The association between serum inflammatory biomarkers and incident hypertension among postmenopausal women in the Buffalo OsteoPerio Study

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Abstract

Several serum inflammatory biomarkers have been associated with blood pressure and hypertension prevalence in cross-sectional studies. Few of these associations have been evaluated prospectively. We examined associations for 10 serum inflammatory biomarkers with incident hypertension among 471 postmenopausal women (mean age = 65) in the Buffalo OsteoPerio Study. Concentrations of C-reactive protein, interleukin (IL)-2, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor (TNF)-\textalpha, monocyte chemoattractant protein (MCP)-1, adiponectin, and leptin were measured using multiplexed sandwich immunoassays on fasting serum samples collected at baseline (1997–2001). Incident hypertension (195 cases) was defined as physician-diagnosed
hypertension and treatment with medication identified on annual mailed health surveys during follow-up (mean 10 years). Cox regression was used to estimate hazard ratios (HR) and 95% confidence intervals (CI) between log-transformed biomarkers (per 1-SD) and hypertension. When adjusted for age, leptin was significantly associated with hypertension risk (HR=1.55, 95% CI: 1.04, 2.29), however, the association was attenuated and not significant after adjustment for demographic and lifestyle factors, including BMI. Significant (P<0.10) interactions were observed for smoking (never, ever) with CRP (HR: Never, 1.31; Ever, 0.91; P=0.06) and MCP-1 (HR: Never, 0.59; Ever, 5.11; P=0.004); for BMI (<25, ≥25) with MCP-1 (HR: <25, 3.45; ≥25, 0.95; P=0.07); for systolic BP with IL-10 (HR: <120, 0.85; 120–139, 1.11; P=0.07); and for diastolic BP with MCP-1 (HR: <80, 1.29; 80–89, 0.84; P=0.03) and with adiponectin (HR: <80, 0.86; 80–89, 1.50; P=0.03). This study adds needed understanding on prospective associations between several serum inflammatory biomarkers and hypertension risk in older postmenopausal women, among whom hypertension burden is substantial.

INTRODUCTION

In the United States, hypertension affects >65% of women ages 65 and older.1 The etiologic mechanisms involved with essential hypertension are not fully understood.2 Understanding the mechanisms for hypertension development is critical given its role as a risk factor for cardiovascular disease and stroke.3 There is mounting evidence in animal models for the involvement of the immune system and inflammation in the pathogenesis of hypertension, with a variety of immune cells and cell signaling molecules thought to play a role.4 Nevertheless, if discrete innate and adaptive immunological mechanisms were primary causes of hypertension, we might expect that genes associated with the immune system would be associated with hypertension. Few of the genetic loci associated with hypertension identified in genome-wide association studies are associated with inflammation and the immune system,5 suggesting that inflammation may be an accelerating rather than initiating factor in the development of hypertension.6

Cross-sectional epidemiologic studies have demonstrated associations between multiple inflammatory biomarkers and hypertension prevalence;7,8 however, these studies cannot distinguish whether inflammation preceded hypertension, is a concurrent phenomenon, or was possibly a consequence of blood pressure dysregulation and the hypertension itself. Prospective studies have reported on serum inflammatory biomarkers including C-reactive protein (CRP), interleukin (IL)-6,9–11 adiponectin,12 and leptin13 in relation to hypertension incidence. However, other cytokines believed to be involved in hypertension development, such as IL-2, IL-4, IL-8, IL-10, and MCP-1, have been less frequently evaluated in prospective study designs. Furthermore, few prospective studies have investigated interactions between concentrations of these inflammatory biomarkers and traditional hypertension risk factors, like age, body mass index (BMI), menopausal hormone therapy (HT) use, and smoking. Stratified analyses may give additional clues to how biomarkers influence hypertension. The goals of the present study were to (1) prospectively evaluate the association for concentrations of 10 serum inflammatory biomarkers (CRP; IL-2, 4, 6, 8, 10; TNFα; MCP-1; leptin; adiponectin) with incident hypertension in postmenopausal women, and (2) determine whether associations between serum inflammatory biomarkers...
and incident hypertension vary according to age, BMI, HT use, smoking, and baseline blood pressure.

