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Angiotensin receptor blockers are not associated with reduced inflammatory markers in the general population

Running title: ARBs and cytokines

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Condensed abstract

The association between angiotensin receptor blockers **(**ARBs) and inflammatory markers was assessed in a population-based prospective study conducted in Lausanne, Switzerland. Data from 933 (baseline, 2003-6) and 1120 (follow-up, 2009-12) participants on antihypertensive drugs was used. No differences were found between participants taking or not taking ARBs for C-reactive protein, interleukins 1β and 6 and tumor necrosis factor alpha. These findings were replicated in the follow-up study, and comparing participants who received ARBs during follow-up with participants on other antihypertensive drugs. We conclude that ARBs are not associated with reduced levels of inflammatory markers in the general population.

Highlights

- We assessed the association between ARBs and inflammatory markers
- The associations were assessed cross-sectionally and prospectively
- A population-based sample of patients treated for hypertension was used
- no association was found between ARBs and inflammatory markers

ABSTRACT

Objective: angiotensin receptor blockers **(**ARBs) have been suggested to reduce inflammation in randomized controlled trials. We assessed the association between ARBs and inflammatory markers in a general population setting.

Methods: population-based prospective study conducted in Lausanne, Switzerland. Baseline data from 933 participants on antihypertensive drugs (424 on ARBs) was collected in 2003-6. Follow-up data from 1120 participants (572 on ARBs) was collected in 2009-12. C-reactive protein (CRP), interleukins 1β (IL-1β) and 6 (IL-6) and tumor necrosis factor alpha (TNF-α) were assessed and categorized in quartiles.

Results: At baseline, no differences were found between participants taking or not taking ARBs for all inflammatory markers studied, and this association persisted after multivariate adjustment: Odds ratios and (95% confidence interval) for being in the highest quartile of IL-1β, IL-6, TNF-α and CRP for participants on ARB compared to participants not on ARB; 1.23 (0.89-1.70); 1.26 (0.93-1.70); 1.14 (0.85-1.53) and 1.27 (0.96-1.69), respectively (P>0.05). These findings were further replicated in the follow-up study: OR and (95% CI) of 1.10 (0.78-1.55); 0.87 (0.64-1.19); 0.83 (0.61-1.14) and 0.91 (0.68-1.22) for IL-1β, IL-6, TNF-α and CRP, respectively (P>0.05). Finally, no effect of ARBs was found when comparing participants who received ARBs throughout the 5.5 years follow-up with participants on other antihypertensive drugs: OR and (95% CI) of 0.93 (0.61-1.42); 0.80 (0.54-1.17); 0.86 (0.59-1.25) and 0.95 (0.67-1.35) for IL-1β, IL-6, TNF-α and CRP, respectively (P>0.05).

Conclusion: ARBs are not associated with reduced levels of inflammatory markers in the general population.

KEYWORDS Angiotensin receptor blockers; cytokines; inflammation; population-based study.

INTRODUCTION

Several studies have suggested that angiotensin receptor blockers (ARB), a class of antihypertensive drugs, could exert an anti-inflammatory effect (for a review, see [1]). Several mechanisms have been proposed, such as reduction of mitochondrial reactive oxygen species [2], cytokine production [3, 4] or inflammatory response of macrophages to lipopolysaccharide [5, 6] via the activation of the peroxisome proliferator-activated receptor-gamma [7]. In humans, ARB have been shown to reduce fibrinogen [8], CRP [8, 9], interleukin-6 [10], TNF-α [11, 12] and vascular cell adhesion molecule-1 levels [12], although these effects have been challenged [13, 14]. Still, most human studies were conducted using a limited sample size or only diseased subjects [1], and it is unclear whether the anti-inflammatory effects of ARBs observed in randomized controlled trials with a considerable selection procedure also apply to the general population.

Thus, our study aimed to assess the impact of ARB on inflammatory markers in a general population setting.

METHODS

Participants

The methodology of the CoLaus study has been described previously [15]. The study was approved by the Institutional Ethic's Committee of the University of Lausanne and all participants provided informed consent prior to being interviewed. Briefly, a simple random sample of the population aged between 35 and 75 years of the city of Lausanne (Switzerland) was drawn from the complete list of Lausanne's inhabitants provided by the population register of the city and invited to participate. Inclusion criteria were 1) living in

Lausanne; 2) age between 35 and 75 years and 3) willingness to participate and to provide informed consent. Participation rate was 41% [15].

After a median follow-up time of 5.4 years (interquartile range: 5.3–5.6 years), participants were invited to attend a second examination, which included the same assessments as for baseline.

Personal and clinical data

All participants attended the outpatient clinic of the University Hospital of Lausanne in the morning after an overnight fast (minimum fasting time 8 hours). Data were collected by trained field interviewers in a single visit lasting about 60 min. The procedures were identical for the baseline and the follow-up surveys.

