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Inflammation Markers and Risk of Developing Hypertension: a Meta-Analysis of Cohort Studies

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	intermediate-risk categories. Conclusions: Higher levels of circulating CRP, hs-CRP, and IL-6, but not IL-1β, were associated with the risk of developing hypertension. The association persisted in subgroups of studies defined by major sources of heterogeneity.

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Inflammation Markers and Risk of Developing Hypertension: a Meta-Analysis of Cohort Studies

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Abstract

Objective: To systematically assess the association of circulating inflammation markers with the future risk of hypertension.

Methods: We did a systematic literature search of PubMed and Scopus, from database inception to July 10, 2018. Prospective and retrospective cohort studies evaluating the association of circulating C-reactive protein (CRP), high-sensitive C-reactive protein (hs-CRP), interleukin-6 (IL-6), and interleukin-1β (IL-1β) with risk of developing hypertension in the general population were included. The relative risks (RRs) for the top vs. bottom tertile of circulating biomarkers were calculated using a fixed-effects/random-effects model. A potential nonlinear dose-response association was tested.

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Conclusions: Higher levels of circulating CRP, hs-CRP, and IL-6, but not IL-1β, were associated with the risk of developing hypertension. The association persisted in subgroups of studies defined by major sources of heterogeneity.

Keywords: C-reactive protein; Hypertension; Inflammation; Interleukins; Meta-analysis.

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Key messages

- **What is already known about this subject?**

Recent studies suggest that systemic inflammation may play a role in the pathogenesis and progression of hypertension. However, existing evidence is inconsistent.

- **What does this study add?**

This meta-analysis of prospective and retrospective cohort studies demonstrated that higher levels of circulating inflammation markers including CRP, hs-CRP, and IL-6, but not IL-1β, were associated with an increased risk of developing hypertension. In the analysis of CRP, the association did not change when the analysis was restricted only to studies that controlled for body mass index. A nonlinear dose-response meta-analysis demonstrated that the risk of hypertension increased significantly and linearly with increasing circulating CRP, even within the low- (<1 mg/L) and intermediate-risk (1-3 mg/L) categories.

- **How might this impact on clinical practice?**

Further studies should assess if levels of these inflammatory markers could be useful as screening test to identify individuals at high-risk of hypertension.

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Introduction

High blood pressure is a major risk factor for cardiovascular morbidity, mortality, and disability worldwide. About a quarter of the world population has hypertension (systolic blood pressure -SBP ≥140 and diastolic blood pressure -DBP ≥90 mm Hg) .[1] Although hypertension is easy to diagnose and effective treatments are available, only a third of those receiving pharmacological treatment for hypertension have their blood pressure controlled.[2] In consequence, there is a pressing need to identify novel risk factors for hypertension, potentially useful for risk stratification and for development of preventive interventions.

Recent studies suggest that systemic inflammation may play a role in the pathogenesis and progression of hypertension.[3] A few cross-sectional studies have shown a positive association between levels of circulating inflammation markers and elevated blood pressure.[4, 5] However, finding from cross-sectional studies could be explained by reverse causality bias, since hypertension may be a driver of inflammation .[6] On the other hand, a significant association has been reported in some [7, 8, 9] but not all cohort studies, [10, 11, 12, 13] while uncertainty in the estimate of the association have compromised the conclusions of other cohort studies.[14, 15, 16, 17, 18]

In spite of current uncertainty, no previous study has summarized the existing evidence on the role of elevated inflammation markers on the risk of hypertension. We therefor conducted a meta-analysis of prospective and retrospective cohort studies to evaluate if high levels of circulating inflammation markers (C-reactive protein -CRP, high-sensitive C-reactive protein -hs-CRP, interleukin-6 -IL-6, and interleukin-1β -IL-1β) are associated with an increased risk of hypertension in the general population.

Methods

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Search strategy

To find potentially relevant studies, we searched for articles in English referenced in PubMed and Scopus, from database inception to July 10, 2018, using a combination of keywords relevant to inflammation, hypertension, and study design (**Online Supplementary file 1**). The reference lists of retrieved articles were reviewed for additional eligible studies.

Eligibility and study selection

Two authors (AJ, MSZ) independently reviewed the title and abstract of all articles retrieved, and selected those meeting the following criteria: (1) based on prospective and retrospective cohort, nested case-control, or case-cohort studies; (2) conducted in adults aged 18 years or older; (3) reporting circulating levels of inflammation markers (CRP, hs-CRP, IL-6, and IL-1β) as exposure; (4) reporting the incidence of hypertension; and (5) reporting the effect of the exposure in the form of a relative risk (RR: risk ratio, odds ratio, or hazard ratio) and its 95% confidence interval (95% CI). Cross-sectional and case-control studies, as well as studies in patient populations were excluded.

Data extraction and quality assessment

Two investigators (AJ, MSZ) independently extracted the following information from eligible studies: first author's name, publication year, study name, country, age range and/or mean age (years), number of participants, hypertension diagnostic criteria, exposure levels, RR and its 95% CI for each exposure category, and confounding factors included in the multivariate analysis. We selected the RR from the model with the most comprehensive covariate adjustment. We used the Newcastle-Ottawa Scale to assess the quality of the studies included.[19] Any discrepancies were resolved through discussion under supervision a senior author (SS-b).

Statistical analysis

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We conducted separate meta-analyses for CRP, hs-CRP, IL-6, and IL-1β. We calculated the average RRs for the third compared to the first tertile of the inflammation markers using a fixed-effects model. For studies that did not define exposed and non-exposed cohorts using tertiles of the marker, for the purpose of data analysis, we translated the effect of the marker on the incidence of hypertension to a risk ratio corresponding to a comparison between individuals in the upper and lower tertile of the marker (**Online Supplementary file 2**).[20] A random-effects model was used [21] if there was evidence of significant heterogeneity (I^2 >50%, *P*-heterogeneity <0.05).[22]

We evaluated the influence of each study on the average RR, by re-estimating the RR after excluding each study at a time. We also conducted subgroup analyses by gender, geographical location, number of hypertension cases, and adjustment for main confounders (baseline blood pressure, physical activity, smoking, body mass index -BMI, and alcohol intake). The I^2 statistic and the Cochrane's Q test of heterogeneity were used to evaluate between-study heterogeneity .[22] Publication bias was assessed using funnel plots asymmetry and tested with Egger's asymmetry test [23] and Begg's test ($P < 0.10$) ,[24] when there were at least 10 studies.

We also tested for a nonlinear dose-response when at least three studies were available. For this purpose, we modeled the inflammation marker using restricted cubic splines with three knots at fixed percentiles (10, 50, and 90%) of the distribution ,[25] fitted a regression model with the RR as dependent variable, and tested whether the coefficient for the second spline was statistically different from zero. For the purpose of this analysis, if the numbers of participants/cases or personyears were not reported in the primary studies, we estimated them by dividing the total numbers by the number of exposure groups, if the exposure was defined as quantiles. All analyses were conducted with Stata software, version 13 (Stata Corp, College Station, Texas, USA).

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Results

The initial systematic search identified 10,235 potentially eligible publications. Of these, 10,103 publications were not relevant based on the review of the abstract (**Online Supplementary file 3**). Eventually, 132 articles were fully reviewed for inclusion in the meta-analysis. Only 18 articles were eligible for the meta-analysis. Reasons for excluding studies are described in **Online Supplementary file 3.** Three articles reported separate results for men and women [9, 11] and blacks and whites [26] and estimates were separately included in the present meta-analysis. Thus, a total of 21 studies (18 articles) with 142,640 participants and 20,676 incident cases of hypertension were included in the meta-analysis.[7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 26, 27, 28, 29, 30, 31] We included in our analysis one study with isolated systolic hypertension in participants with a mean age of 64 years as the outcome ,[27] because isolated systolic hypertension is the most frequent type of hypertension in patients ≥60 years old.

