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ORIGINAL ARTICLE

The effects of angiotensin receptor blockers *vs.* calcium channel blockers on the acute exercise-induced inflammatory and thrombotic response

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Arterial hypertension is an established risk factor for acute coronary syndromes, and physical exertion may trigger the onset of such an event. The mechanisms involved include the rupture of a small, inflamed, coronary plaque and the activation of thrombogenic factors. Blood pressure (BP)–lowering treatment has been associated with beneficial effects on subclinical inflammation and thrombosis at rest and during exercise. This prospective study sought to compare the effect of different antihypertensive drugs on the inflammatory and thrombotic response during exercise. A total of 60 never-treated hypertensive patients were randomized to an angiotensin receptor blocker (ARB)- or non-dihydropyridine calcium channel blocker (CCB)-based regimen. Patients with inflammatory or coronary artery disease were excluded. Six months after pharmaceutical BP normalization, the patients underwent a maximal treadmill exercise testing. High-sensitivity C-reactive protein (hsCRP), serum amyloid A (SAA), white blood cells (WBC), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), total fibrinogen (TF) and von Willebrand factor (vWF) levels, as well as plasminogen activator inhibitor-1 (PAI-1) activity were measured in blood samples taken while the patients were at rest and during peak exercise. All of these biomarkers increased with exercise, except PAI-1, which decreased ($P<0.05$ for the difference between resting and peak exercise for all biomarkers). The ARB group had less marked ($P<0.05$) exercise-induced changes than the CCB group in hsCRP (5.8% *vs.* 7.7%), SAA (4.2% *vs.* 7.2%), WBC (46.8% *vs.* 52.6%), TNF-α (16.3% *vs.* 24.3%), TF (9.5% *vs.* 16.9%) and PAI-1 (−9.5% *vs.* −12.3%) but a similar ($P=$ NS) change in IL-6 (39.4% *vs.* 38.6%) and vWF (29.2% *vs.* 28.6%). In conclusion, ARBs are most likely more effective than CCBs at suppressing the exercise-induced acute phase response. Potential protection against exercise-related coronary events remains to be elucidated.

INTRODUCTION

Large epidemiological studies have demonstrated that vigorous physical activity may trigger the onset of an acute coronary event in asymptomatic individuals, particularly in sedentary adults who engage in high-intensity exercise but are unaccustomed to this type of exercise.[1] Although the precise mechanisms that cause these events are not well defined, the prevailing hypothesis involves increased wall stress from the increased heart rate (HR) and blood pressure (BP), followed by disruption of a small inflamed atherosclerotic coronary plaque, activation of thrombogenic risk factors, clot formation and acute thrombotic occlusion.[1]

Arterial hypertension (AH) is an established cardiovascular (CV) risk factor, whereas inflammatory and prothrombotic biomarkers

implicated in the pathophysiology of AH[2–4] have emerged as novel CV risk factors.[5–11] Of the many promising molecules, C-reactive protein (CRP), serum amyloid A (SAA), white blood cells (WBC) count, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), fibrinogen, von Willebrand factor (vWF) and plasminogen activator inhibitor-1 (PAI-1) have generated considerable attention as predictors of AH, atherosclerosis, plaque destabilization and major CV events.[2–11]

The acute effects of exercise on circulating biomarkers have mainly been studied many hours after exercise in young athletes engaging in exhaustive exercise of long duration (for example, a marathon race).[12–16] The early acute phase response (APR) during short-term, vigorous aerobic exercise (work rate of at least 6 metabolic equivalents, for example, jogging)[1] in older, untrained adults has

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not been well studied.[17–21] In general, the APR seems to be proportional to the amount of activity and/or muscle injury.[12,17] Therefore, it is not clear whether short-duration exercise causes rapid systemic cytokine release. Considering the various biomarkers, the results are controversial; an increase in IL-6 is the earliest and the most prominent of the cytokine responses to exercise, whereas no change or a small, delayed increase has been noted for TNF-α.[12,14,16]

Antihypertensive treatment has been associated with beneficial effects on subclinical inflammation and thrombosis at rest and during exercise.[22–26] Nevertheless, comparative studies between different BP-lowering agents have not been previously reported.

Building on the previous reports,[1–26] we conducted a prospective study to compare the effect of different antihypertensive drugs on mediators of inflammation and coagulation in hypertensive patients exposed to short bouts of exercise-induced physical stress. Angiotensin receptor blockers (ARBs) and non-dihydropyridine calcium channel blockers (CCBs), two of the most commonly used first-line, BP-lowering treatments that are free of adverse metabolic effects were selected for a comparison of their potential effects on exercise-induced APR.

METHODS

Patients and design

This prospective study enrolled Caucasian patients, aged 30–65 years, with uncomplicated, grade 1 or 2 AH that had been never treated. Patients with secondary hypertension, inflammatory diseases, malignancies, coronary artery disease (CAD), cerebrovascular events, diabetes, uncontrolled symptomatic heart failure, atrial fibrillation, conduction disturbances, pre-excitation syndromes, electrolyte abnormalities, renal or liver dysfunction, chronic obstructive pulmonary disease, locomotor disability, current aspirin use, current lipid-lowering treatment and/or other current anti-inflammatory medication use were excluded from the study. Pregnant females were also excluded.