Participants received a questionnaire to record information about their status and lifestyle factors. Marital status was defined as married, divorced, single and widowed. Educational level was stratified into low (primary), middle (apprenticeship, secondary school) and high (university). Smoking status was classified as never, current or former smoker. Physical activity was defined as the practice of leisure time physical activity at least twice per week. Alcohol consumption was assessed by asking the participant how many units of alcoholic beverages (i.e. cans of beer, glasses of wine) he/she had consumed during the previous week, and categorized as drinker/non drinker. Caucasian ethnicity was defined if the parents and grandparents of the participants were born in a selected list of countries (available from the investigators).

Body weight and height were measured in light indoor clothes with shoes off. Body weight was measured in kilograms to the nearest 100g using a Seca® scale, which was calibrated regularly. Height was measured to the nearest 5 mm using a Seca® height gauge.

Body Mass Index (BMI) was calculated as weight (kg) divided by the square of the height (m). Overweight was defined as BMI ≥25 kg/m^2 and <30 kg/m^2, and obesity by a BMI ≥30 kg/m^2.

Diabetes was defined as a fasting plasma glucose ≥7 mmol/L or presence of antidiabetic drug treatment (oral or insulin). Information on the use of prescription and over the counter drugs was collected, together with their main indications. Collection was done by asking the participant to bring the drugs to the visit.

As antihypertensive drug treatment might be prescribed differently according to the presence of other comorbidities or of renal disease, we calculated the Functional Comorbidity Index (FCI) [16] at baseline and estimated the glomerular filtration rate (eGFR) according to the Modification of Diet in Renal Disease (MDRD) equation [17] at baseline and follow-up for each participant.

Antihypertensive drug treatment

All antihypertensive drugs reported and brought by the participants were coded using the Anatomical Therapeutic Chemical classification system [18]. In both baseline and follow-up, antihypertensive drugs were classified into six different binary categories (yes/no): 1) Diuretics (isolated or associated with other drugs); 2) Calcium channel blockers (CCBs); 3) Beta-blockers (BBs); 4) Angiotensin-converting enzyme inhibitors (ACEIs); 5) Angiotensin receptor blockers (ARB) and 6) Other (reserpine). Combinations were split into the drug classes they contained; for example ATC code C08GA01, corresponding to nifedipine and diuretics, was split into “diuretics associated with other drugs” and “calcium channel blockers”. For statistical analysis, another two categories: were created ARBs (irrespective of the presence or absence of other antihypertensive drugs) / other antihypertensive drugs.

Biological data

For both the baseline and the follow-up surveys, most biological assays were performed by the CHUV Clinical Laboratory on fresh blood samples within 2 hours of blood collection. Glucose was measured by glucose dehydrogenase, with a maximum inter-batch coefficient of variation (CV) of 2.1% and a maximum intra-batch CV of 1.0%. High sensitive CRP was measured by immunoassay and latex HS, with a maximum inter-batch CV of 4.6% and a maximum intra-batch CV of 1.3%. Cytokines were measured using a multiplexed particle-based flow cytometric cytokine assay. Milliplex kits were purchased from Millipore (Zug, Switzerland). The procedures closely followed the manufacturer's instructions. The analysis was conducted using a conventional flow cytometer (FC500 MPL, BeckmanCoulter, Nyon, Switzerland). Lower detection limits for IL-1β, IL-6 and TNF-α were 0.2 pg/ml. A good agreement between signal and cytokine was found within the assay range ($R^2 \geq 0.99$). Intra and inter-assay coefficients of variation were 15% and 16.7% for IL-1β, 16.9% and 16.1% for IL-6 and 12.5% and 13.5% for TNF-α, respectively. Repeated measurements were conducted in 80 subjects randomly drawn from the initial sample; Spearman rank correlations between duplicate measurements were 0.914, 0.961 and 0.891 for IL-1β, IL-6 and TNF-α (all $p<0.001$).

Statistical analysis

Participants were excluded from the analysis if they 1) presented an inflammatory status (defined by CRP values ≥20 mg/L) or 2) reported taking any type of anti-inflammatory drug or any type of systemic antibiotic. As the analysis was restricted to participants receiving antihypertensive drug treatment, all untreated participants (exclusion criterion 3) were also excluded.

Statistical analyses were performed using Stata version 13.0 for windows (Stata Corp, College Station, Texas, USA). Due to the skewed distribution of inflammatory markers, a categorization into quartiles was performed, including all values below the detection limit in the first quartile. Categorization was performed using data from the whole sample after excluding participants with exclusion criteria 1) and 2) This procedure was preferred to the log-transformation of the data, which in several cases did not lead to a normal-distributed variable and could not adequately handle results below the detection limit. Descriptive results were expressed as number of participants and (percentage) or as average ± standard deviation. Bivariate analyses were performed using chi-square test for qualitative variables and Student's t-test or analysis of variance for quantitative variables.

Multivariate analysis was performed by logistic regression on baseline data and using non-treated participants as reference. The results were expressed as Odds ratio (OR) and 95% confidence interval (CI). Two models were applied: 1) assessing the likelihood of being in the topmost quartile relative to the other three quartiles and 2) assessing the likelihood of being in the topmost quartile relative to the lowest one. A second set of analysis was conducted using only participants treated for hypertension and comparing participants taking ARB to participants not taking ARB. The whole analytical procedure was replicated using data from the follow-up period. Due to the number of comparisons performed, statistical significance was assessed for $p<0.001$.

