Drugs (2018) 78:99–109
https://doi.org/10.1007/s40265-017-0839-5

SYSTEMATIC REVIEW

Effects of Allopurinol on Endothelial Function: A Systematic Review and Meta-Analysis of Randomized Placebo-Controlled Trials

Arrigo F. G. Cicero[1] · Matteo Pirro[2] · Gerald F. Watts[3] · Dimitri P. Mikhailidis[4] · Maciej Banach[5,6] · Amirhossein Sahebkar[7,8]

Published online: 14 November 2017
© Springer International Publishing AG, part of Springer Nature 2017

Abstract

Introduction Uric acid (UA), the final product of purine catabolism, may be associated with an increased risk of cardiovascular disease.

Aim The aim of this meta-analysis of randomized placebo-controlled trials was to evaluate whether lowering serum UA (SUA) levels with allopurinol is associated with improved flow-mediated dilation (FMD), a validated marker of early vascular damage.

Methods A literature search was carried out from inception until 20 June 2017. Meta-analysis was performed using an inverse variance-weighted, random-effects model with standardized mean difference (SMD) as the effect size estimate.

Results Meta-analysis of data from the ten eligible randomized controlled trials (RCTs), with 670 subjects, suggested a significant increase in FMD following allopurinol treatment (weighted mean difference [WMD] 1.79%, 95% confidence interval [CI] 1.01–2.56, $p < 0.001$; I^2: 86.77%). The effect size was robust and remained significant after omission of each single study. Subgroup analyses of RCTs based on the administered dose or duration of treatment did not reveal any significant impact of these variables on FMD change. Nor was a significant association found between allopurinol-induced changes in SUA levels and FMD (slope 0.46, $p = 0.253$), whereas baseline FMD significantly influenced the degree of FMD improvement following allopurinol treatment (slope 0.52, $p = 0.022$). Nitroglycerin-mediated dilation was not altered by allopurinol treatment (WMD 0.88%, 95% CI −1.15–2.91, $p = 0.395$; I^2: 80.88%).

Conclusion This meta-analysis of available RCTs suggests a significant benefit from allopurinol intake in increasing FMD in humans, independent of its effect on SUA levels.

✉ Amirhossein Sahebkar
sahebkara@mums.ac.ir; amir_saheb2000@yahoo.com; amirhossein.sahebkar@uwa.edu.au

[1] Department of Medical and Surgical Sciences, University of Bologna, Via Albertoni 15, Bologna, Italy

[2] Unit of Internal Medicine, Angiology and Arteriosclerosis Diseases, Department of Medicine, University of Perugia, Perugia, Italy

[3] Department of Cardiology, Lipid Disorders Clinic, Royal Perth Hospital, Perth, WA, Australia

[4] Department of Clinical Biochemistry, Royal Free Hospital Campus, University College London Medical School, University College London, London, United Kingdom

[5] Department of Hypertension, WAM University Hospital in Lodz, Medical University of Lodz, Zeromskiego 113, Lodz, Poland

[6] Polish Mother's Memorial Hospital Research Institute (PMMHRI), Lodz, Poland

[7] Biotechnology Research Center, Institute of Pharmaceutical Technology, Mashhad University of Medical Sciences, Mashhad 9177948564, Iran

[8] School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

Key Points

Uric acid is an emerging risk factor for cardiovascular disease.

Allopurinol, a xanthine oxidase inhibitor, is one of the most used and studied uric acid-lowering drugs.

Allopurinol could improve flow-mediated dilation, an early marker of cardiovascular disease risk.

The effect seems to be independent of allopurinol dose and serum uric acid change.

1 Introduction

Uric acid (UA) is the final product of purine catabolism and is formed from xanthine, mainly in the liver and intestine [1]. This metabolic pathway has been well preserved throughout the evolution of most living species, thus suggesting its importance [1]. Hyperuricemia might result from either overproduction of UA and/or reduced UA degradation and/or renal excretion, thus indicating the large number of factors that can affect serum UA (SUA) levels [2]. These include physiological factors (e.g. age, sex, renal function and cellular turnover) and exogenous/dietary factors (e.g. purine intake, certain drugs, some types of malignant disease, fructose intake and alcohol consumption) [3].

It is well known that SUA excess has pro-oxidative and inflammatory effects and is associated with systemic oxidative stress and, consequently, with metabolic and cardiovascular (CV) disorders [4, 5]. The detrimental effects of UA on the CV system include mediating immune response upon cell injury [6], and increasing endotoxin-stimulated tumor necrosis factor-alpha production and hence proinflammatory immune activation [7]. Therefore, high SUA levels may contribute to CV damage through endothelial injury. In fact, a large body of evidence shows that SUA is associated with early markers of vascular stiffness [8, 9] and is an independent predictor of CV risk [10] in children [11], adolescents [12] and healthy subjects [13].