**METHODS**

**Study design/population**

Participants in this study were postmenopausal women enrolled in the Buffalo Osteoporosis and Periodontitis (OsteoPerio) Study,\textsuperscript{14} an ancillary study to the Women’s Health Initiative Observational Study (WHI-OS).\textsuperscript{15} at the Buffalo WHI clinical center. The OsteoPerio study was comprised of 1,341 postmenopausal women enrolled in 1997–2001 who had ≥6 teeth present, no bilateral hip replacement, no history of bone disease other than osteoporosis, no cancer in the last 10 years, and no other serious illness.\textsuperscript{14} Participants were excluded from the present analysis if they had prevalent hypertension defined by self-reported history of physician-diagnosed hypertension treated with antihypertensive medication (n=518), were missing baseline blood pressure (BP) measurements (n=9), had clinically measured resting systolic BP ≥140 mmHg, or diastolic BP ≥90 mmHg at baseline (n=235), were missing follow-up information (n=1) or did not have available serum samples (n=249). Participants missing serum samples attended baseline study visits prior to initiation of serum collection protocols. After application of exclusion criteria, 471 women were included in the present analysis (Figure 1). All study protocols were approved annually by the University at Buffalo Health Sciences Institutional Review Board. Participants provided written informed consent prior to completing study activities.

**Hypertension Outcome**

Incident hypertension was ascertained yearly during follow-up in the WHI OS using mailed health surveys (Form 33). Participants were asked “Since your last completed questionnaire, has a doctor or other healthcare provider prescribed for the first time pills for high blood pressure or hypertension?”. This case finding question is similar to that used in other prospective epidemiologic studies on well-educated adults,\textsuperscript{9,10} and has shown excellent agreement (>85%) with criterion measures of hypertension.\textsuperscript{16,17} The kappa coefficient between hypertension status based on this question and Medicare claims data is 0.84 in the larger WHI (unpublished data).

**Other Assessments**

Study covariates assessed by questionnaire included age, race-ethnicity, education, smoking status (never, former, current) and intensity (pack-years), recreational physical activity (metabolic equivalent (MET)-hours/week), history cardiovascular disease (myocardial infarction or stroke) and physician-diagnosed diabetes with treatment by medication. Alcohol intake (oz/day), dietary intake (Healthy Eating Index 2005 score) and sodium intake (mg/day) were determined using a food frequency questionnaire.\textsuperscript{18} Neighborhood socioeconomic status was based on information pertaining to education, household income and census tract.\textsuperscript{19} Body mass index (BMI; kg/m\textsuperscript{2}) was calculated from measured height and weight. Resting systolic and diastolic BP (mmHg) were measured in the seated position, following five minutes of quiet sitting, by auscultation with a manual sphygmomanometer.
and cuff size based on measured arm circumference.\textsuperscript{20} The average of two readings taken at least 30 seconds apart, were recorded and used in analysis.

**Biomarker Measures**

**Blood Collection and Storage.**—Following an overnight fast and recommended abstinence from smoking and heavy exertion, participants gave a fasting venous blood sample in the morning at the beginning of the study visit.\textsuperscript{21} Samples for cytokine assessment were collected by venipuncture using 10cc (red top) tubes without anticoagulant. Tubes were left in darkness for 30 minutes so that samples could clot, and were then centrifuged. Serum was removed and transferred to 0.5 mL straws which were frozen in −80°C freezers prior to long-term submersion in liquid nitrogen (−196°C). Quality control pools were created using samples at a single visit in 24 individuals, and stored in straws in liquid nitrogen. Straws were shipped on dry ice to The Forsyth institute, Boston, MA and remained stored in −80°C freezers until analysis.