RESULTS

Characteristics of participants at baseline

Of the 6733 participants at baseline, 933 (13.9%) were included. The reasons for exclusion are summarized in **figure 1**. Of the 933 participants retained, 424 (45.4%) were on

ARBs, but none of them had ARBs on monotherapy; their characteristics according to group are summarized in **table 1**. Participants on ARBs had a higher BMI, and were more frequently obese and less frequently widowed than participants not taking ARBs, while no differences were found for all other characteristics (**table 1**).

Association of ARB treatment with inflammatory markers

The distribution within the different quartiles of inflammatory markers according to antihypertensive drug treatment is summarized in **table 2**. No differences were found between participants taking or not taking ARBs for all inflammatory markers studied. Participants on ARBs had a borderline ($p<0.07$) higher likelihood of being in the highest quartile of CRP than participants not taking ARBs (**table 2**).

Multivariate analysis was conducted adjusting for gender, age, marital status, physical activity, education categories, alcohol drinking (yes/no), smoking categories, body mass index categories and diabetes (yes/no). The results are summarized in **table 3**. No differences were found between participants taking or not taking ARBs. A further analysis assessing the individual effect of each type of antihypertensive drug using diuretics as reference showed no specific effect of ARB on inflammatory markers, although a borderline higher likelihood of being the highest quartile of TNF-α was found for ARB (**table 3**).

Replication in the follow-up survey

Of the 5064 participants (75.2% of baseline) who completed the follow-up, 1120 (22%) were treated for hypertension and thus included in the analysis (**supplementary figure 1**). Replication of the analysis confirmed the lack of specific effect of ARB on all four inflammatory markers (**supplementary tables 1 and 2**).

Of the 1093 participants treated for hypertension and who completed the follow-up, 572 (52%) had no ARB at baseline and follow-up, 268 (25%) had no ARB at baseline but had ARB prescribed during follow-up, and 253 (23%) had ARB at baseline and follow-up. Multivariate analysis comparing the groups with previous or newly introduced ARBs relative to participants who never had ARB showed no specific effect of ARB on inflammatory markers (**table 4**).

DISCUSSION

To our knowledge, this is one of the first studies to assess the anti-inflammatory effect of ARBs in a general population setting. Our results do not confirm a specific anti-inflammatory effect of ARB relative to the other antihypertensive drugs.

Angiotensin II type 1 receptor activation has been shown to increase TNF-α production in rats [19], prompting the hypothesis that ARBs could have anti-inflammatory properties. Indeed, several mechanisms for the anti-inflammatory effect of ARBs have been suggested (for a review, see [1]) and a number of randomized controlled trials (RCTs) have shown that ARBs decrease inflammatory markers such as CRP, TNF-α or IL-6, although this statement has been challenged. Indeed, out of the 27 studies assessing the effect of ARB on CRP levels reviewed in [1], 12 (44%) reported nonsignificant changes, the effect ranging between a 44% decrease [20] and a 90% increase [21] in CRP levels. Similarly, the effect of ARBs on TNF-α and IL-6 ranged between -39% and +6.8% and between -39% and -4%, respectively [1].

Possible explanations for the discrepancy between the results of RCTs and our study are that most RCTs used log-transformed data, while in this study a more conservative approach was preferred. Still, multivariate analysis of the effect of ARBs on inflammatory

markers using log-transformed data failed to show any significant difference (p-values of 0.08, 0.11, 0.22 and 0.12 for IL-1β, IL-6, TNF-α and CRP, respectively). Hence, it is unlikely that the differences observed between our study and previous RCTs are due to differences in statistical methodology. Similarly, the mean age and male / female ratio of our study did not overtly differ from those of most RCTs (see **supplementary table 3**), so it is also unlikely that the differences observed between our study and previous RCTs are due to gender or age.

The most likely explanation for the discrepancy between the results of most RCTs and our study might be the time period. Indeed, most RCTs were conducted for a limited period of time: only one [22] took longer than 6 months. ARBs block angiotensin II receptors, leading to an increase in renin levels via a feedback loop [23]. The resulting increased levels of renin and prorenin would increase the activity of (pro)renin receptors (P)RR [23], leading to an increased inflammatory cytokine production by the kidney [24]. The (P)RR could enhance the production of these inflammatory cytokines through direct stimulation of ERK1/2-NF-kappaB signaling cascade [25]. Interestingly, (P)RR blockers have been suggested to reduce sepsis-induced systemic inflammatory response in a rat model [26]. Thus, the initial decrease in inflammatory markers due to ARBs could be offset in the long run by an increased (P)RR-mediated cytokine production, thus explaining the lack of anti-inflammatory effect of ARBs observed in our study and also in the longest RCT [22]. **Figure 2** summarizes the hypothesis explaining the lack of anti-inflammatory effect of ARBs in the long term. This mechanism could also explain the neutralization of the anti-inflammatory effect of ARBs by hydrochlorothiazide in the Val-MARC study [27], as hydrochlorothiazide has been shown to increase renin levels [28].