Screening of the clinic population to define which patients met the inclusion or/and exclusion criteria was performed by obtaining a thorough medical history and performing a physical examination, repeated BP measurements, resting electrocardiogram, full laboratory exams and an echocardiogram. During the recruitment period, 100 patients were examined, and 84 patients were eligible and accepted to participate in the study (Figure 1). Written informed consent was obtained from all the patients, and the study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of our institutions.

The participants were divided into two groups (Figure 1) and randomized to ARB (irbesartan, titrated to 300 mg once daily) or non-dihydropyridine CCB (diltiazem, titrated to 300 mg once daily) treatment. Hydrochlorothiazide was added, when necessary, and titrated to 25 mg once daily to achieve the BP target (office BP not exceeding 140/90 mm Hg, ambulatory BP < 130/80 mm Hg for a 24-h period, 135/85 mm Hg for day-time BP and 120/70 mm Hg for night-time BP). The mean duration of the total treatment period was 9 months (3 months to achieve adequate BP control in all patients plus 6 months of a fixed-dose medication). Patients who experienced medication side effects, were non-compliant with the medical regimen, initiated any additional treatment, failed to achieve the BP target, became pregnant or were non-compliant with follow-up were withdrawn from the study (Figure 1).

Six months after BP normalization, the patients underwent maximal treadmill exercise testing (ET; Figure 1). The test was indicated owing to a persistent, post-treatment, 10-year CV risk score consistent with at least a moderate risk for serious cardiac events, atypical angina, intention to engage in vigorous exercise or occupations in which impairment might impact public safety.[27] Circulating levels of high-sensitivity CRP (hsCRP), SAA, WBC, TNF-α, IL-6, total fibrinogen (TF) and vWF, as well as PAI-1 activity were measured in blood samples before and immediately after peak exercise. All of the measurements and procedures were performed by personnel blinded to the treatment group. Patients with an inconclusive or submaximal ET were not used in the analysis, while patients with a positive ET were subjected to stress echocardiography or thallium-201 scintigraphy (Figure 1). The absence of wall motion abnormalities or perfusion defects, respectively, ruled out obstructive CAD. Coronary angiography was recommended in patients with
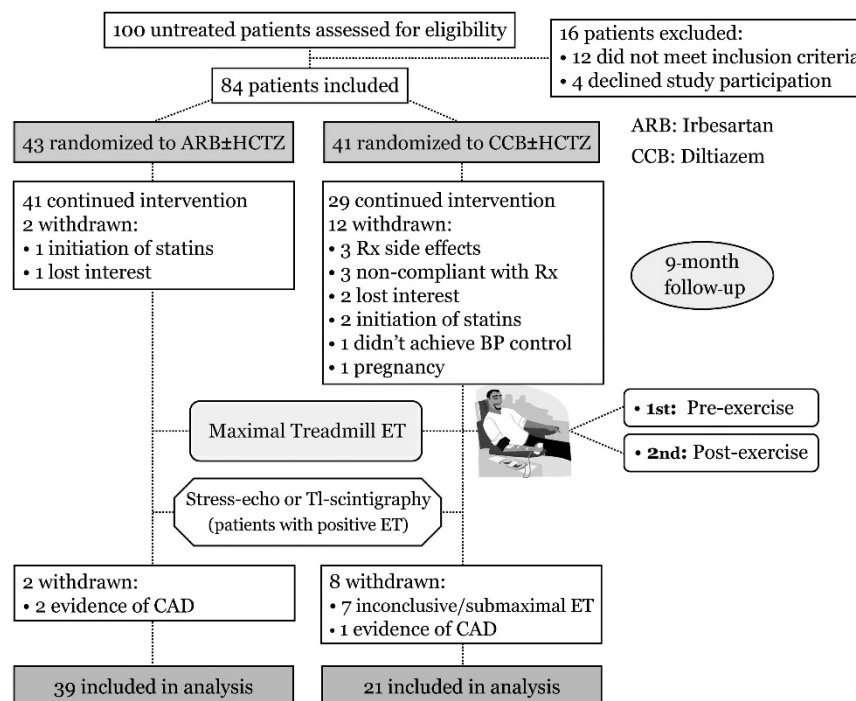

Figure 1 Study design. Flowchart for enrollment, randomization, and follow-up of the participants. ARB, angiotensin receptor blocker; BP, blood pressure; CAD, coronary artery disease; CCB, calcium channel blocker; ET, exercise testing; HCTZ, hydrochlorothiazide; Rx, treatment; TI, thallium.

positive–stress imaging results; however, these patients were excluded from the analysis (Figure 1) because this study targeted non-CAD individuals.

At the end of follow-up, 60 patients were included in the analysis: 39 in the ARB group and 21 in the CCB group (Figure 1). Treatment included hydrochlorothiazide in 5 (12.8%) and 3 (14.3%) patients in the ARB and CCB groups, respectively.