Two studies (one article) were retrospective cohort studies ,[9] five (four articles) were nested case-control studies ,[13, 14, 26, 28] and the rest were prospective cohort studies. Eight studies (six articles) were from Asia, [9, 11, 14, 15, 16, 17], eight (seven articles) from the US, [7, 8, 12, 13, 26, 28, 31] and five from Europe.[10, 18, 27, 29, 30] Of the 21 studies, 16 controlled for smoking, 14 controlled for physical activity and BMI, 13 controlled for alcohol intake, 11 for baseline blood pressure, and two for dietary sodium intake. All studies excluded participants with a history of hypertension at baseline. All but two studies on CRP defined hypertension as SBP/DBP≥140/90 mm Hg or taking antihypertensive medication, the standard approach in epidemiologic studies. One study on CRP[27] focused on isolated hypertension, defined as (SBP ≥ 160 mm Hg and DBP < 90 mm Hg), and another[10] defined hypertension as SBP/DBP≥135/85 mm Hg or using antihypertensive medication. All studies on Hs-CRP and IL-6 used the standard

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definition of hypertension. The general characteristics of the studies are presented in **Online Supplementary file 4**, and the numbers of participants/cases and reported RRs across categories of inflammation markers are presented in **Online Supplementary file 5**.

C-reactive protein

Twelve studies (11 articles) were included in the analysis of CRP and risk of hypertension.[7, 8, 9, 10, 12, 15, 16, 27, 28, 29, 31] The RR of hypertension for the top vs. bottom tertile of CRP was 1.17 (95%CI: 1.12, 1.22) for the fixed-effects model, and 1.23 (95%CI: 1.11, 1.35) for a random-effect model. There was moderate-to-high evidence of heterogeneity, I^2 = 59.3%, $P_{heterogeneity}$ = 0.005; (**Figure 1A**). The influence analysis showed two large studies were the main sources of heterogeneity.[8, 9] Excluding the Women's Health Study [8] changed the RR to 1.14 (95% CI: 1.06, 1.21; I^2 = 8%), and excluding the Sung et al.'s study [9] changed the RR to 1.30 (95% CI: 1.17, 1.42; I^2 = 14%).

Findings were consistent, regardless of the definition of hypertension used in the study ($P_{heterogeneity}$=0.47). However, gender, study region, and adjustment for smoking were major sources of heterogeneity on the effect of CRP on the risk of hypertension (**Table 1**). Indeed, the association was significantly stronger in women (RR: 1.25) than in men (RR: 1.10); in studies conducted in the US (RR: 1.34) than in those conducted in Europe (RR: 1.17) and Asia (RR: 1.13); in studies with participants with a mean age of≥50 years (RR: 1.36) than in those with a mean age of <50 years (RR: 1.11); and in studies that did not adjust for smoking (RR: 1.71) than in those that adjusted (RR: 1.21). A significant positive association persisted even after adjustment for BMI (RR: 1.23, 95%CI: 1.08, 1.37; I^2 = 71%, n = 7 studies). On the other hand, there was no evidence of significant publication or small-studies bias (Egger's test *P*=0.15; Begg's test *P*=0.12; **Online Supplementary file 6**).

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Table 1. Relative risk of hypertension for the third compared with the first tertile of circulating C-reactive protein.

		n	Participants	RR (95%CI)	I^2(%), $P_{heterogeneity}$[1]	$P_{between}$[2]
All studies		12	137,916	1.23 (1.11-1.35)	59%, 0.005	-
Sex						
Men		3	57,746	1.10 (1.03-1.16)	0%, 0.54	0.003
Women		3	64,096	1.25 (1.06-1.44)	69%, 0.04	
Both		8	19,993	1.22 (1.05-1.39)	7%, 0.37	
Age (mean)						
<50 years		7	110,420	1.11 (1.05-1.16)	0%, 0.44	<0.001
≥50 years		5	27,496	1.36 (1.26-1.47)	0%, 0.66	
Geographical region						
US		5	30,235	1.34 (1.24-1.44)	0%, 0.45	<0.001
Europe		3	4853	1.17 (0.77-1.57)	30%, 0.24	
Asia		4	102,828	1.13 (1.02-1.23)	26%, 0.25	
Definition of hypertension						
SBP ≥140, DBP ≥90 mm Hg and/or antihypertensive treatment		10	135,944	1.22 (1.09-1.34)	65%, 0.002	0.47
SBP ≥135, DBP ≥85 mm Hg and/or antihypertensive treatment		1	1637	1.79 (0.68-4.70)	-	
Isolated systolic hypertension		1	335	1.46 (1.02-2.11)	-	
Number of cases						
<500		7	13,133	1.24 (1.03-1.45)	11%, 0.34	0.62
>500		5	124,783	1.22 (1.06-1.37)	80%, < 0.001	
Adjustment for confounders						
Alcohol consumption	Yes	8	130,288	1.21 (1.07-1.35)	71%, 0.001	0.27
	No	4	7628	1.29 (1.07-1.52)	0%, 0.63	
Physical activity	Yes	8	130,288	1.21 (1.07-1.35)	71%, 0.001	0.27
	No	4	7628	1.29 (1.07-1.52)	0%, 0.63	
Smoking	Yes	9	130,288	1.21 (1.09-1.33)	67%, 0.002	0.08
	No	3	7628	1.74 (1.09-2.40)	0%, 0.98	

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Body mass index	Yes	7	123,814	1.23 (1.08-1.37)	71%, 0.002	0.72
	No	5	14,102	1.26 (0.98-1.54)	36%, 0.18	
Baseline blood pressure	Yes	7	107,15	1.11 (1.05-1.17)	0%, 0.44	<0.001
	No	5	30,801	1.37 (1.26-1.47)	0%, 0.53	

Abbreviations**:** DBP, diastolic blood pressure; RR, relative risk; SBP, systolic blood pressure.

[1]*P*-heterogeneity within subgroups with the use of a random-effects model.

[2]*P*-heterogeneity between subgroups with the use of a fixed-effects model.

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Seven studies (six articles) included sufficient data for the dose-response meta-analysis.[7, 8, 9, 12, 16, 28] The risk of hypertension increased linearly with increasing circulating CRP concentration (*P* for nonlinearity = 0.07, **Figure 2A**).

High sensitive C-reactive protein

Seven studies (five articles) were included in the analysis of hs-CRP.[11, 14, 17, 18, 26] The RR for the top vs. bottom tertile of hs-CRP was 1.06 (95%CI: 1.01, 1.07) for the fixed-effects model, and 1.20 (95%CI: 1.02, 1.37) for the random-effects model (**Figure 1B**). There was significant between-study heterogeneity (I^2 = 73.6%, $P_{heterogeneity}$ = 0.001). The RR changed from 1.04 (95%CI: 0.95, 1.13) excluding the Women's Health Initiative Observational Study [26] to 1.78 (95%CI: 1.46, 2.10) excluding the Korean Genome and Epidemiology Study on Atherosclerosis Risk [11]; the two studies with the largest impact on heterogeneity.

