5 (3.1–6.5) | 6.3 (3.3–7.1) | 0.880 |
| 12 weeks | 5.8 (3.36–6.45) | 5.3 (3.0–7.2) | 6.1 (3.5–6.8) | |
| *P*: baseline *vs* 12 weeks | 0.723 | 0.517 | 0.732 | |
| Mean change (%) | −3.5 (−14.0–11.0) | 1.5 (−16.5–32.0) | −0.5 (−8.0–10.0) | 0.754 |
| hsCRP, mg/L | | | | |
| Baseline | 0.32 (0.26–0.50) | 0.75 (0.30–0.99) | 0.43 (0.27–0.90) | 0.020 |
| 12 weeks | 0.39 (0.26–0.57) | 0.41 (0.30–0.79) | 0.38 (0.25–0.90) | |
| *P*: baseline *vs* 12 weeks | 0.306 | 0.538 | 0.446 | |
| Mean change, (%) | 6.0 (−19.0–31.0) | 0 (−43.5–31.5) | −10.5 (−43.0–38.0) | 0.391 |
| IgG anti-ApoB-D peptide Abs, RI | | | | |
| Baseline | 1.52 (1.01–2.94) | 1.12 (0.65–2.15) | 1.14 (0.65–1.86) | 0.246 |
| 12 weeks | 2.30 (1.28–4.96) | 1.40 (1.09–3.40) | 1.37 (1.01–1.89) | |
| *P*: baseline *vs* 12 weeks | 0.053 | 0.116 | 0.534 | |
| Mean change (%) | 53.0 (−23.0–203.5) | 25.5 (−30.5–168.5) | 16.0 (−34.6–100.5) | 0.696 |
| IgM anti-ApoB-D peptide Abs, RI | | | | |
| Baseline | 0.72 (0.53–0.85) | 0.71 (0.42–1.02) | 0.80 (0.51–1.44) | 0.358 |
| 12 weeks | 0.84 (0.43–1.19) | 0.90 (0.70–1.50) | 0.86 (0.54–1.53) | |
| *P*: baseline *vs* 12 weeks | 0.046 | 0.003 | 0.149 | |
| Mean change (%) | 27.0 (−7.5–130.0) | 61.0 (−1.0–114.0) | 26.0 (−17.0–93.5) | 0.525 |

Abbreviations: IgG anti-ApoB-D peptide Abs: IgG class autoantibodies to ApoB-D peptide; IgM anti-ApoB-D peptide Abs: IgM class autoantibodies to ApoB-D peptide; hsCRP: high-sensitivity C-reactive protein; IQR: interquartile range; IL: interleukin; RI: reactivity index. Variables are expressed as median values and IQR. Comparisons between mean changes were analyzed by ANOVA-Tukey or Kruskal–Wallis test. Comparisons between time-points were performed using the Wilcoxon test. $P \leq 0.05$; for hsCRP, perindopril *vs.* hydrochlorothiazide at baseline; for IgM anti-ApoB-[peptide] D Abs, baseline *vs.* 12 weeks.

However, the levels of IgM autoantibodies to ApoB-D peptide were modified in two arms of the treatment. Both perindopril and hydrochlorothiazide promoted an increase in the titers of IgM autoantibodies to ApoB-D at week 12 ($P = 0.046$ and $P = 0.003$, respectively). We did not observe modification in the IgM autoantibodies to ApoB-D in the indapamide group at week 12 ($P = 0.149$) (Table 2).

*3.2.4. Relationship between change in autoantibodies and change in blood pressure or vascular parameters*

The differences between absolute results at 12 weeks after treatment and baseline (12 weeks − baseline) were expressed as delta (Δ). The Δ of IgG antibodies titers to ApoB-D peptide were positively correlated with those for the Δ of the mean 24-h diastolic ABPM ($r = 0.29$, $P = 0.02$). No significant correlation was observed for other parameters (Table S2).

The Δ of IgM antibodies titers to ApoB-D peptide was inversely correlated with that for Δ of FMD ($r = -0.31$; $P = 0.006$) (Fig. 1), but not with the Δ of the mean values of 24-h systolic or diastolic ABPM (Fig. 2). The Δ of IgG autoantibodies titers to ApoB-D peptide did not show any correlation with Δ of FMD ($r = -0.11$, $P = 0.315$) (Fig. 1).