**Inflammatory Biomarker Selection and Measurement.**—Biomarkers were selected for analysis in the present study on the basis of hypothesized associations with hypertension as supported by previous published studies and biological reasoning. The set of biomarkers included herein had previously been measured in a separate investigation in the OsteoPerio cohort.\textsuperscript{22} Biomarker concentrations were measured using multiplexed sandwich immunoassays on the Luminex 100 Bio-Plex Platform (Bio-Rad, Hercules, CA).\textsuperscript{22} The multiplex protocol and quality control experiment results are described in detail elsewhere.\textsuperscript{22} Commercially available biomarker-specific multiplex assay kits were further optimized in our laboratory.\textsuperscript{22} The multiplex panels from which biomarkers in the present study derived were a “10-plex” panel (TNFα, IL-1β, IL-6, IFN-gamma, IL-4, IL-10, IL-2, IL-5, IL-8, GM-CSF) from Invitrogen (Ultrasensitive kit, Invitrogen, Carlsbad, CA); a “bone” panel (osteoprotegerin, leptin, parathyroid hormone, and insulin) from Millipore (EMD Millipore, Billerica, MA); an “obesity” panel (adiponectin and CRP) from R&D Systems (R&D Systems, Minneapolis, MN); and, a “4-plex” panel (VEGF, IL-17, TNFα and MCP-1) from R&D Systems. The biomarkers included in the present study can be generally categorized as pro-inflammatory with respect to endothelium and vasculature (CRP, IL-2, IL-4, IL-6, IL-8, TNFα, MCP-1, leptin) or anti-inflammatory (IL-10, and adiponectin).\textsuperscript{23–25}

**Statistical analysis**

Baseline participant characteristics and biomarker concentrations were compared between incident hypertension cases and non-cases using Student’s t-tests for continuous variables and Chi-squared tests for categorical variables. Correlations between biomarkers were assessed using Pearson Product-Moment coefficients after log(10)-transforming biomarker concentrations. Missing values of biomarkers and covariates were imputed to complete the dataset 10 times. Imputation was used to maximize the study sample size available for analysis and to allow for analysis of biomarkers in continuous format, which enhances statistical efficiency and power. Supplemental Table 1 gives the number of values imputed for all variables studied. Rubin’s rules were used to combine analysis of 10 datasets and the aggregated results are reported. Imputation occurred in two steps. First, for a biomarker value that was missing because the assay indicated the true concentration was
beyond the test’s limits of detection, a biomarker value was generated from the appropriate tail of a lognormal distribution that was fit with censored regression methods (PROC LIFEREG) to the observed and out-of-range (right- and left-censored) biomarker values. Second, biomarker values missing due to assay failure and other missing covariate values were generated by Markov chain Monte Carlo simulation from the covariate’s posterior distribution (PROC MI). The primary analysis relating baseline biomarker concentrations with hypertension incidence was conducted using Cox proportional hazards regression to estimate hazard ratios (HR) and 95% confidence intervals (CI) as measures of association with incident hypertension. The base model was adjusted for age only (Model 1), followed by two additional models adjusting for age, race-ethnicity, education, neighborhood socioeconomic status, smoking history, HT use, alcohol intake, dietary Healthy Eating Index score, and physical activity (Model 2), and additionally for BMI (Model 3). HRs were estimated based on continuous log-transformed biomarker concentrations, and are reported for a 1-SD difference in baseline concentration. Interactions for biomarkers with age (<65 years vs. ≥65 years), smoking status (never vs. ever), current HT use (no, yes), BMI (<25 vs. ≥25 kg/m²), and baseline BP (systolic: <120 vs 120–139; diastolic: <80 vs 80–89) were tested with cross-product terms between each biomarker and the above covariates using Model 2 adjustments. Interaction hypothesis tests were conducted at alpha 0.10 to reduce the likelihood of type 2 error due to relatively small cell sizes. All other statistical tests were two-sided conducted at alpha 0.05. Reported p-values are not corrected for multiple comparisons. Analyses were completed using SAS® software, version 9.4 (Cary, NC, USA).

RESULTS

There were 195 (41.4%) cases incident physician-diagnosed hypertension treated with medication identified during an average follow-up of 10.2 years. Baseline participant characteristics according to incident hypertension status are in Table 1. Women were, on average, 65 years of age, mostly white (98.1%) and the majority were educated beyond high school. Women who developed hypertension had a significantly higher BMI (26.7 vs. 24.9 kg/m²), higher proportion of ever-smokers (57.4% vs. 40.6%), and higher systolic (118.4 vs 109.0 mmHg) and diastolic (70.5 vs. 66.5 mmHg) BP, compared with non-cases. Education, neighborhood socioeconomic status, alcohol intake, dietary healthy eating index and sodium intake, physical activity, and history of diabetes and cardiovascular disease were not significantly different between those with and without incident hypertension.