A significant RR was observed in two studies conducted in the US (RR: 1.72, 95%CI: 1.38, 2.07), but not in four studies in Asian populations (RR: 1.03, 95%CI: 0.95, 1.12; **Table 2**). Also, the association was not significant in studies that controlled for BMI (RR: 1.03, 95%CI: 0.95, 1.12; n=4). On the other hand, the association was stronger in studies with participants with a mean age of<50 years (RR: 1.93) than in those with a mean age of ≥50 years (RR: 1.17), but not significantly so ($P_{heterogeneity}$=0.11).

Publication bias was not assessed, due to the small number of studies (n < 10). Finally, there was a positive linear association between circulating hs-CRP concentration and hypertension risk in three studies (two articles) [17, 26] (*P* for nonlinearity = 0.61, **Figure 2B**).

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Table 2. Relative risk of hypertension for the third compared with the first tertile of circulating high-sensitive C-reactive protein.

		n	Participants	RR (95%CI)	I^2(%), $P_{heterogeneity}$[1]	$P_{between}$[2]
All studies		7	4194	1.20 (1.02-1.37)	73.6%, 0.001	-
Sex						
Men		2	1155	1.38 (0.03-2.78)	39.9%, 0.20	0.07
Women		3	2377	1.43 (0.86-2.00)	87.1%, < 0.001	
Both		2	662	2.05 (1.13-2.97)	0%, 0.70	
Age (mean)						
<50 years		1	210	1.93 (1.12-3.33)	-	0.11
≥50 years		6	3984	1.17 (1.00-1.37)	75%, 0.001	
Geographical region						
US		2	1600	1.72 (1.38-2.07)	0%, 0.41	<0.001
Europe		1	379	2.81 (1.19-6.63)	-	
Asia		4	2215	1.03 (0.95-1.12)	39.9%, 0.17	
Adjustment for confounders						
Alcohol consumption	Yes	4	2431	1.76 (1.43-2.20)	0%, 0.63	<0.001
	No	3	1763	1.03 (0.97-1.09)	23.7%, 0.27	
Physical activity	Yes	6	3984	1.17 (1.00-1.34)	75.2%, 0.001	0.11
	No	1	210	1.93 (1.12-3.33)	-	
Smoking	Yes	6	3984	1.17 (1.00-1.34)	75.2%, 0.001	0.11
	No	1	210	1.93 (1.12-3.33)	-	
Body mass index	Yes	4	2215	1.03 (0.95-1.12)	39.9%, 0.17	<0.001
	No	3	1979	1.74 (1.40-2.08)	0%, 0.53	
Baseline blood pressure	Yes	5	2594	1.04 (0.95-1.13)	39.8%, 0.16	<0.001
	No	2	1600	1.72 (1.38-2.07)	0%, 0.41	

Abbreviations: RR, relative risk.

[1]*P*-heterogeneity within subgroups with the use of a random-effects model.

[2]*P*-heterogeneity between subgroups with the use of a fixed-effects model.

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Interleukin-6

Five studies (four articles) were included in the analysis for IL-6.[7, 13, 28, 30] All of them were from the US, and controlled for smoking and alcohol consumption. Participants in the third tertile of IL-6 had a 51% higher risk of hypertension as compared to those in the first tertile (RR: 1.51, 95%CI: 1.30, 1.71), with no evidence of significant heterogeneity, $I^2 = 0\%$, $P_{heterogeneity} = 0.56$) (**Figure 1C**). The RR changed little when individual studies were excluded (between 1.41 and 1.57). There was a significant association among studies than controlled for physical activity (RR: 1.57, 95%CI: 1.31, 1.84), but not in studies that controlled for BMI (RR: 1.34, 95%CI: 0.90, 1.77; n = 2). There was a monotonic association between circulating IL-6 concentration and hypertension risk, with a somewhat slower trend of increment in the risk within the higher circulating IL-6 concentrations (*P* for nonlinearity = 0.07, **Figure 2C**).

Interleukin-1β

A non-significant association between circulating IL-1β concentration and the risk of developing hypertension (RR: 1.22, 95%CI: 0.92, 1.51; $I^2 = 0\%$) was found in the three available studies (two articles) .[26, 30]

Discussion

In this meta-analysis we gathered all cohort studies of the association between inflammation markers and incident hypertension, and found that a higher levels of CRP, hs-CRP and IL-6, increased the risk of hypertension by 23%, 20%, and 51%, respectively. We also found a linear association between levels of these markers and risk of hypertension. In contrast, IL-1β was not significantly associated to incident hypertension. Although the risk of hypertension increased

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linearly with the level of inflammatory markers, the cut point to identify exposed individuals varied across studies and was undefined in some studies.

Higher levels of CRP could increase the risk of hypertension by decreasing expression and activation of the nitric oxide synthase ,[32] increasing the synthesis of endothelin-1 ,[33] and impairing endothelium-dependent vasorelaxation .[34] IL-6 may increase the risk of hypertension by increasing hepatocyte synthesis of CRP .[35] IL-6 also impairs insulin signaling and activity, contributing to the development of insulin resistance, [36] one of the major mechanisms underlying the development of hypertension. Also, IL-6 plays a key role in the pathogenesis of angiotensin II-mediated hypertension .[37]

Although CRP and IL-6 may partly mediate the effect of obesity on the risk of hypertension, [38] the strength of the CRP-hypertension association changed little when the analysis was restricted to studies that controlled for BMI. For hs-CRP and IL-6 increases in the risk of hypertension were also observed in studies that adjusted for BMI. However, these increases were no longer statistically significant, which was likely due the smaller number of studies included in that analysis (four for hs-CRP, and two for IL-6).

In an analysis by region, CRP and hs-CRP exhibited stronger associations with hypertension risk in the US than in Asia (RRs: 1.34 vs. 1.13 and 1.73 vs. 1.03, respectively). This may be due to differences in the set of factors included as confounders in US and Asian studies. Indeed, none of the US studies in the analysis of hs-CRP controlled for measures of adiposity, and this may have resulted in an overestimate of the effect of this marker. Nevertheless, the issue of regional differences in the effect of inflammation markers should be explored in future studies.

The present meta-analysis had several strengths. We tested the association across different inflammation markers instead of an individual marker, and thereby, presented a relatively

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comprehensive perspective of the possible association of inflammation markers with risk of developing hypertension. The study further shows that the association between circulating CRP and risk of hypertension was independent of BMI, and thereby, could add evidence to the existing evidence about the potential role of inflammatory pathways in the pathogenesis of hypertension. Finally, we showed the shape of the dose-response relations, especially in the analysis of CRP, which indicated that increasing the circulating concentration of CRP, even within the low-risk range, was associated with a significant higher risk of developing hypertension.

Our study had also some important limitations. First, we performed a meta-analysis of observational studies which are subject to residual confounding. As found in some of the original studies, higher levels of systemic inflammation is accompanied by unhealthy life-style related behaviors such as smoking, alcohol consumption, low physical activity, and higher adherence to a western-style dietary patterns; as well as conditions such as general and abdominal adiposity, diabetes, dyslipidemia, and higher blood pressure levels;[12, 13, 16, 17, 28] which in turn are associated with a higher risk. Indeed, higher levels of circulating inflammation markers may reflect an unfavorable health status or an unhealthy life-style related behavior. So, it is difficult to statistically control for the confounding effects of these variables. Second, in the analyses of hs-CRP and IL-6, the association became non-significant when the analysis was restricted to studies that controlled for BMI. However, this was likely due to the smaller number of studies included in that analysis because only four studies in the analysis of hs-CRP and two studies in the analysis of IL-6 controlled for BMI. In addition, in the analysis of CRP, the association did not change in the subgroup of studies that controlled for BMI. Therefore, uncertainty remains regarding to what degree obesity explains the effect of inflammatory markers on the incidence of hypertension. Third, the number of studies was low in the analyses of hs-CRP, IL-6, and IL-1β; and all of the

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studies in the analysis of IL-6 were form the US. In addition, we observed a relatively weak association in the analysis of CRP, which was accompanied by heterogeneity. Finally, inflammatory markers included in our analysis are the ones that have been better studied, so far, though they do not fully illustrate the complex interactions between immune function and arterial blood pressure. Thus, the interpretation of the results should be made with caution.