The stepwise linear regression model was utilized to evaluate the determinants of changes in the titers of IgM autoantibodies to ApoB-D peptide. We observed that the value for Δ FMD was independently associated with that for Δ IgM autoantibodies titers to ApoB-D peptide ($F = 7.65$; $r^2 = 0.14$; $P = 0.01$). In addition, regression analysis showed that baseline BMI and Δ of 24-h diastolic ABPM were independently associated with Δ of IgG autoantibodies titers to ApoB-D peptide ($F = 4.09$; $r^2 = 0.15$; $P = 0.04$) (Table 3).

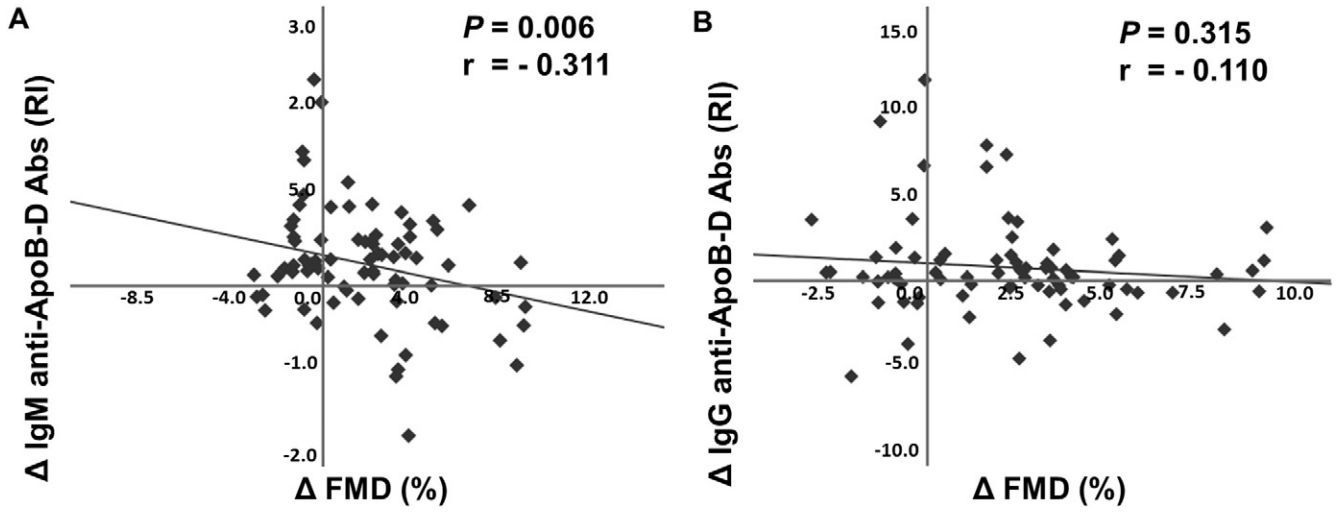


**Fig. 1.** Dot plots representing correlation between the values for Δ (12 weeks − baseline) of titers of IgM (A) and IgG (B) antibodies titers to ApoB-D peptide with those for Δ of flow mediated dilation (FMD) in all arms of the anti-hypertensive therapy. $P < 0.05$, Pearson's correlation coefficient.