Biomarker concentrations according to incident hypertension status are in Table 2. Median CRP, IL-8, TNF-α, MCP-1, Leptin, and IL-10 concentrations at baseline tended to be somewhat higher in women who developed incident hypertension compared with those who did not.

Pearson correlation coefficients among the log-transformed biomarkers are in Table 3. The largest correlations were between IL-2 and IL-4 (r = 0.59), IL-6 and IL-10 (r = 0.56), IL-6 and IL-2 (r = 0.47), and IL-2 and IL-10 (r = 0.40), all P<0.05. The strongest correlation with CRP was leptin (r = 0.24, P<0.05).
Prospective associations between baseline biomarkers and incident hypertension are in Table 4. When adjusted for age, each 1-SD increment in leptin was associated with a 55% higher risk of developing hypertension (HR = 1.55, 95% CI: 1.04, 2.29). Further adjustment for baseline covariates (Model 2, HR = 1.42) modestly attenuated the association, which no longer was statistically significant. This association was markedly attenuated when additionally adjusted for BMI (Model 3, HR = 0.69). Findings for the other proinflammatory biomarkers were inconsistent (Table 4). Positive associations were observed, as expected, for CRP, IL-8, TNFα, and MCP-1, whereas, inverse associations for IL-2, IL-4, and IL-6. None of these associations achieved statistical significance. Both anti-inflammatory biomarkers (IL-10, adiponectin) were inversely associated with hypertension risk, but these associations were not statistically significant.

Interactions were evaluated for each biomarker with baseline age, smoking history, HT use, BMI, and BP (Table 4). Statistically significant interactions (P<0.10) were evident for smoking with CRP (P=0.06) and MCP-1 (P=0.004); for BMI with MCP-1 (P=0.07); for systolic BP with IL-10 (P=0.07); and, for diastolic BP with MCP-1 (P=0.03) and with adiponectin (P=0.03). Supplemental Table 2 gives the HRs and 95% CI for the entire stratified analysis. With respect to the above interactions achieving statistical significance, multivariable adjusted HRs associated with CRP were higher in never (HR = 1.31) compared with ever (HR = 0.91) smokers, and HRs with MCP-1 higher in ever (HR = 5.11) than never (HR = 0.59) smokers. A stronger association with MCP-1 was also observed for BMI <25 (HR = 3.45) than BMI ≥25 (HR = 0.95). Baseline systolic BP of 120–139 was associated with a stronger hypertension risk for IL-10 (HR = 1.11) as compared with systolic BP <120 (IL-10, HR = 0.85). A stronger association for MCP-1 was seen for diastolic BP <80 (HR = 1.29) compared with 80–89 (HR = 0.84). Adiponectin was more strongly associated with hypertension when diastolic BP was 80–89 (HR = 1.50) than <80 (HR = 0.86).