Flow-mediated dilation (FMD) is a validated marker of CV disease (CVD) [14, 15]. Lowering SUA levels via inhibition of xanthine oxidase has resulted in conflicting results in terms of endothelial function improvement. For this reason, we carried out a meta-analysis of randomized controlled trials (RCTs) testing the effect of allopurinol on endothelial function.

2 Methods

2.1 Search Strategy

This study was designed according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines [16]. PubMed–MEDLINE, Scopus and ISI Web of Knowledge databases were searched, without any language restriction, using the following search terms in titles and abstracts: (allopurinol) AND ("flow-mediated dilation" OR "flow-mediated dilatation" OR "flow mediated dilation" OR "flow mediated dilatation" OR FMD) AND (placebo). The wild-card "*" was used at the end of the word "placebo" to consider all interchangeable formats of the word in the search (e.g. "placebo-adjusted" OR "placebo-controlled"). The literature was searched from inception to 20 June 2017. A manual search of the reference lists of identified studies was also performed to find additional relevant studies.

2.2 Study Selection

Original studies were included if they met the following inclusion criteria: (1) a randomized placebo-controlled trial with either parallel or cross-over design, (2) investigating the impact of allopurinol on FMD as a measure of endothelial function, (3) administration of allopurinol for a period of ≥ 2 weeks, and (4) presentation of sufficient information on FMD values at baseline and after allopurinol treatment or net change values. Exclusion criteria were (1) non-randomized studies, (2) not placebo-controlled studies, (3) using endothelial function indices other than FMD, (4) evaluated acute (single-dose) effects of allopurinol, (5) observational studies with case–control, cross-sectional or cohort design, and (6) lack of sufficient information on baseline or follow-up FMD levels. Two authors (AFGC and AS) were responsible for this step, and disagreements were resolved through discussion.

2.3 Data Extraction

Eligible studies were reviewed and the following data were abstracted: first author's name; year of publication; country in which the study was performed; study design; number of participants treated with allopurinol and placebo; underlying disease/condition of the studied population; allopurinol dose; treatment duration; age, sex and body mass index (BMI) of study participants; and data regarding baseline and follow-up FMD. Two authors (AFGC and AS) were responsible for this step, and disagreements were resolved through discussion.

2.4 Quality Assessment

The risk of bias in the studies considered in this meta-analysis was evaluated according to Cochrane Collaboration guidelines [17]. Selection bias, performance bias, attrition bias, detection bias, reporting bias and other sources of bias were categorized as high, low or unclear in each of the included studies.

2.5 Quantitative Data Synthesis

This meta-analysis was conducted using Comprehensive Meta-Analysis (CMA) V2 software (Biostat, NJ, USA) [18]. A random-effects model (using the DerSimonian–Laird method) and the generic inverse variance weighting method were used to compensate for the heterogeneity of studies in terms of study design, treatment duration, and the characteristics of populations being studied [19]. Standard deviations (SDs) of the mean differences were calculated using the following formula: SD = square root $[(SD_{pre\text{-}treatment})^2 + (SD_{post\text{-}treatment})^2 - (2R \times SD_{pre\text{-}treatment} \times SD_{post\text{-}treatment})]$, assuming a correlation coefficient $(R) = 0.5$. Where only the standard error of the mean (SEM) was reported, the SD was estimated using the following formula: SD = SEM × square root (n), where n is the number of subjects. Effect sizes were expressed as weighted mean difference (WMD) and 95% confidence interval (CI). Where values were only presented as a graph, the software GetData Graph Digitizer 2.24 (http://getdata-graph-digitizer.com/) was applied to digitize and extract the data.

Subgroup analyses were performed according to the administered doses and duration of treatment using the CMA V2 software and considering the method described by Bucher et al. [20] for adjusted indirect comparisons with a single common comparator.

2.6 Meta-Regression

As potential confounders of treatment response, duration of treatment with allopurinol, changes in plasma and/or SUA concentrations, and baseline FMD values were entered into a random-effects meta-regression model to explore their association with the estimated effect size on FMD.

2.7 Publication Bias

Funnel plots, Begg's rank correlation and Egger's weighted regression tests were evaluated to assess the presence of publication bias in the meta-analysis. When there was evidence of funnel plot asymmetry, potentially missing studies were imputed using the "trim and fill" method [21]. Where results were significant, the number of potentially