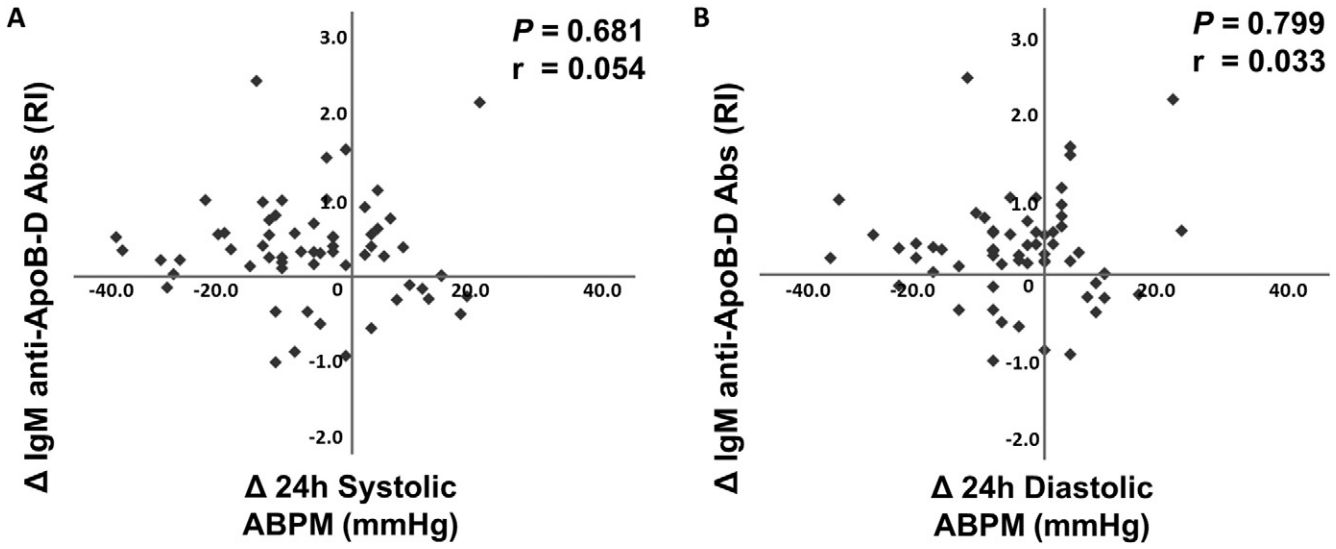


**Fig. 2.** Dot plots representing correlation between the values for Δ (12 weeks − baseline) of titers of IgM autoantibodies to ApoB-D peptide and those for Δ of 24-h systolic (A), and diastolic (B) ambulatory blood pressure monitoring (24 h-ABPM) in all arms of the anti-hypertensive therapy. $P < 0.05$, Pearson's correlation coefficient.

## 4. Discussion

The present study shows that the reduction in mean 24-h ABPM, with improvement in endothelial function 12 weeks after antihypertensive therapy was followed by an increase in the titers of IgM autoantibodies to ApoB-D peptide. However, no changes were observed in the titers of IgG autoantibodies to ApoB-D peptide and plasma cytokines levels after the treatments.

An experimental model conducted by our group revealed that increase in the titers of IgG autoantibodies anti-ApoB-D peptide was associated with progression of atherosclerosis [16]. It has also been shown that high blood pressure levels caused the titers of IgG autoantibodies anti-ApoB-D peptide to increase in association with inflammatory markers [10]. Interestingly, we did not observe any change in the titers of IgG autoantibodies anti-ApoB-D peptide in the present study. Possibly, due to lack of a cytokine level modification, the IgG autoantibodies remained unchanged after the antihypertensive treatment.

In the present study, we could speculate that the action of antihypertensive treatment to lower BP, which improved the endothelial function, could also promote a reduction in endothelial cell apoptosis, with minor release of microparticles [17,18], and decrease in oxidative stress [19]. Apoptosis process of endothelial cells releases microparticles in the blood, and these particles may be targets for IgM antibodies [20,21]. Oxidative-stress derived dysfunctional endothelial cells may promote the exposure and subsequent degradation of ApoB protein, thus generating new ApoB-derived peptide fragments [15,22]. ApoB-D peptide also forms immune complexes with the IgM antibody, as observed in the present study.

As shown in our manuscript, antihypertensive treatment promoted increases in titers of IgM autoantibodies anti-ApoB-D peptide. Such increases could indicate the amount of free, non-complexed antibody in the blood, due to minor consumption of these autoantibodies by ApoB-D peptide, or by microparticles derived from endothelial apoptotic cells. The effects of antihypertensive therapy would reduce microparticles and ApoB-D peptide. However, such speculations need further studies to investigate the effect of different antihypertensive therapies on the humoral response.

We observed that the Δ of endothelial-dependent dilatation were associated with the modifications that occurred in the titers of IgM antibodies to ApoB-D peptide. These findings suggest that the natural immune responses to ApoB-derived peptides can be related with the endothelial response to be explored in the other studies.