Strengths and limitations

This study relied on a large, population-based sample of participants treated for hypertension; it thus reflects the expected, "real-life" effect of ARBs on inflammatory markers rather than the effect observed in a carefully selected group of patients from a RCT. The study was also based in sample considerably larger than most RCTs (**supplementary table 3**), thus enabling the detection of relatively small effect sizes.

This study has several limitations worth acknowledging. First, no data was available regarding compliance, dosage or length of treatment with ARBs. Hence, it is possible that the putative anti-inflammatory effect of ARBs might have been lessened by noncompliers, participants on low dosage or on ARBs for a short period of time. Still, analysis of participants who received ARBs during 5.5 years follow-up and of participants newly prescribed with ARBs during follow-up did not show any significant effect relative to participants who received another class of antihypertensive drug. Overall, our results do not support a specific anti-inflammatory effect of ARBs or, if such an effect is present, its magnitude is too small to be detected using the current sample size. Second, it is possible that participants who were prescribed ARBs differed from participants not prescribed ARBs by other characteristics than those used for adjustment. Hence, we cannot completely rule out that the absence of anti-inflammatory effect of ARBs cannot be explained by unaccounted confounders. Third, it is possible that the other antihypertensive drugs also exert an anti-inflammatory effect, although most RCTs published failed to show such an effect [14, 29, 30]. As there were no participants on ARB monotherapy, the specific effect of ARB devoid of possible confounding by other antihypertensive drugs could not be assessed. Thus, any anti-inflammatory effect detected would be questionable, as it could not be solely attributed to ARBs beyond doubt. Fourth, it is possible that ARBs might exert an anti-inflammatory effect at the local level, which could not be assessed by the circulating

inflammatory biomarkers used in this study. Fifth, no information was collected regarding dosage and duration of antihypertensive treatment; thus, no dose-dependent effect could be assessed and any minor effect on inflammatory markers might just not be recognizable in this data set. Finally, the CoLaus study recruited mainly participants of Caucasian origin (93% of the participants in this study), and it is currently unknown if our results also apply to other ethnicities. It would be of interest that this analysis be replicated in other studies conducted in other ethnicities to confirm or infirm our findings.

CONCLUSION

In a population-based setting, angiotensin receptor blockers are not associated with decreased levels of inflammatory markers.

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CONFLICT OF INTEREST

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TABLES

Table 1: characteristics at baseline of participants according to treatment category

	ARB	No ARB	p-value
Sample size	424	509	
Women (%)	187 (44.1)	226 (44.4)	0.93
Age (years)	59.7 ± 9.1	60.9 ± 9.3	0.07
Marital status (%)			0.03
Married	260 (61.3)	302 (59.3)	
Divorced	87 (20.5)	96 (18.9)	
Single	55 (13.0)	58 (11.4)	
Widowed	22 (5.2)	53 (10.4)	
Physical activity (%)	212 (50.0)	248 (48.7)	0.70
Education (%)			0.56
High	57 (13.5)	61 (12.1)	
Middle	85 (20.1)	115 (22.6)	
Low	282 (66.5)	332 (65.4)	
Alcohol drinker (%)	305 (71.9)	366 (71.9)	0.99
Smoking status (%)			0.89
Never	170 (40.1)	197 (38.8)	
Former	169 (39.9)	210 (41.3)	
Current	85 (20.0)	101 (19.9)	
BMI (kg/m^2)	28.9 ± 5.0	27.7 ± 4.4	<0.001
BMI categories (%)			0.005
Normal	90 (21.2)	142 (27.9)	
Overweight	182 (42.9)	231 (45.4)	

Obese	152 (35.9)	136 (26.7)	
Caucasian origin	397 (93.6)	474 (93.1)	0.76
Diabetes (%)	84 (19.8)	90 (17.7)	0.41
FCI	1.64 ± 1.38	1.52 ± 1.31	0.18
SBP (mm Hg)	141 ± 19	139 ± 17	0.17
DBP (mm Hg)	84 ± 11	83 ± 11	0.05
eGFR (mL / min / 1.73m^2)	73.8 ± 15.4	76.1 ± 19.2	0.05

Results are expressed as mean ± standard deviation or as number of participants (percentage). ARB, angiotension-receptor blockers; BMI, body mass index; FCI, functional comorbidity index; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate. Statistical analysis by Student's t-test or chi-square test.

Table 2: distribution within cytokine quartiles of participants treated with angiotensin receptor blockers and treated with other antihypertensive drugs, CoLaus baseline data.

Quartile	First	Second	Third	Fourth	p-value
Interleukin 1β					
ARB	59 (25.3)	58 (24.9)	59 (25.3)	57 (24.5)	0.59
No ARB	83 (30.7)	55 (20.4)	68 (25.2)	64 (23.7)	
Interleukin 6 (%)					
ARB	72 (18.5)	84 (21.5)	118 (30.3)	116 (29.7)	0.32
No ARB	80 (17.8)	114 (25.3)	143 (31.8)	113 (25.1)	
Tumor necrosis factor α					
ARB	74 (18.3)	90 (22.2)	110 (27.2)	131 (32.4)	0.68
No ARB	99 (20.8)	106 (22.2)	130 (27.3)	142 (29.8)	
C-reactive protein					
ARB	61 (14.4)	71 (16.8)	122 (28.8)	170 (40.1)	0.07
No ARB	79 (15.5)	111 (21.8)	154 (30.3)	165 (32.4)	

Results are expressed as number of subjects and (percentage). ARB, angiotension-receptor blockers. Statistical analysis by chi-square.