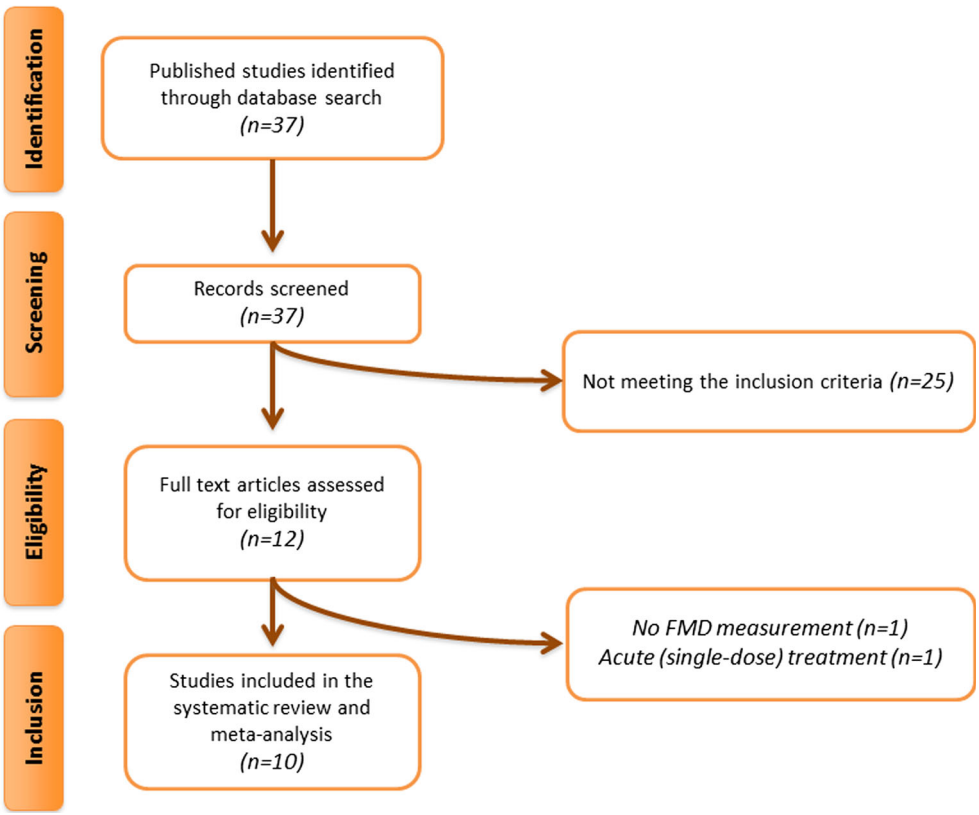


Fig. 1 Flow chart of the number of studies identified and included in the meta-analysis

Table 1 Demographic characteristics of the included studies

Author	Study design	Target population	Treatment duration	*n*	Study groups	Age, years	Female (*n*, %)	SUA (mg/dl)	FMD (%)
El Solh et al. [22]	Ran, DB, PC, CO	Sleep apnea pts	2 weeks	12	ALL 300 mg/day	47.3 ± 8.7	7 (58)	4.6 ± 0.9	6.9 ± 2.4
				12	PL	47.3 ± 8.7	7 (58)	–	7.4 ± 2.8
Yiginer et al. [23]	Ran, DB, PC, PG	Metabolic syndrome	4 weeks	28	ALL 300 mg/day	65 ± 7	21 (75)	6.6 ± 0.6	8.0 ± 0.5
				22	PL	64 ± 7	15 (68)	6.5 ± 0.6	7.8 ± 0.5
Dogan et al. [26]	Ran, SB, PC	Diabetic normotensive pts	12 weeks	50	ALL 900 mg/day	50.5 ± 5.0	25 (50)	5.0 ± 0.8	5.6 ± 2.1
				50	PL	50.0 ± 6.0	24 (48)	4.8 ± 1.1	5.8 ± 1.8
Kao et al. [24]	Ran, DB, PC, PG	Stage 3 CKD and LVH pts	9 months	27	ALL 300 mg/day	70.6 ± 6.9	11 (41)	7.4 ± 0.9	5.0 ± 1.9
				26	PL	73.7 ± 5.3	14 (54)	7.1 ± 0.9	4.9 ± 2.5
Rajendra et al. [28]	Ran, DB, PC, CO	CAD pts	8 weeks	80	ALL 600 mg/day	65.5 ± 7.73	16 (20)	–	4.2 ± 1.8
				80	PL	65.5 ± 7.73	16 (20)		4.2 ± 1.8
Tousoulis et al. [30]	Ran, DB, PC, PG	CHF pts	4 weeks	21	ALL 300 mg/day	65 ± 12	1 (5)	7.4 ± 2.4	3.3 ± 1.3
				18	PL	66 ± 11	2 (11)	7.5 ± 2.0	3.1 ± 1.3
Rekhraj et al. [29]	Ran, DB, PC, PG	CAD and LVH pts	9 months	31	ALL 600 mg/day	65.0 ± 6.7	5 (16)	9.9 ± 1.5	4.1 ± 2.1
				29	PL	64.0 ± 7.2	1 (3)	9.4 ± 2.3	5.7 ± 2.4
Szwejkowski et al. [27]	Ran, DB PC clinical PG	T2DM pts, LVH	9 months	29	ALL 600 mg/day	63.2 ± 8.6	9 (30)	9.1 ± 1.7	4.1 ± 0.6
				30	PL	66.0 ± 8.9	13 (45)	9.2 ± 2.5	4.2 ± 0.6
Robertson et al. [31]	Ran, DB PC clinical PG	PAD pts	6 months	25	ALL 600 mg/day	69.6 ± 9.1	4 (16)	6.0 ± 1.5	8.3 ± 2.3
				25	PL	67.3 ± 7.5	7 (28)	5.7 ± 1.5	9.61 ± 3.4
Jalal et al. [25]	Ran, DB, PC, PG	Stage 3 CKD and asymptomatic hyperuricemia	12 weeks	39	ALL 300 mg/day	58.9 ± 9.3	7 (18)	8.7 ± 1.6	6.0 ± 5.0
				41	PL	55.9 ± 13.7	9 (22)	8.3 ± 1.4	4.8 ± 5.0