Conclusions

In summary, our study shows that high levels of CRP, hs-CRP, and IL-6 increase the risk of hypertension. Additional studies are needed to elucidate the potential role of these markers in prevention and management of hypertension. Specifically, further studies should assess if levels of these inflammatory markers could be useful as screening test to identify individuals at high-risk of hypertension, and should provide a more comprehensive view of the role of immune function on the development of hypertension.

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Figure captions:

Figure 1 Relative risk of hypertension for the top vs. bottom tertile of circulating inflammation markers: A: C-reactive protein; B: high-sensitive C-reactive protein; C: Interleukin-6.

Figure 2 Dose-response associations between circulating inflammation markers and risk of developing hypertension. CRP, C-reactive protein; hs-CRP, high-sensitive C-reactive protein; IL-6, interlukin-6.

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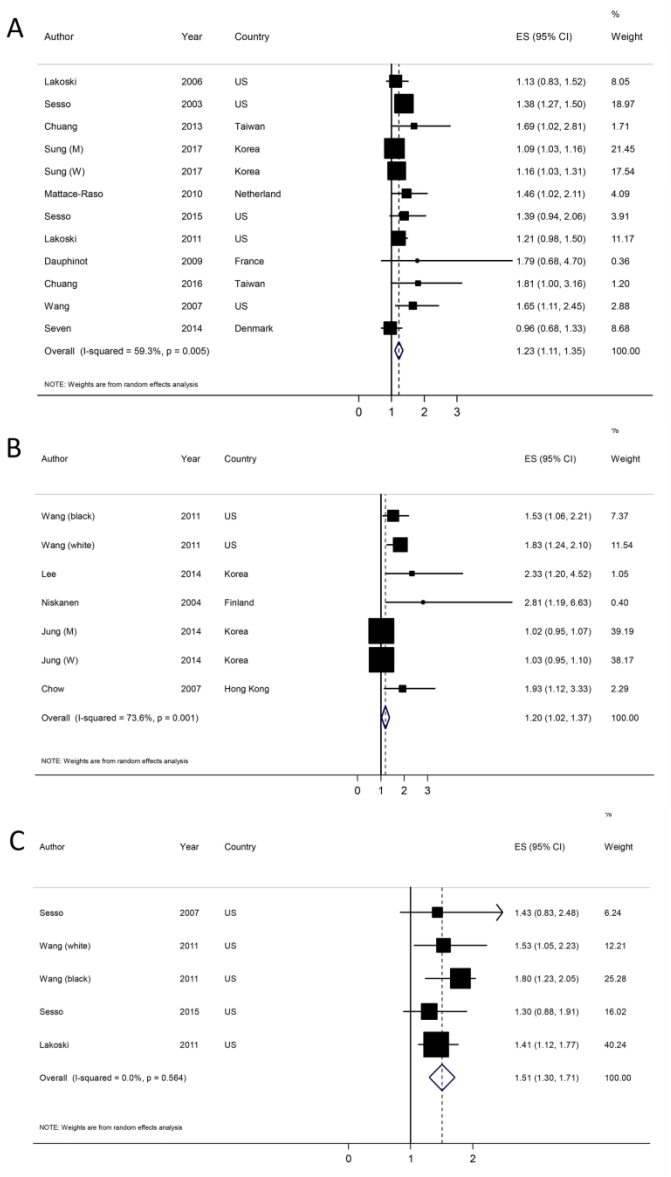


Figure 1 Relative risk of hypertension for the top vs. bottom tertile of circulating inflammation markers: A: C-reactive protein; B: high-sensitive C-reactive protein; C: Interleukin-6.

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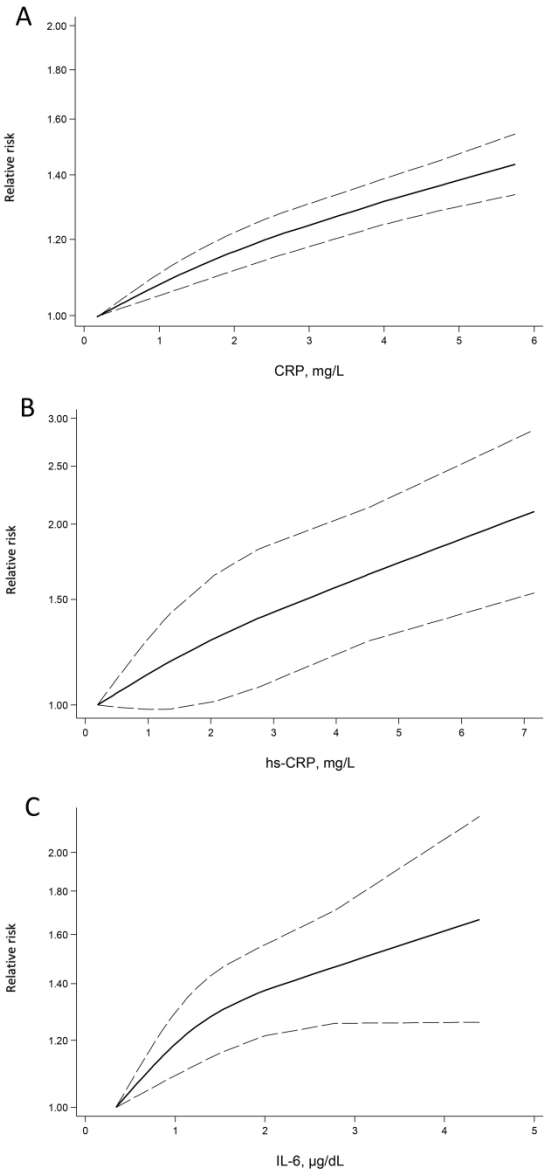


Figure 2 Dose-response associations between circulating inflammation markers and risk of developing hypertension. CRP, C-reactive protein; hs-CRP, high-sensitive C-reactive protein; IL-6, interlukin-6.

159x339mm (600 x 600 DPI)

Supplementary Files

Inflammation Markers and Risk of Developing Hypertension: a Meta-Analysis of Cohort Studies

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Supplementary Files

Online Supplementary file 1. Search strategy to find the relevant articles for inclusion in meta-analysis of inflammation markers and risk of hypertension.