Definition of AH

Hypertension was diagnosed if the office BP was $\geqslant 140/90$ mm Hg on at least three visits, in accordance with the European Society of Hypertension guidelines.[28] An office BP 140–159/90–99 mm Hg was defined as grade 1, whereas 160–179/100–109 mm Hg was defined as grade 2 hypertension. The office BP was measured as the average of three consecutive measurements with 1-min intervals and the patient sitting, after a 15-min relaxation period. Patients with a borderline office BP or a home BP not corresponding to the office BP were subjected to 24-h ambulatory BP monitoring (ABPM) using certified equipment (Spacelabs 90207, Redmond, WA, USA). The BP thresholds for the diagnosis of hypertension, according to ABPM recordings, were 130/80 mm Hg over 24 h, 135/85 mm Hg for the day-time BP and 120/70 mm Hg for the night-time BP. The achievement of the target BP after treatment was determined with office BP measurements and, if necessary, with ABPM, as previously described.

CV risk assessment

The total 10-year CV risk was calculated during the baseline evaluation of the patients and at the end of follow-up using the Framingham Risk Score (FRS) and the Greek version of the HeartScore (HS). The FRS was considered low at $<10\%$, intermediate at 10–20% and high at $>20\%$.[29] The HS was considered low at $<1\%$, intermediate at 1–5% and high at $>5\%$.[30] The algorithms for the FRS and HS calculations accounted for traditional risk factors (age, sex, systolic BP, total cholesterol, high-density lipoprotein, smoking, presence of diabetes or/and antihypertensive treatment).[29,30]

Physical activity assessment

Physical activity was estimated during the baseline evaluation and at the end of follow-up. A translated version of the validated International Physical Activity Questionnaire, which is suitable for assessing levels of self-reported physical activity, was used.[31] Participants were instructed to refer to all the domains of activity during a usual week over the past year (time spent walking and in moderate- and vigorous-intensity exercise). Continuous (metabolic equivalents times minutes of exercise per week) and categorical (low, moderate or high level of activity) scores were calculated. The criteria for grouping in the categorical score have been previously described.[32]

Treadmill ET

All patients underwent a maximal ET (achieved HR $>90\%$ of the target HR (220 beats per min minus age in years)) at the end of follow-up, according to the multistage Bruce protocol, between 0730 and 0830 hrs after a 12-h overnight fast. Medication was not discontinued before the test to preserve BP control and the potential anti-inflammatory/anti-thrombotic effects. A 15-lead, electrocardiogram analysis, treadmill system (GE CaseT-2000 Series, Milwaukee, WI, USA) was used. Electrocardiogram was recorded continuously, and the BP was measured every minute at the rest, during exercise and for up to 10 min after exercise. The exam was terminated when the target HR was reached. An ischemic ST-segment response was defined according to the standard ST deviation criteria.[27] Tests without ischemic ST changes and achieving a HR $<85\%$ of the target HR were considered to be inconclusive.

Laboratory measurements

Blood samples were collected pre- and immediately post-exercise, between 0730 and 0830 hrs, after a 12-h overnight fast, to avoid the diurnal variation in the measured variables. The WBC count was measured on the same day from whole-blood specimens using a CelltacMEK-6410 automatic hematology analyzer (Nihon Kohden, Rosbach v.d.H., Germany). The blood samples used to measure all of the other biomarkers were immediately placed on ice and

centrifuged at 1500 g, at 4 °C for 10 min, and the supernatants were stored in 1-ml aliquots at -80 °C until further analyses. Each sample was labeled with a code and assayed in duplicate with a maximum of one freeze-thaw cycle. The analysts were not aware of the status of the samples, and the reported results are the mean of the duplicate measurements. Serum concentrations of hsCRP and SAA were determined by means of particle-enhanced immunonephelometry using the BNII system (Siemens Healthcare Diagnostics, Marburg, Germany). Serum TNF-α and IL-6 levels were measured with a high-sensitivity, enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (R&D Systems Europe, Abingdon, UK). TF was measured using a modified Clauss method on a BCS coagulation system (Siemens Healthcare Diagnostics). A quantitative determination of the vWF antigen was performed using an automated latex particle-enhanced immuno-turbidimetric assay with IL coagulation systems (Instrumentation Laboratory Company—Lexington, MA, USA). PAI-1 activity was determined with an amidolytic method using a synthetic chromogenic substrate (Diagnostica Stago S.A.S., Asnières sur Seine, France). The intra-assay coefficients of variation for these tests ranged between 2.1% and 4.3%. To eliminate inter-assay variability, all of the samples from the same patient were tested in a single assay.

Measurement of routine chemistries was performed during the baseline evaluation and at the end of follow-up. Blood samples were collected at rest and analyzed in the central study laboratory using the standard equipment and certified methods.

Statistical analysis

Continuous variables are presented as the mean ± s.d. and categorical variables as the observed number (n, %). Student's t-test (paired or un-paired, as appropriate) was used to evaluate differences in continuous variables once normality was demonstrated (Kolmogorov–Smirnov or Shapiro–Wilk test); otherwise, a nonparametric test (Wilcoxon or Mann–Whitney, respectively) was used. Differences in the categorical variables were analyzed using the chi-square test (or Fisher's exact test, if applicable). A general linear model, multivariate analysis of covariance, was used to adjust the possible exercise-induced APR differences between the groups for the effects of relevant confounders (that is, office HR during the baseline evaluation and office BP at the end of follow-up). Differences were considered significant for a two-sided $P < 0.05$. The data analysis was performed using IBM SPSS 19 statistical software (2010, IBM, Route 100 Somers, NY, USA).