ALL allopurinol, *CAD* coronary artery disease, *CHF* chronic heart failure, *CKD* chronic kidney disease, *CO* crossover, *DB* double-blind, *FMD* flow-mediated dilation, *LVH* left ventricular hypertrophy, *PAD* peripheral artery disease, *PC* placebo-controlled, *PG* parallel-group, *PL* placebo, *pts* patients, *Ran* randomized, *SB* single-blind, *SUA* serum uric acid

Table 2 Risk-of-bias assessment according to the Cochrane Collaboration's tool for randomized controlled trials and ROBINS-I tool for non-randomized and observational studies

Study	Sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective outcome reporting	Other potential threats to validity
El Solh et al. [22]	L	L	L	L	L	L	H[a]
Yiginer et al. [23]	U	L	L	L	L	L	L
Dogan et al. [26]	U	L	L	L	L	L	L
Kao et al. [24]	U	L	L	L	L	L	L
Rajendra et al. [28]		L	L	L	L	L	H[a]
Tousoulis et al. [30]		L	L	L	L	L	L
Rekhraj et al. [29]		L	L	L	L	L	L
Szwejkowski et al. [27]		L	L	L	L	L	L
Robertson et al. [31]		L	L	L	L	L	L
Jalal et al. [25]		L	L	L	L	L	L

H high risk of bias, *L* low risk of bias, *ROBINS-I* Risk Of Bias In Non-randomized Studies of Interventions, *SUA* serum uric acid, *U* unclear risk of bias

[a]El Solh [22] and Rajendra [28] did not report complete SUA data

missing studies required to make the p value non-significant was estimated using the "fail-safe N" method as another marker of publication bias.

3 Results

3.1 Main Results

Overall, the multi-database search found 37 articles. After screening the titles and abstracts, 12 articles were assessed as full text. One of these articles was excluded because FMD values were not reported, and another was excluded because the acute (single-dose) effects of allopurinol were not evaluated, leaving ten eligible articles for meta-analysis (Fig. 1). Table 1 provides the main characteristics of the included studies, and Table 2 shows the risk-of-bias assessment.

Two studies enrolled patients receiving primary prevention for CVD but affected by sleep apnoea [22] or metabolic syndrome [23]. Four studies included subjects with high CV risk without overt CVD: two included patients affected by stage 3 chronic kidney disease [24, 25] and two included patients affected by type 2 diabetes mellitus [26, 27]. The remaining trials enrolled patients receiving secondary prevention for CVD who were already affected by coronary artery disease [28, 29], chronic heart failure [30] or peripheral artery disease [31].

Meta-analysis of data from ten RCTs, including 670 subjects, suggested a significant elevation of FMD following allopurinol treatment (WMD 1.79%, 95% CI 1.01–2.56, $p < 0.001$; I^2: 86.77%) (Fig. 2). The effect size was robust in the leave-one-out sensitivity analysis (Fig. 2), and the effect size remained significant after omission of each single study from the meta-analysis.

Subgroup analyses of RCTs based on the administered dose of allopurinol did not reveal any significant difference between studies administering a daily dose of allopurinol ≤ 300 mg (WMD 1.90%, 95% CI 0.77–3.04, $p = 0.001$; I^2: 80.74%) and those administering a daily dose of allopurinol > 300 mg/day (WMD 1.65%, 95% CI 0.40–2.91, $p = 0.010$; I^2: 90.12%) ($p = 0.772$ for between-group comparison) (Fig. 3).

Subgroup analyses of RCTs based on the duration of allopurinol treatment did not reveal any significant difference between studies administering allopurinol for < 12 weeks (WMD 1.87%, 95% CI 0.73–3.00, $p = 0.001$; I^2: 90.21%) and those administering allopurinol for ≥ 12 weeks (WMD 1.67%, 95% CI 0.35–2.99, $p = 0.013$; I^2: 86.60%) ($p = 0.826$ for between-group comparison) (Fig. 4).