Table 3: multivariate analysis of the effect of angiotensin receptor blockers on inflammatory markers, CoLaus baseline data.

	Interleukin-1β	Interleukin-6	TNF-α	CRP
Last vs. all other quartiles				
Model 1				
No angiotensin receptor blockers	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
Angiotensin receptor blockers	1.26 (0.91 - 1.75)	1.27 (0.94 - 1.71)	1.14 (0.85 - 1.53)	1.26 (0.95 - 1.68)
Model 2				
Diuretics	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
Beta-blockers	1.08 (0.75 - 1.57)	1.12 (0.8 - 1.57)	1.04 (0.75 - 1.44)	1.16 (0.84 - 1.60)
Calcium channel blockers	0.87 (0.57 - 1.34)	1.19 (0.82 - 1.72)	1.19 (0.83 - 1.71)	1.57 (1.11 - 2.23) *
ACEIs	1.27 (0.86 - 1.87)	0.83 (0.58 - 1.20)	1.18 (0.83 - 1.67)	0.98 (0.69 - 1.37)
Angiotensin receptor blockers	1.41 (0.96 - 2.07)	1.23 (0.86 - 1.75)	1.24 (0.88 - 1.74)	1.34 (0.96 - 1.87)
Last vs. first quartile				
Model 1				
No angiotensin receptor blockers	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
Angiotensin receptor blockers	1.24 (0.87 - 1.79)	1.34 (0.86 - 2.09)	1.20 (0.81 - 1.79)	1.24 (0.79 - 1.94)

Model 2				
Diuretics	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
Beta-blockers	1.15 (0.77 - 1.74)	1.46 (0.87 - 2.43)	1.26 (0.79 - 2.01)	1.53 (0.9 - 2.59)
Calcium channel blockers	0.96 (0.60 - 1.55)	1.17 (0.67 - 2.03)	1.40 (0.83 - 2.34)	2.07 (1.12 - 3.82) *
ACEIs	1.21 (0.79 - 1.85)	1.03 (0.60 - 1.76)	1.54 (0.94 - 2.52)	0.98 (0.56 - 1.70)
Angiotensin receptor blockers	1.39 (0.91 - 2.12)	1.51 (0.89 - 2.56)	1.53 (0.95 - 2.46)	1.39 (0.81 - 2.38)

Results are expressed as odds-ratio (95% confidence interval) of being in the highest quartile. TNF-α, tumor necrosis factor α; CRP, C-reactive protein; ACEI; angiotensin-converting enzyme inhibitor. Statistical analysis by logistic regression adjusting for gender, age, marital status, physical activity, education categories, alcohol drinking (yes/no), smoking categories, body mass index categories, diabetes (yes/no), functional comorbidity index and estimated glomerular filtration rate ; *, $p<0.05$.

Table 4: multivariate analysis of the effect of changing antihypertensive treatment on inflammatory markers.

	Interleukin-1β	Interleukin-6	TNF-α	CRP
Last vs. all other quartiles				
No ARB at baseline and follow-up	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
No ARB at baseline, ARB at follow-up	1.13 (0.76 - 1.67)	0.94 (0.65 - 1.35)	0.70 (0.48 - 1.02)	0.92 (0.64 - 1.30)
ARB at baseline and follow-up	0.91 (0.59 - 1.39)	0.78 (0.53 - 1.15)	0.82 (0.56 - 1.19)	0.93 (0.65 - 1.32)
Last vs. first quartile				
No ARB at baseline and follow-up	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
No ARB at baseline, ARB at follow-up	1.60 (0.96 - 2.67)	1.13 (0.69 - 1.86)	0.81 (0.49 - 1.34)	1.05 (0.63 - 1.73)
ARB at baseline and follow-up	0.91 (0.55 - 1.50)	0.79 (0.48 - 1.31)	0.79 (0.48 - 1.29)	0.91 (0.54 - 1.52)

Data for all participants who completed the follow-up and who were retained for analysis. Results are expressed as odds-ratio (95% confidence interval) of being in the highest quartile. TNF-α, tumor necrosis factor α; CRP, C-reactive protein; ARB, angiotensin-receptor blockers. Statistical analysis by logistic regression adjusting for gender, age, marital status, physical activity, education categories, alcohol drinking (yes/no), smoking categories, body mass index categories, diabetes (yes/no) and estimated glomerular filtration rate.

Figure 1: flowchart of the participant's selection, baseline survey.