RESULTS

A total of 60 hypertensive patients (65.0% males) were included in the analysis. At the baseline evaluation, the mean age of the participants was 46.9 years; the mean office BP was 141.4/94.0 mm Hg; and the mean office HR was 78.8 beats per min. The mean FRS and HS were 13.0% and 1.47%, respectively (Table 1). None of the patients fulfilled the electrocardiographic criteria for left ventricular hypertrophy or had ST depression at rest. With the exception of office HR, the treatment groups were comparable at baseline ($p = $ NS) with regard to clinical, biochemical and echocardiographic parameters (Table 1).

At the end of follow-up, the goal BP was achieved with treatment in all of the patients (mean office BP 118.4/77.4 mm Hg, $P < 0.001$ vs. baseline), and the 10-year CV risk was significantly reduced (mean FRS 12.3%, $P < 0.05$ vs. baseline and mean HS 0.95%, $P < 0.05$ vs. baseline) (Table 2). Meanwhile, the obesity indices, the smoking habits and the level of physical activity were not improved ($p = $ NS) at the end of follow-up. Minimal changes ($p = $ NS) were also observed in basic laboratory exams. Thus, lifestyle modifications, although recommended, were not adopted by the treated population. Consequently, BP normalization should be mainly attributed to the pharmacological treatment. With the exception of office BP, the treatment groups did not differ to a significant degree ($p = $ NS) in clinical, biochemical or echocardiographic parameters at the end of follow-up (Table 2). The groups were also comparable ($p = $ NS) with regard to exercise parameters (Table 2) and resting values of the

Table 1 Patient characteristics at baseline

	All patients (n = 60)	ARB group (n = 39)	CCB group (n = 21)	P value
Clinical parameters				
Age (years)	46.9 ± 9.2	46.6 ± 10.1	47.5 ± 6.1	0.752
Male sex, n (%)	39 (65.0)	25 (64.1)	14 (66.7)	0.736
Office systolic blood pressure (mm Hg)	141.4 ± 11.3	142.7 ± 12.5	139.1 ± 6.1	0.326
Office diastolic blood pressure (mm Hg)	94.0 ± 7.5	94.8 ± 8.0	92.6 ± 5.9	0.378
Office heart rate (beats min^{-1})	78.8 ± 9.0	76.7 ± 8.3	82.8 ± 9.9	0.035
Obesity markers				
Body mass index (kg m^{-2})	27.5 ± 3.9	27.1 ± 4.2	28.1 ± 2.7	0.431
Waist-to-hip ratio	0.918 ± 0.075	0.918 ± 0.076	0.918 ± 0.075	0.999
Smoking				
Current smokers, n (%)	29 (48.3)	18 (46.2)	11 (52.4)	0.631
Pack-years (in smokers)	32.7 ± 20.0	30.7 ± 22.2	36.3 ± 13.5	0.543
Physical activity				
Low, n (%)	18 (30.0)	11 (28.2)	7 (33.3)	0.332
Moderate, n (%)	26 (43.3)	17 (43.6)	9 (42.9)	0.815
High, n (%)	16 (26.7)	11 (28.2)	5 (23.8)	0.718
MET-min/week	1726 ± 1589	1794 ± 1634	1600 ± 1496	0.704
10-year CV risk				
Framingham risk score (%)	13.0 ± 9.6	12.7 ± 10.1	13.5 ± 8.4	0.796
HeartScore (%)	1.47 ± 1.75	1.57 ± 1.94	1.28 ± 1.01	0.61
Laboratory parameters				
Fasting plasma glucose (mg dl^{-1})	95.2 ± 8.6	95.7 ± 8.0	94.3 ± 10.4	0.618
HbA$_1$c (%)	5.4 ± 0.5	5.4 ± 0.5	5.3 ± 0.3	0.343
eGFR (ml min^{-1} 1.73 m^{-2})	111.1 ± 30.3	111.0 ± 31.0	111.3 ± 29.7	0.979
Total cholesterol (mg dl^{-1})	214.4 ± 39.8	218.0 ± 39.7	207.8 ± 40.7	0.429
Triglycerides (mg dl^{-1})	124.3 ± 64.7	131.4 ± 69.4	111.2 ± 47.4	0.335
HDL cholesterol (mg dl^{-1})	48.3 ± 13.8	50.3 ± 14.1	44.6 ± 12.2	0.204
LDL cholesterol (mg dl^{-1})	134.3 ± 40.3	137.6 ± 38.7	128.3 ± 45.9	0.477
Echocardiographic parameters				
Ejection fraction (%)	65.9 ± 5.0	66.5 ± 4.6	64.8 ± 5.6	0.222
Normal left ventricular geometry, n (%)	31 (51.6)	21 (53.8)	10 (47.6)	0.676
Transmitral early-to-late velocity ratio	1.02 ± 0.28	1.01 ± 0.31	1.04 ± 0.18	0.748

Abbreviations: ARB, angiotensin receptor blocker (irbesartan); CCB, calcium channel blocker (diltiazem); CV, cardiovascular; eGFR, estimated glomerular filtration rate; HbA$_1$c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MET, metabolic equivalent (3.5 ml O$_2$ kg^{-1} min^{-1}); n, number of patients.

measured circulating biomarkers (Table 3). None of the participants complained about angina-like symptoms during exercise.