Moreover *in vitro* studies have shown that angiotensin-converting enzyme inhibitors (ACEI) can prevent endothelial cells damage due to pro-inflammatory receptors by modulating or controlling the innate response by neutrophils that are important mediators for endothelial injury [23]. This can explain in part the improvement of endothelial function in our study, considering that the ACEI perindopril has been added to participants that failed to achieve BP goals at week 6.

Another study, in which the humoral response to ApoB-peptides was evaluated, showed that statins or beta blockers do not promote changes in the titers of autoantibodies to other ApoB-derived peptides [24]. Some data show that the response by autoantibodies to ApoB-derived peptides is discrepant between antibody classes, because the autoimmune response depends on previous clinical factors and other chemical elements associated with peptides [25,26].

Elevation in the titers of IgM autoantibodies to autoantigens is presented at an early stage of life as first mechanisms of immune protection [27] and is a protective mechanism of natural immunity in different clinical settings [28], in which atherosclerosis can be included [29]. The anti-atherosclerotic effects of the autoantibodies may be related to the clearance of immune complexes of IgM and autoantigens derived from apoptotic cells and peptide fragments. However, the role of the immune complexes of IgM antibodies with "self" peptides in atherosclerosis appears to be related to the acute or chronic manifestation of the

**Table 3**
Multiple stepwise regression analysis of variables independently associated with values for differences (Δ) of IgG anti-ApoB-D peptide Abs (model 1) and delta of IgM anti-ApoB-D Abs (model 2).

| Independent variables | β | Standardized β | t | *P*-values |
|---|---|---|---|---|
| Model 1 | | | | |
| Δ 24 h systolic ABPM | 0.097 | 0.289 | 2.495 | 0.020 |
| Baseline BMI | −0.172 | −0.261 | −2.124 | 0.048 |
| Model 2 | | | | |
| Δ FMD | −0.070 | −0.347 | −2.645 | 0.011 |

Abbreviations: Δ 24-h systolic ABPM: mean change in 24-h ambulatory systolic blood pressure monitoring; BMI: body mass index; Δ FMD: mean change in flow-mediated dilatation. The values for Δ were calculated as the difference between those for 12 weeks of treatment and baseline values. The analyses included age, Δ systolic and diastolic 24-h ABPM, Δ office systolic and diastolic blood pressure, baseline BMI, baseline interleukin-8 and -10, which were all excluded in the final models. Parameters for model 1: $F = 4.09$; $r^2 = 0.15$, $P = 0.04$. Parameters for model 2: $F = 7.65$; $r^2 = 0.14$, $P = 0.01$.

disease [30]. Thus, the role of these IgM autoantibodies in the progression of atherosclerosis is not yet clear.

A study used experimental models and showed the role of ApoB-derived peptides in altering the vascular endothelial permeability; in previous clinical studies, we have shown that other ApoB-derived peptide could activate a specific proinflammatory response within the human atherosclerotic plaque [31,32].

The IgM autoantibodies to ApoB-D peptide can form immune-complexes that were assigned a key role in the reduction of chronic inflammation by clearance of the peptide from the blood stream leading to reduced activation of the inflammatory pathway and not contributing to the vascular dysfunction [23,32]. Therapies with potential to modulate the humoral response can be viewed as promising strategies to address atherosclerotic disease in the future [33].

## 5. Conclusion

In conclusion, this study shows for the first time that antihypertensive treatments effective in lowering BP and improving the endothelial function are associated with the elevation of IgM autoantibodies to an ApoB-derived peptide. Based on our findings, it is possible to assert that the antihypertensive therapy may have a modulatory effect in stimulating B-cells and autoimmune responses to different ApoB-derived epitopes associated with endothelium integrity.

### Conflict of interest statement

Izar MC: Consultant and speaker for Abbott, Genzyme, and Aegerion; speaker for MSD, Amgen, Aegerion, and Ache; Fonseca FA: Consultant and speaker for MSD, Astra Zeneca, Bayer, Amgen, Novartis, Novo Nordisk, Ache, and Abbott.

Other authors: None.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.lfs.2015.10.030.

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