PubMed
1. C-reactive protein (MeSH)/ OR interleukins (MeSH)/ OR interleukin-6 (MeSH)
2. interleukin-1beta (MeSH)/ OR IL-6 (Title/Abstract)/ OR IL-1β (Ti/Ab)
3. CRP (Ti/Ab)/ OR high-sensitive c-reactive protein (Ti/Ab)/ OR hs-CRP (Ti/Ab)
4. inflammatory (Ti/Ab)
5. Hypertension (MeSH)/ OR blood pressure (MeSH)
6. HTN (Ti/Ab) or high blood pressure (Ti/Ab)
7. cohort studies (MeSH)/ OR longitudinal studies (MeSH)/ OR observational studies (MeSH)/
8. prospective studies (MeSH)/ OR retrospective studies (MeSH)
9. prospective (Ti/Ab)/ OR r prospectively (Ti/Ab) OR longitudinal (Ti/Ab)
10. cohort (Ti/Ab)/ OR cohorts (Ti/Ab)/ OR observation (Ti/Ab)
11. observational (Ti/Ab)/ OR follow up (Ti/Ab)/ OR nested (Ti/Ab)
12. retrospective (Ti/Ab)/ OR relative risk (Ti/Ab)/ OR RR (Ti/Ab)
13. hazard ratio (Ti/Ab)/ OR HR (Ti/Ab) OR odds ratio (Ti/Ab)
14. 1 OR 2 OR 3 OR 4
15. 5 OR 6
16. 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13
17. 14 AND 15
18. 16 AND 17
Scopus
1. Inflammatory (Article title, Abstract, Keywords)/ OR inflammation (ti/Ab/Ke)
2. C-reactive protein (ti/Ab/Ke)/ OR CRP (ti/Ab/Ke)/ OR hs-CRP (ti/Ab/Ke)
3. high-sensitive c-reactive protein (ti/Ab/Ke)/ OR interleukin-6 (ti/Ab/Ke)/ OR IL-6 (ti/Ab/Ke)
4. interleukin-1 beta (ti/Ab/Ke)/ OR IL-1β (ti/Ab/Ke)
5. hypertension (ti/Ab/Ke)/ OR HTN (ti/Ab/Ke)/ OR blood pressure (ti/Ab/Ke)
6. prospective (ti/Ab/Ke)/ OR prospectively (ti/Ab/Ke)/ OR longitudinal (ti/Ab/Ke)
7. cohort (ti/Ab/Ke)/ OR cohorts (ti/Ab/Ke)/ OR observation (ti/Ab/Ke)
8. observational (ti/Ab/Ke)/ OR follow-up (ti/Ab/Ke)/ OR nested (ti/Ab/Ke)
9. retrospective (ti/Ab/Ke)/ OR relative risk (ti/Ab/Ke)/ OR RR (ti/Ab/Ke)
10. hazard ratio (ti/Ab/Ke)/ OR HR (ti/Ab/Ke)/ OR odds ratio (ti/Ab/Ke)
11. 1 OR 2 OR 3 OR 4
12. 6 OR 7 OR 8 OR 9 OR 10
13. 5 AND 11
14. 12 AND 13

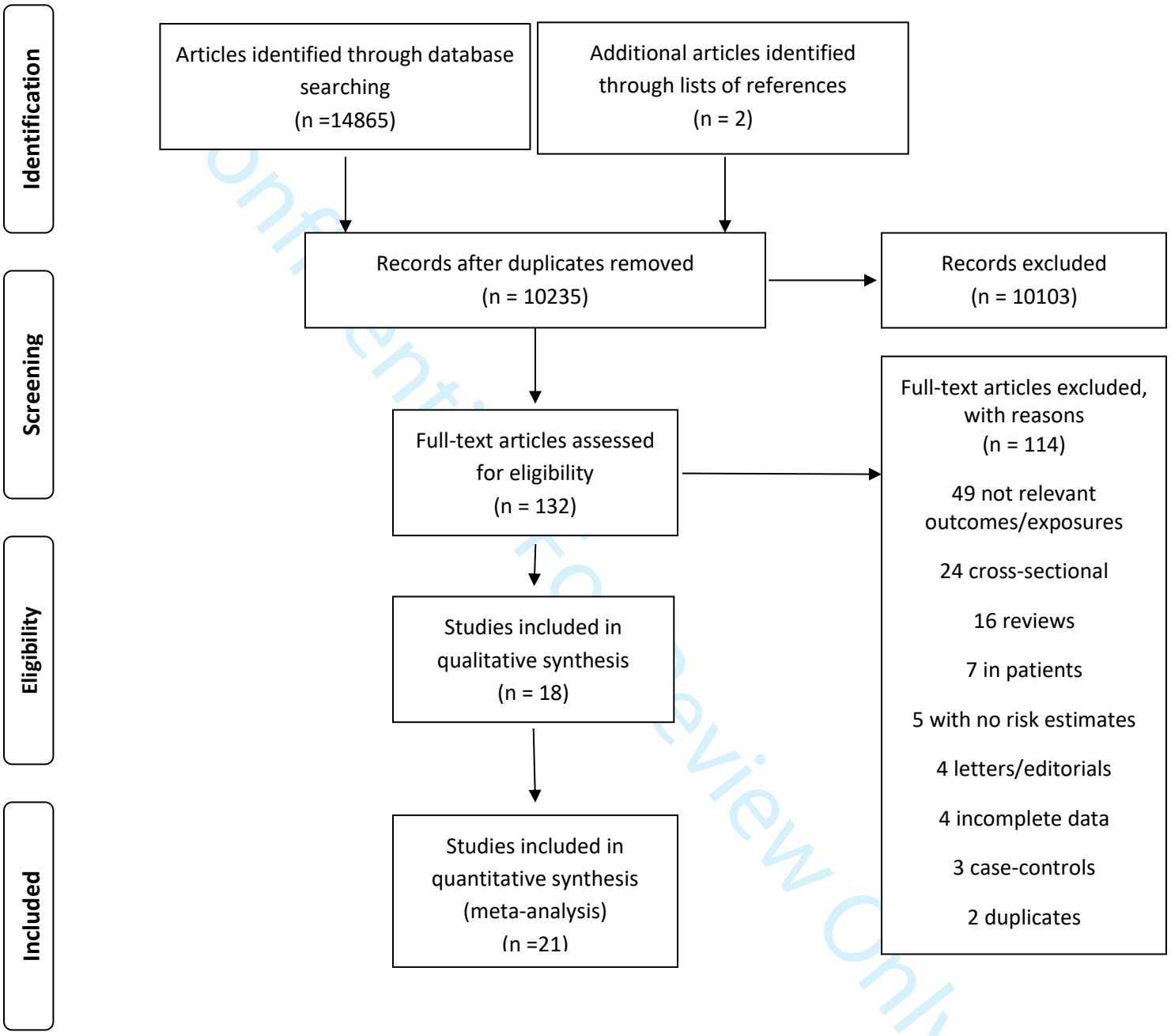
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Online Supplementary file 2. Detailed method to translate reported risk estimates in the primary studies to the third versus first tertile.

For studies that categorized the exposure in tertiles of the inflammation marker, the risk ratios (RRs) were included in the meta-analysis as reported. For studies that categorized the exposure in quartile/quintile of the marker, or reported the results as continuous, we used method introduced by Danesh and colleagues to convert the RRs from original studies to the RR for the third vs. first tertile. [20] According to this method, for studies that reported the RR for an increment of 1-SD in the level of the inflammation marker, we used a scaling factor of 2.18 to obtain the RR for the third vs. first tertile. The factor of 2.18 is the difference in the means of the upper and lower tertile of the standard normal distribution. Also, the factors of 2.54 and 2.80 are the difference in the means of the upper and lower quartiles and quintiles of the standard normal distribution, respectively. Thus, this scaling method assumes that the exposure follows a normal distribution and the association with disease risk (hypertension) is log-linear. For studies that grouped the exposure in quartiles, we used a scaling factor of 2.18/2.54 to translate the RR for the top vs. the bottom quartile to the RR for the third vs. first tertile. Similarly, a scaling factor of 2.18/2.80 was used to translate the RR for the top vs. the bottom quintiles to the RR for the third vs. first tertile. For studies that reported the RR per 1-unit increment of the marker, we exponentiated the log (RR) times the study-specific SD of the marker, to get the RR for an increment of 1-SD in the level of the marker, and then converted this RR to a RR for the third vs. first tertile using a scaling factor of 2.18. If the SD was not reported, we calculated the average difference between the mid-point of the marker in the third and first tertiles, from other studies, and then applied the per-unit RR to the average difference to obtain the RR for the third vs the first tertile.