All of the inflammatory and prothrombotic mediators significantly increased with exercise in the studied population, except PAI-1, which notably decreased ($P < 0.05$ for all) (Table 3). Regarding the two treatment groups, the exercise-related changes in biomarkers were also significant ($P < 0.05$), with the exception of TNF-α in the ARB group and PAI-1 in the CCB group ($p = NS$) (Table 3). However, the irbesartan group compared with the diltiazem group had a less marked ($P < 0.05$) exercise-induced percentage change in hsCRP (5.8 ± 0.8% *vs.* 7.7 ± 1.0%), SAA (4.2 ± 0.6% *vs.* 7.2 ± 0.8%), WBC (46.8 ± 2.8% *vs.* 52.6 ± 3.0%), TNF-α (16.3 ± 2.7% *vs.* 24.3 ± 3.5%), TF (9.5 ± 1.8% *vs.* 16.9 ± 2.1%) and PAI-1 ($-9.5 \pm 1.3\%$ *vs.* $-12.3 \pm 1.7\%$), and a similar ($p = NS$) percentage change in IL-6 (39.4 ± 5.0% *vs.* 38.6 ± 4.9%) and vWF (29.2 ± 4.9% *vs.* 28.6 ± 4.6%; Figure 2). These differences between the treatment groups remained significant ($P < 0.05$) after adjusting for office BP and HR. The data concerning the total duration of the exercise-related APR indicate that approximately 3 h post-exercise, all of the factors almost returned to baseline levels. However, the detailed presentation of these data is beyond the scope of this study.

DISCUSSION

The beneficial longitudinal effects of habitual exercise on subclinical inflammation have been well studied; the underlying mechanisms include the following: decreased cytokine production by adipose tissue, skeletal muscle, endothelial and mononuclear cells; improved endothelial function and insulin sensitivity; and, possibly, an anti-oxidant effect.[12,33] By contrast, the APR to exercise and the parameters involved in this response are still poorly understood. The majority of available data have been obtained from athletes after prolonged strenuous exercise (for example, a marathon race).[12–16] This prospective study was conducted in non-CAD, hypertensive patients with a moderate CV risk undergoing short-term vigorous aerobic exercise (that is, treadmill ET). The main findings of this study indicate that the levels of most of the inflammatory acute phase markers (hsCRP, SAA and WBC), cytokines (IL-6 and TNF-α) and prothrombotic factors (TF and vWF) increase acutely during exercise, whereas PAI-1 activity decreases. Thus, along with the acute inflammatory and thrombotic reaction, there is a parallel 'protective' anti-thrombotic counter-regulation that is part of the APR to exercise. Whether 'protective' anti-inflammatory cytokines are also released into the circulation as a regulatory mode of the cytokine