DISCUSSION

The present study evaluated the associations between 10 inflammatory biomarkers and incident hypertension in a well-characterized cohort of postmenopausal women residing in the community. While CRP, IL-6, adiponectin, and leptin had previously been examined in studies of incident hypertension, similar data was less available on the association between IL-2, IL-4, IL-8, IL-10, MCP-1, and TNFα. Furthermore, few previous studies have assessed for potential interaction with age, BMI, HT use, and smoking status. In cross-sectional correlation analyses (Table 3), we observed several correlations among measured biomarkers that would be expected. For instance, we observed a significant positive correlation between CRP and leptin, as well as significant inverse correlation between leptin and adiponectin. In our prospective analysis, each 1-SD increment in baseline leptin concentration was associated with a significant 55% higher risk of hypertension in an age-adjusted model, but the association was attenuated and no longer significant when additional covariate adjustments were made. In the Rancho Bernardo cohort of older women (mean age, 66), each 1-unit increment in log-transformed leptin was associated with a significant 3-fold higher multivariable odds of incident hypertension. In the Copenhagen Heart Study, which included middle-aged women (mean age, 45), each 1-SD increment in log-transformed leptin was associated with a significant sex and covariate adjusted odds...
ratio of 1.34, when baseline BP and BMI were not in the model and were about 1.20 when these factors also were controlled.\textsuperscript{13} Findings similar to these were reported in a middle-aged Danish cohort.\textsuperscript{29} It is not clear why the association between leptin and hypertension risk did not remain significant beyond the age-adjusted model when further controlling for several of the same covariates as in the above studies where a significant multivariable association was reported. Endothelial dysfunction and arterial wall remodeling leading to vascular stiffening are mechanisms through which higher circulating leptin concentrations affect BP control and hypertension onset.\textsuperscript{30} We also evaluated adiponectin, an adipokine with anti-inflammatory properties that has been evaluated with risk of hypertension.\textsuperscript{29, 31, 32} In the Dallas Heart Study, the risk ratio associated with a 1-SD increment in log-transformed adiponectin was 0.83 (P<0.05) in sex-adjusted analyses controlling for covariates including BP and BMI, with no difference in the association between middle-aged white and black adults.\textsuperscript{32} In a previous nested case-control study in the WHI, a significant inverse multivariable association was seen in older black (quartile 4 vs 1: risk ratio = 0.49, Trend, P<0.001) but not white (risk ratio = 0.82, Trend, P = 0.14) women.\textsuperscript{31} Adiponectin was not associated with hypertension risk in a large middle-aged Danish cohort, where the non-significant multivariable odds ratio of 0.93 was reported.\textsuperscript{29} This is comparable to the result observed in our present study (Model 3: HR = 0.97, Table 4). Unlike the previous WHI study,\textsuperscript{31} our study did not have sufficient numbers of women from racial-ethnic subgroups to permit evaluating a race-ethnicity interaction. Hypoadiponectinemia is thought to contribute to arterial stiffening in adults,\textsuperscript{30} a mechanism that might explain an inverse association with hypertension risk. Unlike previous studies that reported significant strong positive associations (multivariable risk ratios 1.64–4.19) between CRP and hypertension risk\textsuperscript{9–11, 16, 33} including an earlier study in WHI women,\textsuperscript{9} this association was modest (HR = 1.11, Model 2, Table 4) and not statistically significant in our present study. Our result is, however, the same as the non-significant positive age- and sex-adjusted association (OR = 1.11) for CRP with incident hypertension reported in Danish adults.\textsuperscript{29} Associations with hypertension risk for the other pro-inflammatory biomarkers (IL-2, IL-4, IL-6, IL-8, TNFα, MCP-1) were inconsistent. Inverse associations were evident for IL-2, 4, and 6, whereas, positive associations were observed for IL-8, TNFα, and MCP-1. Of those positively associated with hypertension, the strongest was MCP-1, although the association did not achieve statistical significance. Even when adjusted for all covariates, including BMI, a 43% higher risk of hypertension was associated with each 1-SD increment in MCP-1. This result need be interpreted cautiously, nevertheless, it points to a pro-inflammatory biomarker for which an association with hypertension is not nearly as attenuated by adiposity as is several other pro-inflammatory biomarkers including CRP and leptin. MCP-1 is a potent pro-inflammatory cytokine pathway initiated by CRP as part of the response to tissue injury, such as vascular endothelial injury during atherogenesis that results in continuous activation of monocytes and vascular inflammation.\textsuperscript{34} Even in individuals without clinical evidence of atherosclerosis, MCP-1 is higher in hypertensive than non-hypertensive adults.\textsuperscript{35}
To further understand how inflammatory biomarkers might influence hypertension risk in older women, we explored interactions between the biomarkers and several factors known to be particularly influential on BP with aging (Table 4; Supplemental Table 2). No evidence of interaction was evident with age or HT use. Significant interactions with MCP-1 were present for smoking, BMI and diastolic BP. Women who were former or current (ever) smokers had a significant 5-fold higher risk associated with each 1-SD increment in MCP-1, whereas risk among never smokers was not significantly elevated. This is consistent with cross-sectional results showing a strong correlation between MCP-1 and BP in smokers.36 Surprisingly, a significant increased hypertension risk associated with MCP-1 was seen among women with normal BMI (<25 kg/m^2; HR = 3.45) and diastolic BP (<80 mmHg; HR = 1.29) at baseline (Supplemental Table 2). We anticipated that the pro-inflammatory nature of obesity would potentially work synergistically with pro-inflammatory biomarkers to increase hypertension risk, which was not observed in our present study. MCP-1 concentrations have been shown to be higher in non-obese compared with obese adults,37 suggesting that factors other than adiposity might contribute to activation of the MCP-1 pathway. Controlling for BMI in our primary analysis had only a modest influence on the association between MCP-1 and hypertension (Table 4). The explanation for why we observed increased hypertension risk associated with MCP-1 in women with normal diastolic BP is unclear and requires further investigation. Lastly, we observed significantly higher hypertension risk associated with adiponectin among women with elevated diastolic BP (80–89 mmHg; HR 1.50). This too is contrary to what might be expected seeing that adiponectin typically is associated with anti-inflammatory properties and lower BP,30 and requires further investigation.