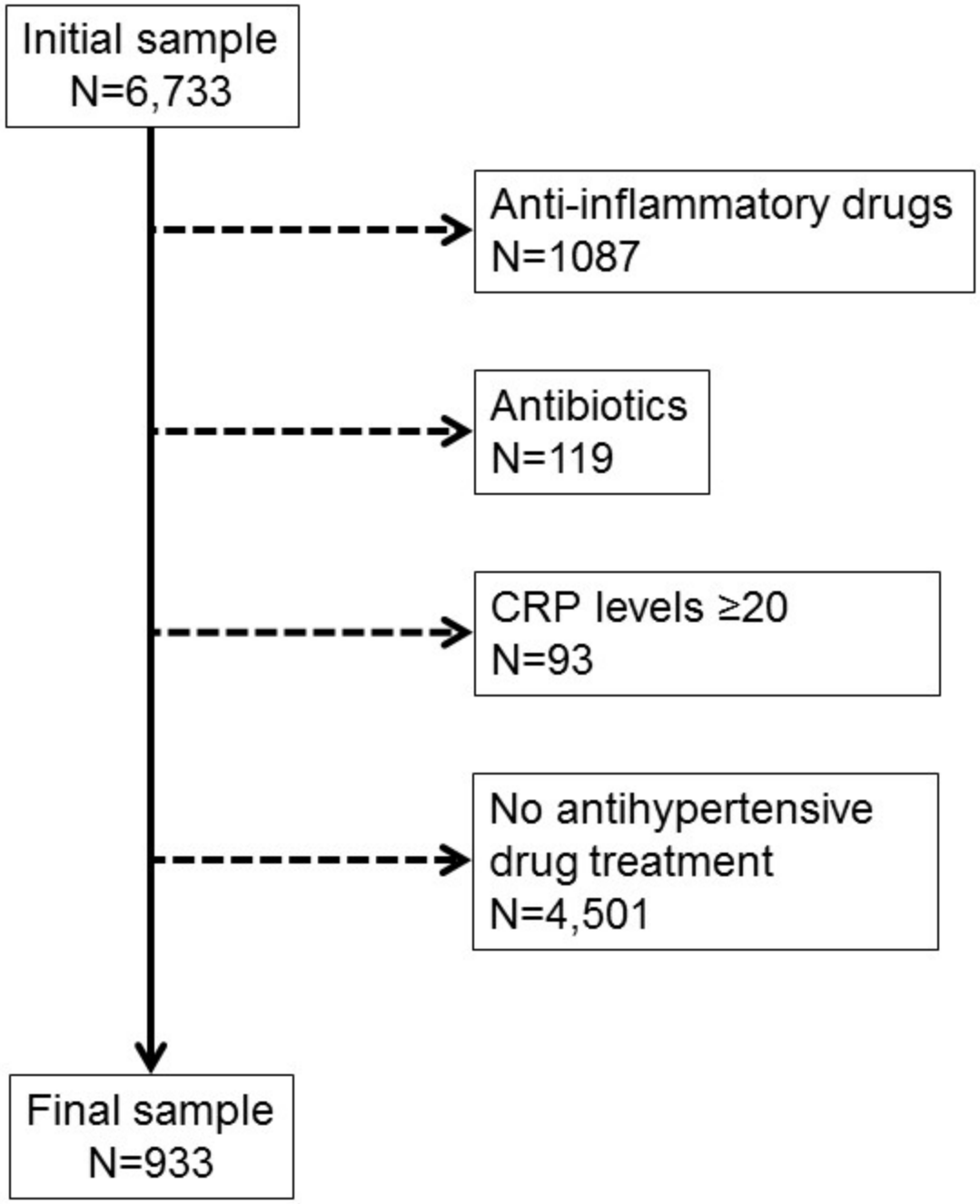
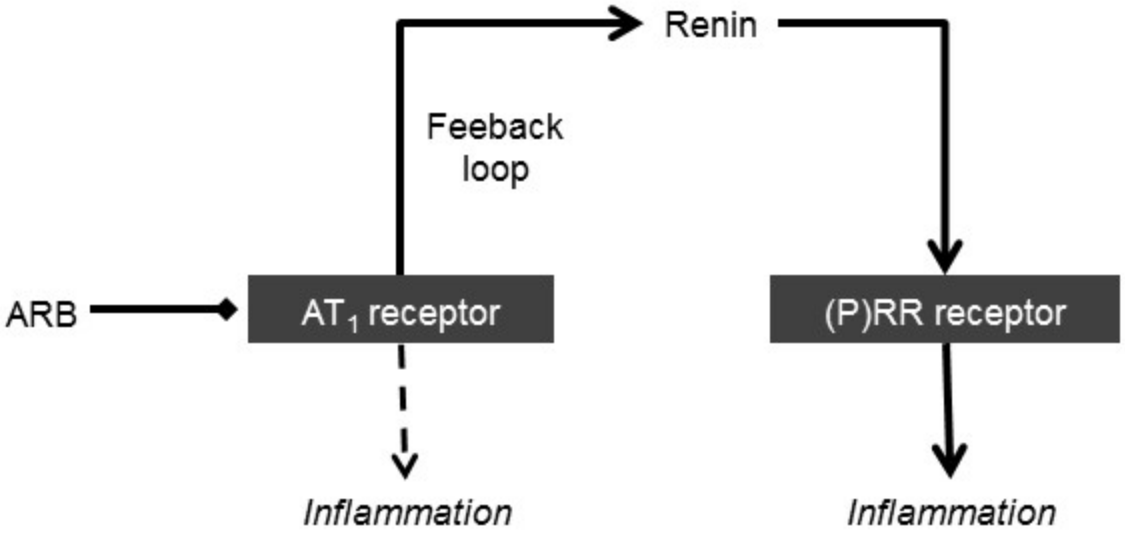
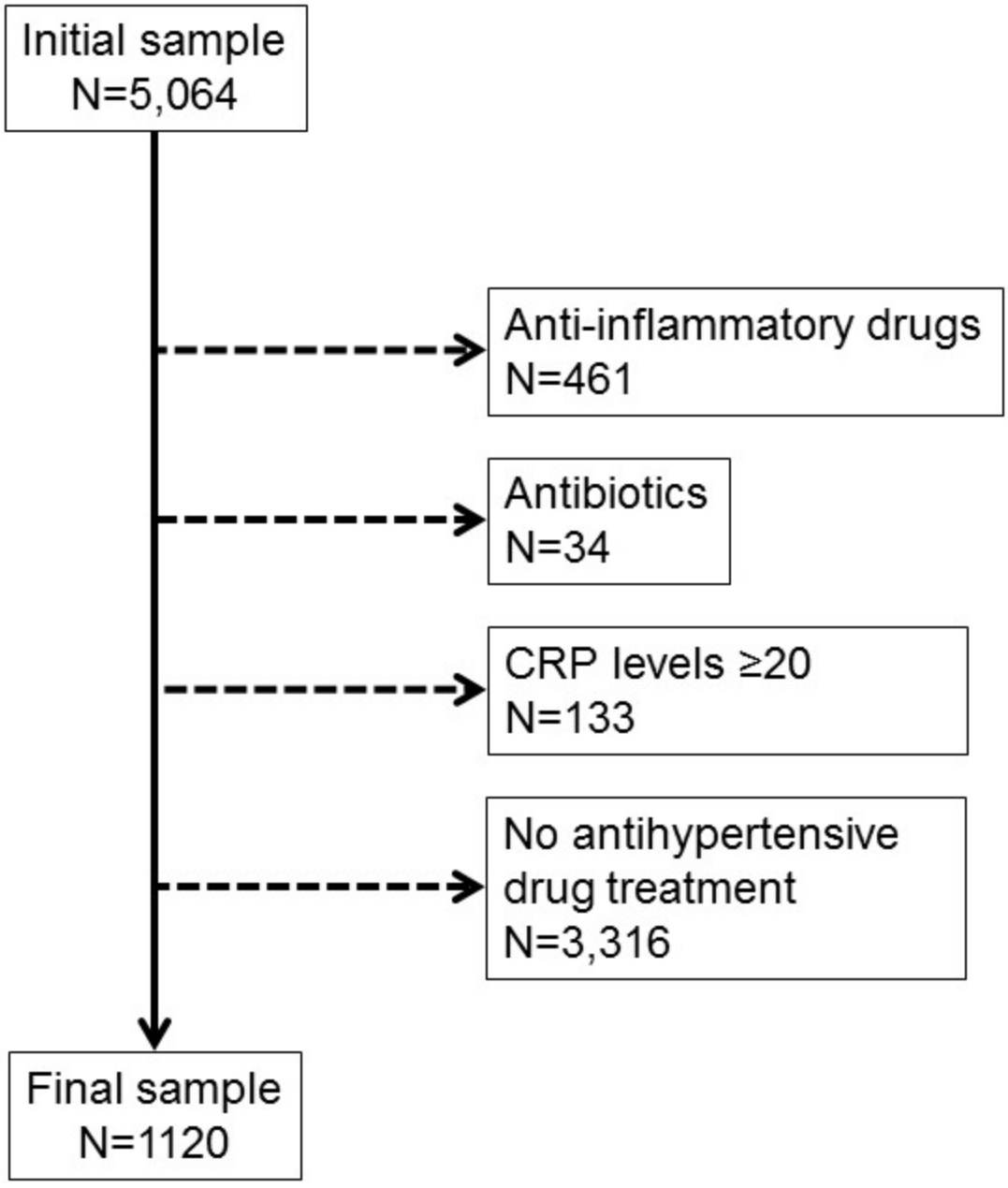