Table 2 Patient characteristics at the end of follow-up

	All patients (n = 60)	ARB group (n = 39)	CCB group (n = 21)	P value
Clinical parameters				
Office systolic blood pressure (mm Hg)	118.4 ± 9.0	116.4 ± 9.4	122.2 ± 6.0	0.014
Office diastolic blood pressure (mm Hg)	77.4 ± 8.7	75.5 ± 8.9	80.9 ± 6.9	0.032
Office heart rate (beats min^{-1})	73.6 ± 6.4	72.7 ± 6.4	75.2 ± 6.4	0.215
HCTZ added in treatment, *n* (%)	8 (13.3)	5 (12.8)	3 (14.3)	0.998
Obesity markers				
Body mass index (kg m^{-2})	27.6 ± 4.2	27.3 ± 4.5	28.1 ± 3.2	0.563
Waist-to-hip ratio	0.919 ± 0.077	0.919 ± 0.079	0.918 ± 0.075	0.947
Smoking				
Current smokers, *n* (%)	29 (48.3)	18 (46.2)	11 (52.4)	0.631
Pack-years (in smokers)	33.5 ± 20.4	31.6 ± 22.6	37.1 ± 13.8	0.559
Physical activity				
Low, *n* (%)	16 (26.7)	9 (23.1)	7 (33.3)	0.212
Moderate, *n* (%)	31 (51.7)	22 (56.4)	9 (42.9)	0.209
High, *n* (%)	13 (21.7)	8 (20.5)	5 (23.8)	0.845
MET-min/week	1759 ± 1536	1758 ± 1548	1761 ± 1560	0.996
10-year CV risk				
Framingham risk score (%)	12.3 ± 9.6	11.4 ± 9.9	14.0 ± 8.9	0.406
HeartScore (%)	0.95 ± 1.02	0.95 ± 1.10	0.95 ± 0.78	0.998
Laboratory parameters				
Fasting plasma glucose (mg dl^{-1})	98.2 ± 11.6	99.6 ± 10.5	95.7 ± 14.5	0.297
HbA$_1$c (%)	5.4 ± 0.5	5.5 ± 0.5	5.3 ± 0.6	0.108
eGFR (ml min^{-1} 1.73 m^{-2})	110.4 ± 28.4	108.4 ± 30.1	114.0 ± 23.2	0.549
Total cholesterol (mg dl^{-1})	216.7 ± 42.9	213.7 ± 45.7	222.4 ± 33.7	0.531
Triglycerides (mg dl^{-1})	129.8 ± 72.5	134.6 ± 75.9	120.9 ± 62.7	0.561
HDL cholesterol (mg dl^{-1})	48.0 ± 12.7	48.3 ± 12.6	47.4 ± 13.6	0.832
LDL cholesterol (mg dl^{-1})	142.8 ± 40.2	138.5 ± 41.8	150.8 ± 34.8	0.343
Echocardiographic parameters				
Ejection fraction (%)	67.3 ± 5.4	68.2 ± 5.3	65.7 ± 5.5	0.164
Normal left ventricular geometry, *n* (%)	33 (55.0)	22 (56.4)	11 (52.4)	0.79
Transmitral early-to-late velocity ratio	1.05 ± 0.27	1.04 ± 0.28	1.06 ± 0.21	0.866
Exercice parameters				
Duration (sec)	627.8 ± 113.7	633.9 ± 121.9	616.5 ± 87.7	0.637
METs	12.3 ± 2.1	12.5 ± 2.3	12.0 ± 1.6	0.468
Achieved heart rate (% of target)	98.5 ± 4.8	99.2 ± 3.6	97.3 ± 7.4	0.395
Maximal heart rate (beats min^{-1})	169.8 ± 12.1	171.2 ± 11.6	167.2 ± 13.7	0.31
Maximal systolic blood pressure (mm Hg)	179.6 ± 19.8	178.0 ± 19.1	182.6 ± 20.4	0.197
Rate-pressure product (beats min^{-1} × mm Hg)	30494 ± 3966	30490 ± 4048	30502 ± 3767	0.75
Pseudo-ischemic ST-segment response, *n* (%)	13 (21.7)	9 (23.1)	4 (19.0)	0.656

Abbreviations: ARB, angiotensin receptor blocker (irbesartan); CCB, calcium channel blocker (diltiazem); CV, cardiovascular; eGFR, estimated glomerular filtration rate; HbA$_1$c, glycated hemoglobin; HCTZ, hydrochlorothiazide; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MET, metabolic equivalent (3.5 ml O$_2$ kg^{-1} min^{-1}); *n*, number of patients.

network for adaptation to systemic inflammatory stress is not clear from this study.

These findings are in agreement with the current literature. Mendham *et al.*[17] found an acute, exercise-induced elevation in IL-6, CRP and WBC in a sedentary, overweight population, with the main determinant of the IL-6 response being exercise intensity. By contrast, Tartibian *et al.*[18] indicated that the same biomarkers significantly increased after 30 min of treadmill running in young untrained males, regardless of the exercise intensity. Desouza *et al.*[21] reported that PAI-1 activity was significantly lower immediately after exercise and remained lower for at least 1 h and that the fibrinolytic response to acute exercise was not impaired in sedentary, older, hypertensive men. The literature also suggests that cytokine inhibitors (for example, IL-1 receptor antagonist) and anti-inflammatory

cytokines (for example, IL-10) restrict the magnitude and duration of the inflammatory response to strenuous exercise.[12,14]

Mechanisms explaining the rapid change in the concentrations of the circulating cytokines and thrombotic factors after short-duration exercise are ambiguous.[14] The most plausible scenario involves mobilization, systemic release and functional augmentation rather than *de novo* synthesis of neutrophils, monocytes, cytokines and hemostatic factors. Hemoconcentration, concerning the macromolecules, could partly be involved as well.

To our knowledge, this is the first head-to-head comparison trial aiming to compare the effect of different antihypertensive drugs on the exercise-induced acute response of numerous circulating inflammatory and thrombotic mediators. The results indicate that irbesartan is most likely more effective than diltiazem at suppressing the

inflammatory and coagulative reaction during exercise; the percentage increase in hsCRP, SAA, WBC, TNF-α and TF levels was lower in the irbesartan group, whereas the changes in TNF-α in the irbesartan group and PAI-1 in the diltiazem group were not significant. The explanation of this differentiation is not clear because the exercise-induced APR differences between the treatment groups remained significant ($P < 0.05$) after controlling for office BP and HR and thus could be only partially attributed to the different drugs' effect on BP and HR.