Our study has both strengths and limitation to consider when interpreting its results. Strengths include the prospective design, which allows us to evaluate temporality between inflammatory biomarkers and hypertension development. Another strength is investigation of 10 biomarkers, including both pro- and anti-inflammatory markers, which provides a broad perspective on associations with hypertension. Several of these biomarkers had not been previously evaluated prospectively with hypertension risk, therefore, we present novel findings. We were able to evaluate interactions with age, smoking, HT use, BMI, and BP at baseline, which provides additional insight into how inflammatory biomarkers might influence hypertension risk. Limitations include the somewhat sample size, which may have limited statistical precision and/or power of some analyses. Additional limitations include ascertainment of incident hypertension cases based on self-reported information, however, WHI participants have shown to reliably self-report history of diagnosed hypertension treated with medication.15 Other information used in this analysis also was obtained by questionnaire (e.g., physical activity, dietary intake, smoking), which could lead to misclassification and potentially bias measures of association. Because covariate information was collected prior to hypertension diagnosis, potential biases would most likely be toward the null.38 We did not have information on certain covariates that could be relevant in testing a hypothesis on inflammation and hypertension. These include plasma catecholamine concentrations or other measures of sympathetic activation, changes in endogenous estrogen concentrations, direct measures of visceral adiposity which likely is a significant pro-inflammatory reservoir, conditions such as allergies and asthma, or use
of steroid medication that might be associated with these pro-inflammatory conditions. Findings reported herein likely are most generalizable to women of similar age and sociodemographic characteristics as those in the present study.

In conclusion, this prospective cohort study on postmenopausal women suggests that higher leptin concentrations were associated with greater risk of developing hypertension, however significance of the association was no longer evident when adjusting for covariates beyond age alone. Other pro-inflammatory biomarkers were positively associated with hypertension risk, albeit non-significantly, and these included TNFα and MCP-1. The anti-inflammatory biomarkers adiponectin and IL-10 were associated non-significantly with lower hypertension risk. Smoking status, BMI, and baseline BP appear to be potential modifiers of associations with certain biomarkers, and should be explored further in studies with larger sample size and greater racial-ethnic diversity than was available in the present investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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SUMMARY

What is known about topic?

• Hypertension burden is especially high in postmenopausal women (an understudied group).
• Inflammation is thought to play a role in hypertension development, however understanding is limited to selected inflammatory biomarkers and is particularly sparse in older women.

What this study adds

• Proinflammatory biomarkers Leptin, TNFα, and MCP-1 were positively associated, and anti-inflammatory biomarkers adiponectin and IL-10 were inversely associated with hypertension risk, although significance of associations was inconsistent.
• Smoking history and body mass index appeared to modify some associations.
• Additional studies with larger sample sizes and range in biomarker concentrations are needed to further understand the impact of inflammation on hypertension development in later life.
Figure 1.
Participant Enrollment and Timeline.
Table 1.
Baseline participant characteristics according to incident hypertension status.