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Supplementary Files



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Online Supplementary file 3. Literature search and study selection process to find the potential relevant studies for inclusion in the meta-analysis of inflammation markers and risk of developing hypertension.

Supplementary Files

Online Supplementary file 4. General characteristics of the studies included in meta-analysis of circulating inflammation markers and risk of developing hypertension.

Author name, publication year	Study name, country	Study design, (Follow-up duration, years)	Age (range, mean; years)	Cases/ participants (controls)	Gender	Marker (comparison)	Hypertension definition	Quality score (max. 9 points)
Lakoski, 2011 [7]	Multi-Ethnic Study of Atherosclerosis (MESA), US	Prospective cohort (5)	45-84 (59)	714/3543	W/M	CRP (≥3.69 vs. 0.71 mg/L) IL-6 (≥1.69 vs. <0.68 pg/mL)	BP ≥140/90 mm Hg or use of blood pressure medications	8
Sesso, 2003 [8]	Women's Health Study, US	Prospective cohort (7.8)	≥ 45 (53)	5365/20525	W	CRP (5.75 vs. 0.27 mg/L)	New physician diagnosis of hypertension, the initiation of medication for elevated BP, or self-reported SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg	8
Sung, 2017 [9]	Samsung Hospital Health Screening Center study, Korea	Retrospective cohort (3.3)	≥ 18 (37)	10405/ 96,606	W/M	CRP (M: ≥1.2 vs. <0.4 mg/L, W: ≥0.9 vs. <0.2 mg/L)	Use of antihypertensive medications, or if the average of two measurements of SBP was ≥140 mm Hg or DBP ≥ 90 mm Hg	8

Supplementary Files

Dauphinot, 2009 [10]	Proof study, France	Prospective cohort (2)	66	33/335	W/M	CRP* (1 mg/L)	Systolic ABMP of at least 135 mm Hg or diastolic ABMP of at least 85 mm Hg or use of antihypertensive medications	6
Jung, 2014 [11]	Korean Genome and Epidemiology Study on Atherosclerosis Risk, Korea	Prospective cohort (2.6)	40-70 (55)	178/1553	W/M	hs-CRP* (1 mg/L)	SBP ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg, and/or the use of antihypertensive medication	7
Lakoski, 2006 [12]	Coronary Artery Risk Development in Young Adults (CARDIA) study, US	Prospective cohort (7)	25-37 (32)	413/3919	W/M	CRP (>3 vs. <1 mg/L)	BP of 140/90 mm Hg or greater, or use of BP medications	8
Sesso, 2007 [13]	Women's Health Study, US	Nested case-control (10)	≥ 45 (55)	400/400	W	IL-6 (1.93 vs. 0.48 μg/dL)	Self-reports of a physician diagnosis of hypertension/ self-reports of antihypertensive treatment/ self-reports of SBP ≥140 mm Hg, self-reports of DBP ≥ 90 mm Hg	8

Chow, 2007 [14]	Hong Kong Cardiovascular Risk Factor Prevalence Study, Hong Kong	Nested case-control (5)	25-74 (50)	70/140	W/M	hs-CRP* (1 mg/L)	Sitting BP ≥ 140/90 mm Hg or on regular antihypertensive drugs	7
Chuang, 2016 [15]	MJ Health Screening Database, Taiwan	Prospective matched cohort (1.6)	≥ 20 (45)	543/4109	W/M	CRP* (1 nmol/L)	SBP ≥ 140 mm Hg, DBP ≥ 90 mm Hg, or the use of antihypertensive drugs	7
Chuang, 2013 [16]	Cardiovascular Disease Risk Factors Two-Township Study, Taiwan	Prospective cohort (3.27)	≥ 20 (45)	145/2113	W/M	CRP (4.84 vs. 0.46 mg/L)	SBP ≥ 140 mm Hg, a DBP ≥ 90 mm Hg, or the use of antihypertensive drugs	7
Lee, 2014 [17]	HYpertension-Diabetes Daegu Initiative (HYDDI) study, Korea	Prospective cohort (5)	≥ 20 (53)	99/452	W/M	hs-CRP (>0.96 vs. <0.38 mg/L)	SBP ≥ 140 mm Hg or a DBP ≥ 90 mm Hg, or use of anti-hypertensive medication	7

Supplementary Files

Niskanen, 2004 [18]	Kuopio Ischemic Heart Disease Risk Factor Study, Finland	Prospective cohort (11)	42-60 (51)	124/379	M	hs-CRP (>3 vs <1 mg/L)	SBP ≥ 140 mm Hg, DBP ≥ 90 mm Hg, or use of antihypertensive medication	7
Wang, 2011 [26]	Women's Health Initiative Observational Study, US	Nested case-control (5.9)	< 70 (59)	800/800	W	IL-6 (White: 2.78 vs. 0.58 pg/mL, Black: 4.39 vs. 0.74) IL-1β (White: 0.48 vs. 0.13 pg/mL, Black: 0.47 vs. 0.13 pg/mL) hs-CRP (White: 4.5 vs. 0.37 mg/L, Black: 7.16 vs. 1.37 mg/L)	The initiation of medication for elevated BP of either SBP ≥140 mmHg or DBP ≥90 mm Hg	9
Mattace-Raso, 2010 [27]	Rotterdam study, Netherland	Prospective cohort (1990-1993 to 2002-2004)	64	252/1637	W/M	CRP* (1 SD)	Isolated systolic hypertension (SBP ≥ 160 mm Hg and a DBP < 90 mm Hg)	8
Sesso, 2015 [28]	Physician's Health Study, US	Nested case-control (14)	40-84 (47)	396/396	M	CRP (≥1.50 vs. 0.35 mg/L) IL-6 (≥1.31 vs. <0.71 pg/mL)	Self-reported initiation of antihypertensive treatment, SBP ≥ 140 mm Hg, or DBP ≥ 90 mm Hg	8

Seven, 2014 [29]	Inter99 study, Denmark	Prospective cohort (5.4)	46	379/2881	W/M	CRP* (1 SD)	Self-reported use of antihypertensive medication or SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg	8
Vanhala, 2008 [30]	Pieks¨am¨aki study, Finland	Prospective cohort (6.5)	51	128/396	W/M	IL-1β (0.82 vs. 0.25 pg/mL)	BP ≥ 140/90 mm Hg, or antihypertensive drug	7
Wang, 2007 [31]	Framingham Offspring Study, US	Prospective cohort (3)	56	232/1456	W/M	CRP* (1 SD)	SBP ≥ 140 mm Hg, DBP ≥ 90 mm Hg, or use of antihypertensive medications	8

* Inflammation markers were reported as continuous.

Abbreviations: BP, blood pressure; CRP, C-reactive protein; DBP, diastolic blood pressure; hs-CRP, high-sensitive C-reactive protein; IL-1β, interleukin--1β; IL-6, interleukin-6; M, men; SBP, systolic blood pressure; SD, standard deviation; W, women.

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Supplementary Files

Online Supplementary file 5.Reported risk estimates of hypertension in the primary studies in meta-analysis of circulating inflammation markers and risk of developing hypertension.