Figure 2: hypothetical schema of the lack of anti-inflammatory effect of angiotensin receptor blockers.



SUPPLEMENTAL MATERIAL

Supplementary figure 1: flowchart of the participant's selection, follow-up survey.



Supplemental table 1: distribution within cytokine quartiles of participants treated with angiotensin receptor blockers and with other antihypertensive drugs, CoLaus follow-up data.

Quartile	First	Second	Third	Fourth	p-value
Interleukin 1β					
ARB	132 (25.3)	139 (26.7)	137 (26.3)	113 (21.7)	0.35
No ARB	156 (30.2)	126 (24.3)	124 (24.0)	111 (21.5)	
Interleukin 6					
ARB	102 (19.5)	126 (24.1)	161 (30.9)	133 (25.5)	0.44
No ARB	114 (22.0)	125 (24.1)	138 (26.5)	142 (27.4)	
Tumor necrosis factor α					
ARB	103 (19.7)	124 (23.8)	158 (30.3)	137 (26.2)	0.31
No ARB	113 (21.8)	105 (20.2)	146 (28.1)	155 (29.9)	
C-reactive protein					
ARB	100 (17.9)	112 (20.0)	147 (26.3)	200 (35.8)	0.90
No ARB	110 (19.6)	111 (19.8)	146 (26.0)	194 (34.6)	

Results are expressed as number of subjects and (percentage). ARB, angiotensin-receptor blockers. Statistical analysis by chi-square.