Five RCTs assessed nitroglycerin-mediated dilation (NMD). Meta-analysis of the NMD changes in these RCTs did not indicate any significant effect of allopurinol versus placebo (WMD 0.88%, 95% CI -1.15–2.91, $p = 0.395$; I^2: 80.88%) (Fig. 5).

3.2 Meta-Regression

Random-effects meta-regression was performed to assess the impact of potential confounders on the effects of allopurinol on FMD. The results did not suggest any significant association between the changes in FMD with either treatment duration (slope -0.02; 95% CI -0.08–0.04; $p = 0.518$) or changes in SUA concentrations (slope 0.46; 95% CI -0.33–1.25; $p = 0.253$) (Fig. 6). However, baseline FMD values were found to be positively

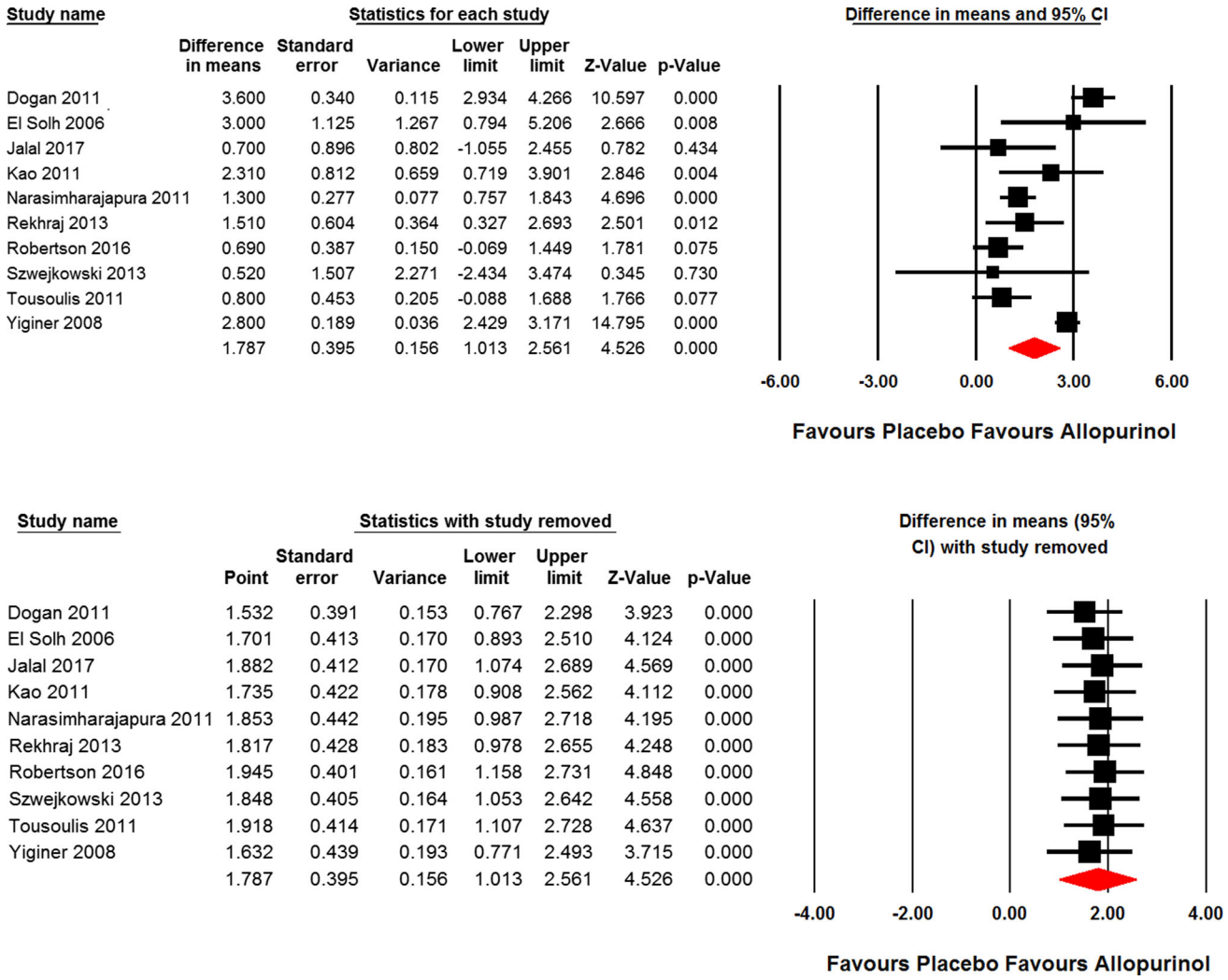


Fig. 2 Forest plot displaying weighted mean difference and 95% confidence intervals (CIs) for the impact of allopurinol treatment on flow-mediated dilation. The lower plot shows the leave-one-out sensitivity analysis