| Characteristic                        | Incident Hypertension | P-value |
|--------------------------------------|-----------------------|---------|
|                                      | No (N=276)            | Yes (N=195) |
| Age (years), mean (SD)               | 65.1 (6.6)            | 65.5 (6.8) | 0.53     |
| White, %                             | 97.8                  | 98.5     | 0.81     |
| High school education or less, %     | 19.6                  | 20.0     | 0.66     |
| Neighborhood SES score, mean (SD)    | 76.5 (7.1)            | 76.5 (6.5) | 0.89     |
| Ever smoker, %                       | 40.6                  | 57.4     | <0.001   |
| Current hormone therapy use, %       | 50.7                  | 45.2     | 0.002    |
| Alcohol intake (oz/day), mean (SD)   | 0.5 (0.6)             | 0.5 (0.8) | 0.38     |
| Dietary Healthy Eating Index score, mean (SD) | 69.6 (11.0) | 68.3 (10.2) | 0.10 |
| Dietary sodium intake (mg/day), mean (SD) | 2,516.9 (950.2) | 2,536.6 (920.5) | 0.66 |
| Systolic blood pressure (mmHg), mean (SD) | 109.0 (11.4) | 118.4 (11.0) | <0.001 |
| Diastolic blood pressure (mmHg), mean (SD) | 66.5 (6.9) | 70.5 (7.7) | <0.001 |
| Body Mass Index (kg/m^2), mean (SD)  | 24.9 (4.0)            | 26.7 (4.6) | <0.001 |
| Physical activity (MET-hr/wk), mean (SD) | 16.0 (14.8) | 14.5 (14.8) | 0.07 |
| Diabetes*, %                         | 0.7                   | 1.0      | 0.72     |
| CVD*, %                              | 2.5                   | 5.1      | 0.13     |

* Diabetes, history of physician-diagnosis with treatment by medication; CVD, cardiovascular disease; history of myocardial infarction or stroke.
Table 2.
Baseline biomarker concentrations according to incident hypertension status.

| Biomarker | Units | Incident Hypertension |
|-----------|-------|-----------------------|
|           |       | No (N=276) | Yes (N=195)   |
| CRP       | ng/mL | 2.536 (1.047–5.375) | 3.125 (1.381–6.284) |
| IL-2      | pg/mL | 0.76 (0.49–1.44) | 0.75 (0.43–1.53) |
| IL-4      | pg/mL | 1.8 (1.3–3.6) | 1.7 (1.2–3.2) |
| IL-6      | pg/mL | 0.92 (0.50–2.18) | 0.88 (0.48–2.21) |
| IL-8      | pg/mL | 9.0 (6.4–13.4) | 9.9 (6.9–15.4) |
| TNF-α     | pg/mL | 1.5 (1.2–1.9) | 1.6 (1.2–2.0) |
| MCP-1     | pg/mL | 130.5 (97.6–174.5) | 140.4 (98.5–188.2) |
| Leptin    | ng/mL | 2.4 (1.3–4.0) | 3.1 (1.5–4.8) |
| IL-10     | pg/mL | 0.83 (0.49–2.32) | 0.85 (0.40–2.35) |
| Adiponectin | ng/mL | 19,126 (13,645–26,514) | 17,390 (12,906–25,213) |

Data are median (25th - 75th percentile).

Pro-inflammatory biomarkers: CRP, IL-2, IL-4, IL-6, IL-8, TNFα, MCP-1, leptin.

Anti-inflammatory biomarkers: IL-10, adiponectin.
### Table 3.

Pearson correlations (r) between biomarkers (N=471).

| Biomarker | IL-2 | IL-4 | IL-6 | IL-8 | TNF-α | MCP-1 | Leptin | IL-10 | Adiponectin |
|-----------|------|------|------|------|-------|-------|--------|-------|-------------|
| CRP       | 0.03 | 0.01 | 0.08 | 0.03 | 0.07  | -0.02 | 0.24   | 0.08  | -0.08       |
| IL-2      | 1.0  | 0.59 | 0.47 | 0.23 | -0.12 | -0.09 | <0.01  | 0.40  | -0.02       |
| IL-4      | 1.0  | 0.26 | 0.16 | -0.07| -0.15 | 0.01  | 0.23   | -0.09 |             |
| IL-6      | 1.0  | 0.19 |    | -0.07| -0.10 | 0.06  | 0.56   | 0.04  |             |
| IL-8      | 1.0  | 0.08 | 0.05 | -0.01| 0.13  | -0.03 |        |       |             |
| TNF-α     | 1.0  | 0.14 | 0.07 | -0.15| 0.01  |       |        |       |             |
| MCP-1     | 1.0  | 0.09 |    |     | <0.01 |       |        |       | -0.09       |
| Leptin    | 1.0  |    | <0.01| -0.27|       |       |        |       |             |
| IL-10     | 1.0  | 0.03 |    |     |       |       |        |       |             |

Correlations are unadjusted and based on log(10)-transformed biomarkers.