Supplemental table 2: multivariate analysis of the effect of angiotensin receptor blockers on inflammatory markers, CoLaus follow-up data.

	Interleukin-1β	Interleukin-6	TNF-α	CRP
Last vs. all other quartiles				
Model 1				
No angiotensin receptor blockers	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
Angiotensin receptor blockers	1.08 (0.77 - 1.53)	0.86 (0.63 - 1.17)	0.80 (0.59 - 1.10)	0.90 (0.67 - 1.20)
Model 2				
Diuretics	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
Beta-blockers	0.86 (0.56 - 1.31)	0.80 (0.55 - 1.17)	0.97 (0.66 - 1.40)	0.89 (0.62 - 1.26)
Calcium channel blockers	0.95 (0.59 - 1.51)	0.68 (0.44 - 1.06)	1.32 (0.88 - 1.98)	1.11 (0.76 - 1.63)
ACEIs	0.83 (0.49 - 1.39)	0.93 (0.58 - 1.47)	1.15 (0.73 - 1.83)	0.89 (0.58 - 1.37)
Angiotensin receptor blockers	0.93 (0.57 - 1.51)	0.74 (0.48 - 1.16)	0.87 (0.56 - 1.35)	0.81 (0.54 - 1.23)
Last vs. first quartile				
Model 1				
No angiotensin receptor blockers	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
Angiotensin receptor blockers	1.29 (0.85 - 1.96)	0.94 (0.62 - 1.42)	0.84 (0.56 - 1.27)	0.95 (0.62 - 1.46)

Model 2				
Diuretics	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
Beta-blockers	0.84 (0.50 - 1.43)	0.82 (0.49 - 1.37)	0.98 (0.60 - 1.57)	0.95 (0.56 - 1.60)
Calcium channel blockers	1.15 (0.64 - 2.08)	0.68 (0.38 - 1.23)	1.24 (0.72 - 2.14)	1.12 (0.63 - 1.98)
ACEIs	0.97 (0.52 - 1.82)	0.85 (0.46 - 1.59)	1.04 (0.58 - 1.87)	0.72 (0.39 - 1.34)
Angiotensin receptor blockers	1.19 (0.65 - 2.19)	0.79 (0.43 - 1.45)	0.87 (0.49 - 1.55)	0.79 (0.43 - 1.46)

Results are expressed as odds-ratio (95% confidence interval) of being in the highest quartile. ACEI, angiotensin-converting enzyme inhibitor; TNF-α, tumor necrosis factor α; CRP, C-reactive protein. Statistical analysis by logistic regression adjusting for gender, age, marital status, physical activity, education categories, alcohol drinking (yes/no), smoking categories, body mass index categories, diabetes (yes/no) and estimated glomerular filtration rate.

Supplemental table 3: comparison of the characteristics of the CoLaus sample with those from randomized controlled trials exploring the effect of angiotensin receptor blockers relative to other antihypertensive treatment on inflammatory markers

Author (ref)	**Year**	**Number of patients under ARB / total**	**Mean age ± SD (ARB group)**	**Male / Female ratio (ARB group)**	**Duration of treatment**
Jilma [1]	2002	15 / 32	59 ± 13	19 / 13	8 weeks
Rahman [2]	2002	19 / 38	43 ± 8.1	11 / 8	4 weeks
Koh [3]	2003	NR / 45	50 ± 2	33 / 12	2 months
Fliser [4]	2004	100 / 199	58 ± 9.8	47 / 53	12 weeks
Koh [5]	2004	NR / 47	57 ± 2	20 / 27	2 months
Sardo [6]	2004	20 / 40	49 ± 7.2	12 / 8	4 weeks
Schieffer [7]	2004	21 / 48	56 ± 8	16 / 5	3 months
Manabe [8]	2005	29 / 45	59 ± 14	16 / 13	3 months
Rosei [9]	2005	61 / 118	59 ± 7	41 / 20	24 weeks
Schram [10]	2005	24 / 70	60 ± 7	13 / 11	12 months
Link [11]	2006	21 / 42	58 ± 11.6	17 / 4	12 weeks

Nagel [12]	2006	20 / 20	36.8 ± 11.2	NR	12 weeks
Nomura [13]	2006	53 / 73	61 ± 7	9 / 16	8 weeks
Ogawa [14]	2006	33 / 66	58.7 ± 1.6	16 / 17	8 weeks
Rajagopalan [15]	2007	137 / 404	62.9 ± 8.1	71 / 66	12 weeks
Ogawa [16]	2009	13 / 13	NR	7 / 6	16 weeks
All studies *	2002-9	666 / 1208	56.8 ± 8.7	348 / 279	
Current study	2006	424 / 509	60.3 ± 9.2	237 / 187	

NR; not reported; *, using available data

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