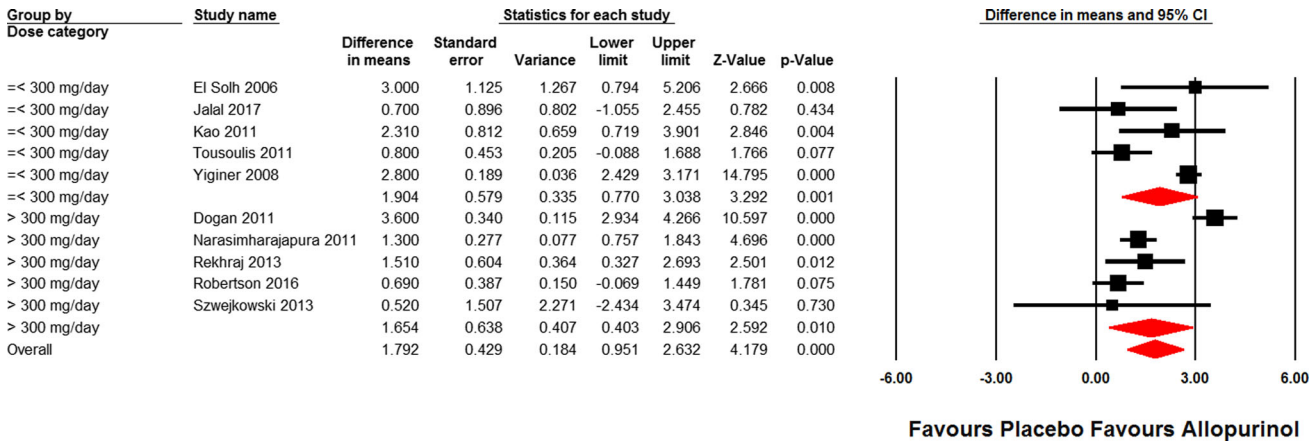


Fig. 3 Forest plot displaying weighted mean difference and 95% confidence intervals (CIs) for the impact of allopurinol treatment on flow-mediated dilation in the subsets of trials administering allopurinol at a daily dose of ≤ 300 mg and > 300 mg

Group by Duration category	Study name	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
< 12 weeks	El Solh 2006	3.000	1.125	1.267	0.794	5.206	2.666	0.008
< 12 weeks	Narasimharajapura 2011	1.300	0.277	0.077	0.757	1.843	4.696	0.000
< 12 weeks	Tousoulis 2011	0.800	0.453	0.205	-0.088	1.688	1.766	0.077
< 12 weeks	Yiginer 2008	2.800	0.189	0.036	2.429	3.171	14.795	0.000
< 12 weeks		1.866	0.579	0.336	0.731	3.002	3.221	0.001
>= 12 weeks	Dogan 2011	3.600	0.340	0.115	2.934	4.266	10.597	0.000
>= 12 weeks	Jalal 2017	0.700	0.896	0.802	-1.055	2.455	0.782	0.434
>= 12 weeks	Kao 2011	2.310	0.812	0.659	0.719	3.901	2.846	0.004
>= 12 weeks	Rekhraj 2013	1.510	0.604	0.364	0.327	2.693	2.501	0.012
>= 12 weeks	Robertson 2016	0.690	0.387	0.150	-0.069	1.449	1.781	0.075
>= 12 weeks	Szwejkowski 2013	0.520	1.507	2.271	-2.434	3.474	0.345	0.730
>= 12 weeks		1.671	0.673	0.453	0.351	2.991	2.482	0.013
Overall		1.783	0.439	0.193	0.922	2.644	4.060	0.000

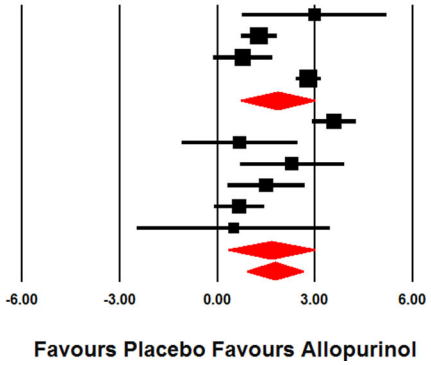


Fig. 4 Forest plot displaying weighted mean difference and 95% confidence intervals (CIs) for the impact of allopurinol treatment on flow-mediated dilation in the subsets of trials administering allopurinol for ≤ 12 weeks and > 12 weeks

Study name	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Dogan 2011	6.000	1.482	2.195	3.096	8.904	4.050	0.000
Kao 2011	0.560	1.721	2.963	-2.814	3.934	0.325	0.745
Rekhraj 2013	-1.510	0.604	0.364	-2.693	-0.327	-2.501	0.012
Szwejkowski 2013	-0.260	1.410	1.988	-3.023	2.503	-0.184	0.854
Yiginer 2008	-0.600	0.850	0.722	-2.265	1.065	-0.706	0.480
Jalal 2017	2.200	1.276	1.628	-0.301	4.701	1.724	0.085
	0.880	1.035	1.070	-1.147	2.908	0.851	0.395