Circulating biomarkers categories	Number of cases/ participants (personyears)	Relative risk (95%CI)	Covariates
Chow, 2007 [14] Hong Kong Cardiovascular Risk Factor Prevalence Study, hs-CRP			
log, 1 mg/L	70/140	1.48 (1.07-2.05)	Age, BMI, mean arterial pressure, triglycerides, fasting insulin, and sex-specific adiponectin.
Chuang, 2013 [16] the Cardiovascular Disease Risk Factors Two-Township Study, CRP			
0.46 mg/L (median)	9.3 (IR/1.000 PY)/ 683	1.00	Age, sex, number of metabolic syndrome components, high sodium, and family history of hypertension.
1.56	19.0 (IR/1.000 PY)/ 714	1.43 (0.84-2.41)	
4.84	33.0 (IR/1.000 PY)/ 716	1.69 (1.02-2.81)	
Chuang, 2016 [15] MJ Health Screening Database, CRP			
1 nmol/L increment	543/4109	1.02 (1.00-1.04)	Matched for age and sex and adjusted for physical activity, drinking habit, and study site.
Dauphinot, 2009 [10] the Proof study, CRP			
1 mg/L increment	33/302	1.17 (0.90-1.52)	Adjusted for BMI, change in CRP, low density lipoprotein cholesterol, 24-h systolic ambulatory blood pressure, and Change in 24-h systolic ambulatory blood pressure.
Jung, 2014 [11] Korean Genome and Epidemiology Study on Atherosclerosis Risk, hs-CRP			
1 mg/L increment, Men	79/584	1.01 (0.97-1.04)	Age, baseline BMI, systolic blood pressure, diastolic blood pressure, smoking, regular exercise, fasting blood glucose, and triglyceride.
1 mg/L increment, Women	99/969	1.02 (0.97-1.06)	
Lakoski, 2006 [12] The Coronary Artery Risk Development in Young Adults, CRP			
< 1 mg/L	157/1528	1.00	Age, race, BMI, smoking status, ethanol use, low- and high-density lipoprotein cholesterol, history of diabetes, cholesterol-lowering medication use, physical activity, and clinic site
1-3	112/876	1.00 (0.76-1.33)	
> 3	144/848	1.13 (0.83-1.52)	
Lakoski, 2011 [7] Multi-Ethnic Study of Atherosclerosis, CRP			
< 0.71 mg/L	132/Q1	1.00	Age, gender, ethnicity, smoking, diabetes, statin use, aspirin use, alcohol use, and MESA study site.
0.71 to < 1.55	162/Q2	1.00 (0.79-1.26)	
1.55 to < 3.69	172/Q3	0.97 (0.77-1.23)	
≥ 3.69	243/Q4	1.25 (0.98-1.60)	
Lakoski, 2011 [7] Multi-Ethnic Study of Atherosclerosis, IL-6			
< 0.68 pg/mL	93/Q1	1.00	Age, gender, ethnicity, smoking, diabetes, statin use, aspirin use, alcohol use, and MESA study site.
0.68 to 1.05	175/Q2	1.44 (1.11-1.86)	
1.05 to 1.69	197/Q3	1.54 (1.19-1.99)	
≥ 1.69	225/Q4	1.49 (1.14-1.95)	
Lee, 2014 [17] The HYpertension-Diabetes Daegu Initiative (HYDDI) study, hs-CRP			
< 0.38 mg/L	1.32 (IR)/151	1.00	

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0.38-0.96	23.2/151	1.83 (0.94-3.57)	Age, gender, body mass index, systolic and diastolic blood pressure, diabetes mellitus, total cholesterol, smoking, drinking and exercise.
> 0.96	29.3/150	2.33 (1.20-4.52)	
Mattace-Raso, 2010 [27] Rotterdam study, CRP			
1 SD increment	252/1637	1.19 (1.01-1.41)	Age, sex, systolic blood pressure, BMI, total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus, smoking and leukocyte count.
Niskanen, 2004 [18] Kuopio Ischemic Heart Disease Risk Factor Study, hs-CRP			
0.1-0.99 mg/L	210 (n)	1.00	Age, smoking, alcohol intake, adult socioeconomic status, leisure-time physical activity, presence of cardiovascular disease, intake of energy-adjusted dietary factors (dietary intake of saturated fat, sodium, potassium, and fruits and vegetables), baseline systolic blood pressure, waist girth, concentrations of insulin, glucose, high-density lipoprotein cholesterol, and changes in waist girth, smoking , and alcohol intake.
1.0-2.99	130	1.78 (1.00-3.17)	
3.0-9.99	39	2.81 (1.19-6.63)	
Sesso, 2003 [8] Women's Health Study, CRP			
0.27 mg/L (median)	511/3131	1.00	Age, randomized aspirin treatment, randomized vitamin E treatment, randomized beta-carotene treatment, BMI, smoking, exercise, alcohol intake, postmenopausal hormone use, parenteral history of myocardial infarction at younger than 60 years, history of diabetes, and history of high cholesterol.
0.63	673/3379	1.07 (0.95-1.20)	
1.28	912/3899	1.17 (1.04-1.31)	
2.44	1307/4581	1.30 (1.17-1.45)	
5.75	1962/5535	1.52 (1.36-1.69)	
Sesso, 2007 [13] Women's Health Study, IL-6			
0.48 μg/dL (median)	59/100	1.00	Matched on age and follow-up time, and adjusted for smoking status, alcohol use, exercise, postmenopausal, postmenopausal hormone use, parental history of myocardial infarction < 60 y, personal history of hypercholesterolemia, history of diabetes, circulating CRP, and BMI.
0.69	76/100	1.35 (0.78-2.33)	
0.94	124/100	2.01 (1.13-3.57)	
1.93	141/100	1.52 (0.81-2.88)	
Sesso, 2015 [28] Physician's Health Study, CRP			
< 0.35 mg/L	Q1	1.00	Matched on age, follow-up time, and date of hypertension, plus adjusted for randomized treatments, smoking status, physical activity, alcohol consumption, parental history of myocardial infarction <60 y, history of high cholesterol and diabetes mellitus, and BMI.
0.35- to < 0.72	Q2	1.24 (0.80-1.91)	
0.73 to < 1.50	Q3	1.60 (1.02-2.51)	
≥ 1.50	Q4	1.47 (0.93-2.32)	
Sesso, 2015 [28] Physician's Health Study, IL-6			
< 0.71 pg/mL	Q1	1.00	Matched on age, follow-up time, and date of hypertension, plus adjusted for randomized treatments, smoking status, physical activity,
0.71 to < 0.94	Q2	1.08 (0.69-1.69)	
0.94 to < 1.31	Q3	0.92 (0.59-1.44)	