The anti-inflammatory effects of ARBs in hypertensive patients with microinflammation have been previously reported in the EUTOPIA study; olmesartan significantly reduced resting levels of hsCRP (-15.1%, $P < 0.05$), TNF-α (-8.9%, $P < 0.02$) and IL-6 (-14.0%, $P < 0.05$) within 6 weeks of treatment.[26] Similar findings for dihydropyridines have been reported in hypertensive patients with metabolic syndrome (MARCADOR and OLAS studies),[23,24] whereas there are no respective reports for non-dihydropyridines. Furthermore, perindopril has shown to have beneficial effects on inflammation indicators during exercise in patients with metabolic syndrome.[25] Similar favorable effects on vascular markers of inflammation have lately been reported for nebivolol in obese hypertensive patients exposed to exercise-induced stress.[22]

The choice of the non-dihydropyridine diltiazem as a representative CCB in this study was based primarily on the fact that the anti-inflammatory effects of this CCB have not been previously studied. Moreover, patients who are treated with dihydropyridines have a high prevalence of peripheral edema in countries with warmer climates, such as Greece, whereas those treated with verapamil have a higher prevalence of constipation. Besides, monotherapy with verapamil is limited in clinical practice in Greece.

Table 3 Exercise-induced changes in inflammatory and thrombotic biomarkers

	Pre-exercise	Post-exercise	P value
All patients (n = 60)			
hsCRP (mg l^{-1})	1.91 ± 1.63	2.04 ± 1.70	<0.001
SAA (mg l^{-1})	4.32 ± 2.97	4.55 ± 3.10	0.001
WBC (μl^{-1})	7151 ± 1967	10655 ± 2672	<0.001
TNF-α (pg ml^{-1})	0.99 ± 0.41	1.18 ± 0.46	0.044
IL-6 (pg ml^{-1})	1.67 ± 1.12	2.33 ± 1.67	<0.001
TF (mg l^{-1})	348.2 ± 89.1	389.4 ± 109.1	0.003
vWF (%)	109.1 ± 46.6	140.7 ± 58.4	<0.001
PAI-1 (AU ml^{-1})	18.80 ± 12.87	16.83 ± 13.51	0.002
ARB group (n = 39)			
hsCRP (mg l^{-1})	1.89 ± 1.70	2.00 ± 1.76	<0.001
SAA (mg l^{-1})	4.53 ± 3.16	4.72 ± 3.32	0.005
WBC (μl^{-1})	6810 ± 1802	9997 ± 2450	<0.001
TNF-α (pg ml^{-1})	1.04 ± 0.43	1.21 ± 0.50	0.141
IL-6 (pg ml^{-1})	1.45 ± 1.00	2.03 ± 1.43	<0.001
TF (mg l^{-1})	366.1 ± 83.1	400.7 ± 109.6	0.027
vWF (%)	109.3 ± 46.8	141.2 ± 61.8	<0.001
PAI-1 (AU ml^{-1})	18.86 ± 12.8	17.06 ± 13.62	0.01
CCB group (n = 21)			
hsCRP (mg l^{-1})	1.95 ± 1.43	2.10 ± 1.58	0.009
SAA (mg l^{-1})	3.93 ± 2.34	4.22 ± 2.41	0.043
WBC (μl^{-1})	7785 ± 2320	11877 ± 2910	<0.001
TNF-α (pg ml^{-1})	0.89 ± 0.31	1.11 ± 0.25	0.013
IL-6 (pg ml^{-1})	2.09 ± 1.35	2.88 ± 2.05	0.028
TF (mg l^{-1})	315.0 ± 98.3	368.4 ± 108.1	0.035
vWF (%)	108.8 ± 47.8	139.9 ± 49.4	0.005
PAI-1 (AU ml^{-1})	18.70 ± 13.62	16.40 ± 13.74	0.1

Abbreviations: ARB, angiotensin receptor blocker (irbesartan); CCB, calcium channel blocker (diltiazem); hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; n, number of patients; PAI-1, plasminogen activator inhibitor-1; SAA, serum amyloid A; TF, total fibrinogen; TNF-α, tumor necrosis factor-α; vWF, von Willebrand factor; WBC, white blood cells.
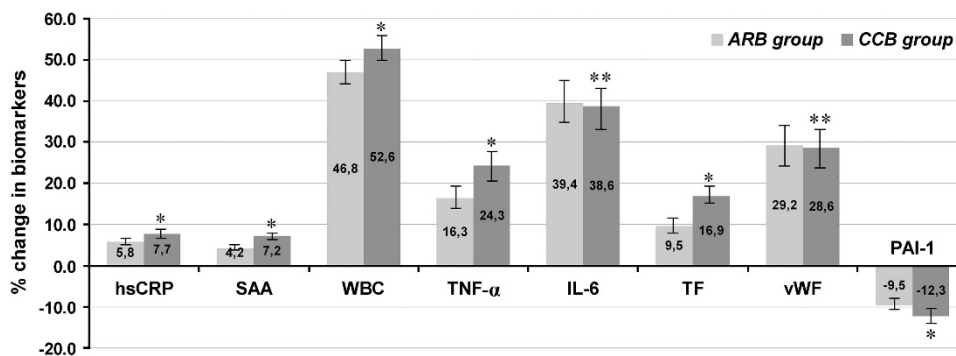
Clinical implications

The finding that even short-term physical activity causes a rapid systemic cytokine release may be of significant clinical importance, particularly in higher CV risk individuals, such as hypertensive patients,[7,28] because inflammatory and thrombotic mechanisms are involved in exercise-related acute vascular events.[1] Elevated levels of IL-6 have been found within the shoulder region of unstable coronary plaques,[5,6] where it colocalizes with the angiotensin II type 1 receptor.[5] Increased PAI-1 gene expression has been observed in the culprit lesions of atherosclerotic coronary arteries.[11] The exercise-induced decrease in PAI-1 activity observed in this study may eliminate the exercise-associated thrombotic risk by, at least in part, maintaining an individual's fibrinolytic capacity during the period of greatest risk.