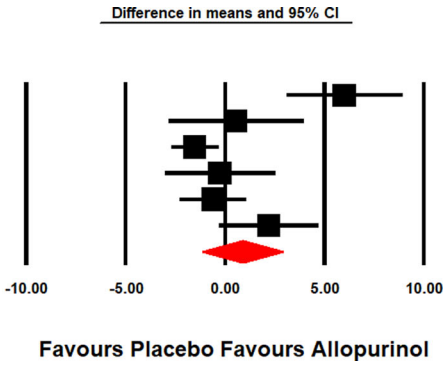


Fig. 5 Forest plot displaying weighted mean difference and 95% confidence intervals (CIs) for the impact of allopurinol treatment on nitroglycerine-mediated dilation

associated with change in FMD following allopurinol treatment (slope 0.52; 95% CI 0.08–0.97; $p = 0.022$).

3.3 Publication Bias

Visual inspection of Begg's funnel plots did not reveal any sign of asymmetry and potential publication bias in the meta-analyses of the effect of allopurinol on FMD (Fig. 7). The results of Egger's linear regression test and Begg's rank correlation tests also confirmed a lack of publication bias. The "fail-safe N" test suggested that 467 potentially missing studies with negative results would be required to make the observed effect size non-significant.

4 Discussion

The biochemical process leading to hyperuricemia increases the generation of free oxygen radicals in the proportion of one molecule of superoxide for each single molecule of UA produced, with a negative impact on the microcirculation and the development of arteriolar disease, particularly at the renal level [32]. Experimental evidence suggests that hyperuricemia can impair endothelial function by promoting an increased oxidative state, which in turn downregulates endothelial nitric oxide (NO) production, in a condition where NO has a central role in the modulation of vascular flow and blood pressure [33]. Hyperuricemia also inhibits endothelial cell proliferation and migration and stimulates the release of inflammatory C-reactive protein (CRP), growth factors and free oxygen radicals [34]. In addition, the negative effects of UA also involve smooth muscle cells, on which it is capable of stimulating cellular proliferation via the mitogen-activated protein kinase (MAPK) pathway and inducing the synthesis of pro-inflammatory substances such as chemokine, monocyte chemo-attractant protein-1 (MCP-1) and CRP. Finally, UA has also been proven to strongly activate the renin-angiotensin system, promoting both angiotensin II receptor type 1 (AT_1) and type 2 (AT_2) and angiotensin II expression, with demonstration of the ability of angiotensin II to inhibit cell proliferation and promote endothelial senescence and apoptosis [35]. SUA can then induce arterial stiffness, as an advanced marker of endothelial dysfunction [36].

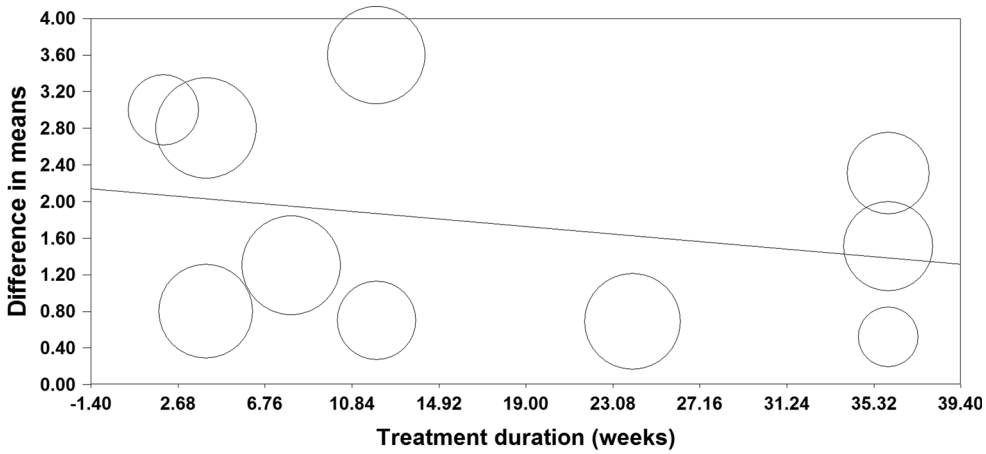
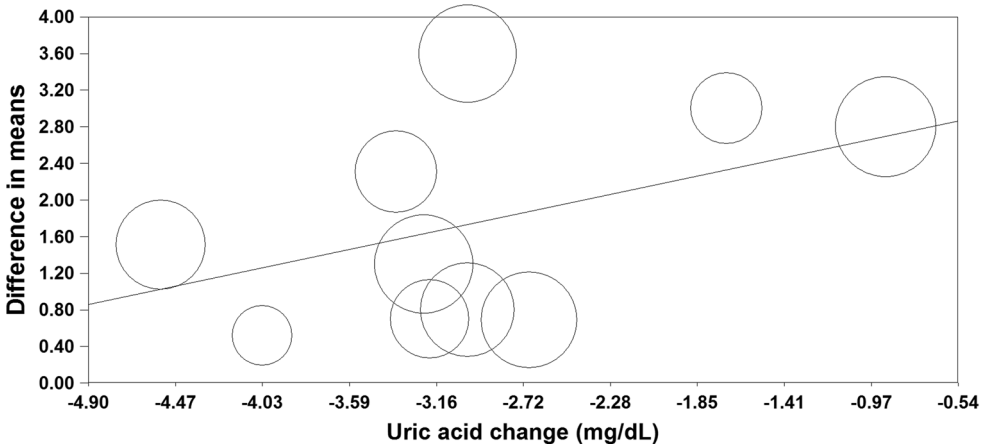