For n=471, |r| ≥0.10 is statistically significant at P<0.05.

Pro-inflammatory biomarkers: CRP, IL-2, IL-4, IL-6, IL-8, TNF-α, MCP-1, leptin.

Anti-inflammatory biomarkers: IL-10, adiponectin.
Table 4.

Hazard ratio and 95% CI for incident HTN (195 cases) in the OsteoPerio Study (N=471).

| Biomarker | Age Adjusted | Multivariable-adjusted Models | Interaction P-value* |
|-----------|--------------|-------------------------------|----------------------|
|           | Model 1      | Model 2                      | Model 3              | Age | Smoking | HT | BMI | SBP | DBP |
| CRP       | 1.11 (0.87-1.41) | 1.11 (0.86-1.44)          | 0.86 (0.66-1.13)     | 0.18 | 0.06    | 0.72 | 0.59 | 0.37 | 0.57 |
| IL-2      | 0.96 (0.71-1.30) | 0.93 (0.68-1.26)          | 0.93 (0.69-1.27)     | 0.65 | 0.78    | 0.54 | 0.26 | 0.63 | 0.71 |
| IL-4      | 0.89 (0.65-1.22) | 0.92 (0.66-1.26)          | 0.93 (0.67-1.29)     | 0.29 | 0.89    | 0.36 | 0.38 | 0.69 | 0.65 |
| IL-6      | 0.93 (0.72-1.20) | 0.92 (0.71-1.19)          | 0.84 (0.64-1.10)     | 0.67 | 0.38    | 0.13 | 0.25 | 0.46 | 0.48 |
| IL-8      | 1.40 (0.88-2.21) | 1.42 (0.88-2.31)          | 1.31 (0.81-2.12)     | 0.29 | 0.20    | 0.37 | 0.89 | 0.28 | 0.16 |
| TNF-α     | 1.27 (0.61-2.66) | 1.44 (0.67-3.09)          | 1.38 (0.64-2.98)     | 0.73 | 0.38    | 0.64 | 0.87 | 0.50 | 0.15 |
| MCP-1     | 1.54 (0.85-2.80) | 1.73 (0.93-3.20)          | 1.43 (0.77-2.62)     | 0.40 | 0.004   | 0.58 | 0.07 | 0.41 | 0.03 |
| Leptin    | 1.55 (1.04-2.29) | 1.42 (0.95-2.13)          | 0.69 (0.42-1.13)     | 0.86 | 0.79    | 0.73 | 0.83 | 0.56 | 0.26 |
| IL-10     | 0.92 (0.76-1.13) | 0.94 (0.76-1.15)          | 0.92 (0.75-1.13)     | 0.42 | 0.39    | 0.62 | 0.71 | 0.07 | 0.13 |
| Adiponectin| 0.60 (0.32-1.12) | 0.68 (0.36-1.27)          | 0.97 (0.51-1.82)     | 0.85 | 0.21    | 0.40 | 0.28 | 0.58 | 0.03 |

HT, current menopausal hormone therapy use; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Biomarkers were log(10) transformed prior to analysis in the Cox regression model.

Hazard ratios are for a 1-SD unit difference in baseline biomarker concentration.

Model 1 adjusted for age.

Model 2 adjusted for age, race, education, nSES, smoking, HT use, alcohol, dietary HEI, physical activity.

Model 3 adjusted for variables in model 2 and BMI.

*Interactions were tested at alpha 0.10 using a cross-product term for the biomarker and covariate, adjusting for model 2 covariates.

Pro-inflammatory biomarkers: CRP, IL-2, IL-4, IL-6, IL-8, TNFα, MCP-1, leptin.

Anti-inflammatory biomarkers: IL-10, adiponectin.