Another interesting finding is that the acute *vs.* the longitudinal effect of exercise on proatherogenic markers of inflammation is similar to that on BP.[34] Acutely, both biomarkers and BP increase, but they decrease over time. The potential interplay between the effect of exercise on biomarkers and the one on BP remains to be elucidated.

Figure 2 Exercise-induced percentage changes (± s.d.) in circulating biomarkers. ARB, angiotensin receptor blocker (irbesartan); CCB, calcium channel blocker (diltiazem); hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; PAI-1, plasminogen activator inhibitor-1; SAA, serum amyloid A; TF, total fibrinogen; TNF-α, tumor necrosis factor-a; vWF, von Willebrand factor; WBC, white blood cells. *$P < 0.05$, **P = NS vs. ARB group (after adjusting for office blood pressure and heart rate).

Central mechanisms up- or downregulated in response to short- or long-term physiological stimuli are most likely implicated.

The emerging relative superiority of ARBs over non-dihydropyridine CCBs at suppressing the exercise-related APR could be accompanied by a significant clinical impact; it can be speculated that ARBs are more effective than CCBs at protecting hypertensive patients who do not have significant coronary stenosis against the destabilization of small vulnerable atherosclerotic plaques, rupture, thrombosis, and the triggering of acute coronary syndrome during vigorous exercise. Nevertheless, this hypothesis needs to be proven by future studies with hard endpoints in terms of morbidity and mortality.

Limitations

It is important to consider specific potential limitations in this study. First, this was a narrow-scale study with a limited number of patients. Especially in the CCB-arm, there was a greater number of patients who withdrew from the study, thus having a potential impact on the results, although this outcome was partially expected because diltiazem was not discontinued before the exercise test, leading to 17% of the participants having a submaximal/inconclusive test (Figure 1). However, there was a high homogeneity among the patients who completed the study (Tables 1 and 2). In any case, it should be noted that the results of this study are only suggestive, but not definitive, for the greater benefit of ARBs compared with non-dihydropyridine CCBs. Moreover, it is not clear whether this is a class effect or an agent-specific effect. Second, given that no ET was performed during the baseline evaluation and that there was not a non-medicated control group, comparisons to untreated hypertensive patients and healthy individuals are not feasible. Third, the exclusion of CAD and the definition of false-positive ET results were based on non-invasive methods, whereas coronary angiography, the gold standard for the diagnosis of CAD, was not performed in the study population. Nevertheless, angiography is not indicated in subjects with a negative ET, absence of wall motion abnormalities during a stress echocardiogram or a normal perfusion thallium-imaging test. Fourth, considering that achievement of target BP in grade 3 hypertensive patients would most likely require multiple medications in most of the participants, only patients with grade 1 or 2 hypertension were eligible for enrollment. Moreover, it should be mentioned that because diltiazem has negative chronotropic properties, patients on diltiazem may have had to exercise more vigorously to achieve the target HR compared with patients on irbesartan. Therefore, the APRs of these patients could have been potentially augmented; however, this hypothesis was not confirmed by the results of this study as the treatment groups were comparable ($P = NS$) with regard to exercise duration and metabolic equivalents.

Conclusions and future perspectives

This study presents novel findings concerning the APR in non-CAD hypertensive patients during short-term vigorous exercise and the potential anti-inflammatory/anti-thrombotic effects of ARBs and non-dihydropyridine CCBs. The activation of inflammatory and thrombotic mechanisms during exercise is clear, although this activation is, in a way, compensated by a concomitant homeostatic fibronolytic response. The pleiotropic effects of ARBs during exercise seem to be more pronounced than those of CCBs in terms of suppressing the APR.

Larger-scale studies are required to confirm these findings and comparably evaluate the effects of different BP-lowering agents and drug categories over a wider age spectrum. Future research should

also investigate the potential superiority of ARBs vs. CCBs in terms of hard endpoints, such as protection from exercise-triggered coronary events or/and mortality. Moreover, the amount of activity, the type of exercise and the muscles involved, the duration of the acute inflammatory activation and the effect of genetic variability on the APR and on the effects of exercise training[35] also warrant examination in future studies. Given the complexity of the inflammatory, coagulative and fibrinolytic systems, future research should continue to investigate the acute effects of physical activity on the thrombogenicity and low-grade inflammation in different at-risk populations to gain further insight into the mechanisms involved in exercise-triggered acute vascular events. The advances in our knowledge of the pathophysiology of atherosclerosis may improve clinical understanding and provide practical tools for better patient treatment and management.

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