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Supplementary Files

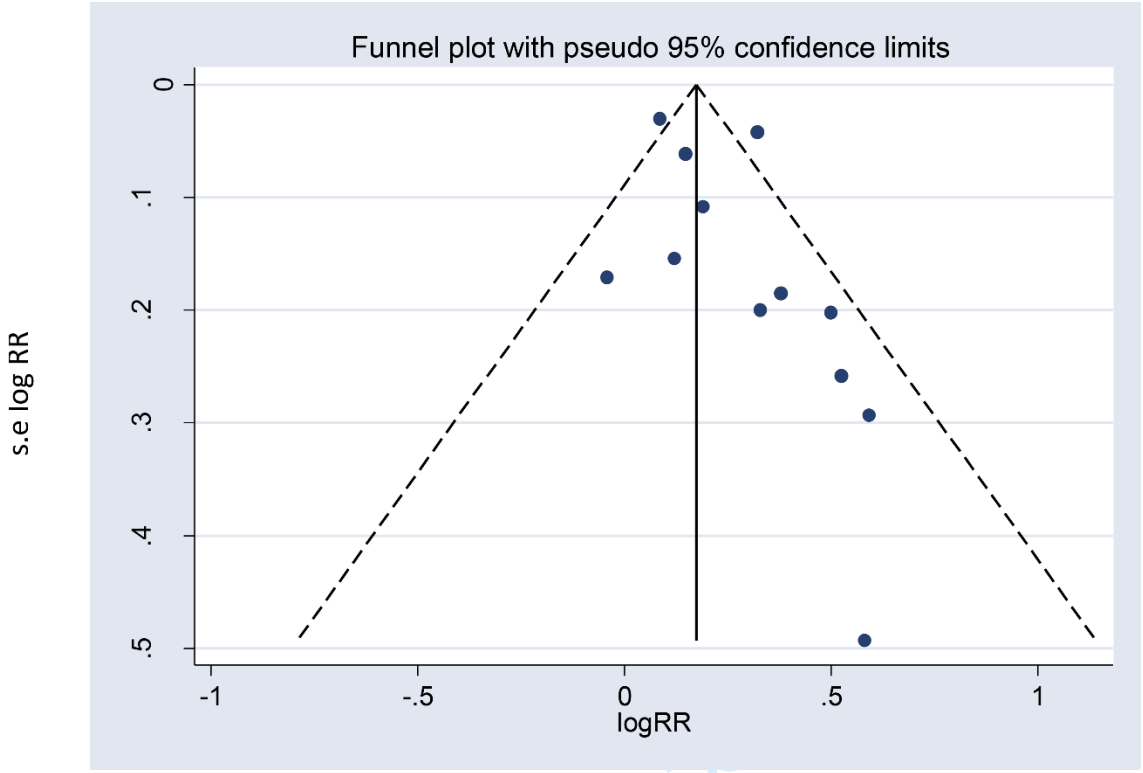
≥ 1.31	Q4	1.36 (0.86-2.13)	alcohol consumption, parental history of myocardial infarction <60 y, history of high cholesterol and diabetes mellitus, and BMI.
Seven, 2014 [29] Inter99 study, CRP			
log, 1 SD increment	379/2881	0.98 (0.84-1.14)	Age, sex, leptin, adiponectin, alcohol intake, smoking, physical activity, dietary habits, educational level, parental history of hypertension, total cholesterol, triglycerides, insulin, HbA1c, baseline heart rate, and systolic and diastolic blood pressure.
Sung, 2017 [9] Samsung Hospital Health Screening Center study, CRP, Men			
< 0.4 mg/L	2482/72,773 PY	1.00	Age, smoking status, alcohol intake, regular exercise, education level, systolic blood pressure, BMI, and insulin.
0.4-0.5	1494/33,877	1.01 (1.02-1.08)	
0.6-1.1	2168/44,725	1.12 (1.04-1.19)	
≥ 1.2	2085/42,645	1.11 (1.04-1.19)	
Sung, 2017 [9] Samsung Hospital Health Screening Center study, CRP, Women			
< 0.2 mg/L	479/44,939 PY	1.00	Age, smoking status, alcohol intake, regular exercise, education level, systolic blood pressure, BMI, and insulin.
0.2-0.3	496/37,287	1.06 (0.93-1.22)	
0.4-0.8	532/28,274	1.06 (1.01-1.33)	
≥ 0.9	669/31,943	1.19 (1.04-1.37)	
Vanhala, 2008 [30] Pieks¨am¨aki study, IL-1β			
0.25 pg/mL (median)	Q1	1.00	Age, gender, follow-up time, and baseline BMI.
0.48	Q2	1.47 (0.79-2.73)	
0.63	Q3	0.83 (0.43-1.58)	
0.82	Q4	1.55 (0.84-2.89)	
Wang, 2007 [31] Framingham Offspring Study, CRP			
log, 1 SD increment	232/1456	1.26 (1.05-1.51)	Age, sex, baseline systolic and diastolic blood pressure, baseline blood pressure category, baseline serum creatinine, diabetes mellitus, cigarette smoking, baseline BMI and percentage of weight change from baseline to follow-up.
Wang, 2011 [26] Women's Health Initiative Observational Study, hs-CRP (white)			
0.37 mg/L (median)	65/100	1.00	Age, clinical center, time of enrollment, smoking, alcohol intake, physical activity, and hormone replacement therapy.
0.99	84/100	1.20 (0.74-1.93)	
2.06	96/100	1.30 (0.82-2.04)	
4.50	154/100	2.02 (1.29-3.14)	
Wang, 2011 [26] Women's Health Initiative Observational Study, hs-CRP (black)			
0.48 mg/L (median)	82/100	1.00	Age, clinical center, time of enrollment, smoking, alcohol intake, physical activity, and hormone replacement therapy.
1.37	76/101	0.94 (0.60-1.45)	
2.76	101/99	1.09 (0.69-1.72)	
7.16	140/100	1.64 (1.07-2.52)	
Wang, 2011 [26] Women's Health Initiative Observational Study, IL-6 (white)			
0.58 pg/mL (median)	74/100	1.00	

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0.97	99/101	1.27 (0.84-1.92)	Age, clinical center, time of enrollment, smoking, alcohol intake, physical activity, and hormone replacement therapy.
1.54	106/99	1.37 (0.88-2.13)	
2.78	118/99	1.64 (1.06-2.55)	
Wang, 2011 [26] Women's Health Initiative Observational Study, IL-6 (black)			
0.74 pg/mL (median)	73/100	1.00	Age, clinical center, time of enrollment, smoking, alcohol intake, physical activity, and hormone replacement therapy.
1.23	91/100	1.40 (0.89-2.19)	
1.94	101/100	1.59 (0.98-2.57)	
4.39	135/100	1.99 (1.27-3.12)	
Wang, 2011 [26] Women's Health Initiative Observational Study, IL-1β (white)			
0.13 pg/mL (median)	91/89	1.00	Age, clinical center, time of enrollment, smoking, alcohol intake, physical activity, and hormone replacement therapy.
0.20	84/90	0.93 (0.65-1.54)	
0.26	91/88	0.99 (0.58-1.68)	
0.48	89/88	1.06 (0.60-1.87)	
Wang, 2011 [26] Women's Health Initiative Observational Study, IL-1β (black)			
0.13 pg/mL (median)	65/90	1.00	Age, clinical center, time of enrollment, smoking, alcohol intake, physical activity, and hormone replacement therapy.
0.20	106/91	1.31 (0.81-2.12)	
0.28	98/89	1.42 (0.84-2.39)	
0.47	89/90	1.30 (0.73-2.33)	
Abbreviations: BMI, body mass index; CRP, C-reactive protein; HbA1c, hemoglobin A1c; hs-CRP, high-sensitive C-reactive protein; IL-1β, interleukin-1β; IL-6, interleukin -6; IR, incidence rate; MESA, Multi-Ethnic Study of Atherosclerosis; PY, personyear; Q, quantile; SD, standard deviation.			

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Supplementary Files



Online Supplementary file 6. Funnel plot of the relative risks of 12 studies on circulating C-reactive protein and risk of developing hypertension. Begg's test *P*=0.12, Egger's test *P*=0.15. Log RR: natural logarithm of relative risk. s.e: standard error.

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	-
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplementary files
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	8,9
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7,8

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7,8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7,8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8,9
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9,10,11
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figures 1-2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9,10,11
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9,10,11
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	9,10,11
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11,12,13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	14,15
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	15

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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