Fig. 6 Meta-regression bubble plots of the association between mean changes in flow-mediated dilation with treatment duration, respective changes in plasma uric acid concentrations and baseline flow-mediated dilation values. The size of each circle is inversely proportional to the variance of change



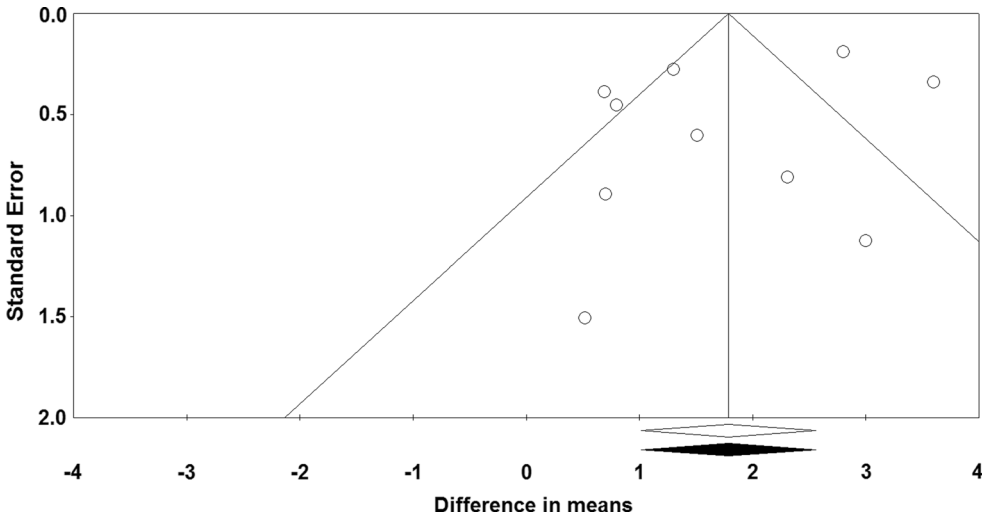


Fig. 7 Funnel plot detailing publication bias in the studies reporting the impact of allopurinol treatment on flow-mediated dilation

On the other hand, reduction of SUA has been associated with the amelioration of a number of risk factors for endothelial dysfunction, including hypertension, hyperglycemia and inflammation. Thus, in a general population sample, allopurinol was able to counteract the age-related development of hypertension and impaired fasting glucose [37]. Furthermore, historical as well as more recent evidence reports the anti-inflammatory effects of allopurinol in preclinical and clinical studies [38, 39]. Other drugs are also able to improve SUA levels, but they have not been deeply investigated for their effects on vascular health independent of SUA reduction [40].

Although this meta-analysis does not provide a mechanistic clue to allopurinol-induced FMD improvement, these latter data [28, 29] may provide a pathophysiological explanation of our main result, that is, an average 1.79% increase in FMD following allopurinol treatment compared with placebo. This result may be relevant from a clinical perspective, as a recent meta-analysis of 35 FMD studies in 17,280 participants concluded that even small increases in FMD could be associated with a relatively small but significant reduction of CV event risk [41]. However, whether the reported allopurinol-induced FMD amelioration would translate into a reproducible CVD risk reduction needs to be further explored in specific long-term trials, thus possibly resolving the debate on the controversial association between allopurinol treatment and CVD risk protection [42–45]. It would also be interesting to test whether other more selective and powerful xanthine oxidase inhibitors such as febuxostat could exert an effect similar to or stronger than that of allopurinol [46]. A preliminary trial carried out in patients undergoing hemodialysis showed that treatment with febuxostat for 4 weeks was associated with an FMD increase of about 3.6% [47]. Similar FMD improvements have also been confirmed in a very recent trial in patients with type 1 diabetes mellitus [48]. However, these preliminary data need to be confirmed in larger patient populations and in long-term clinical trials.

4.1 Limitations

Our meta-analysis has some limitations. First, the number of available studies investigating the effect of allopurinol on FMD is relatively small, and studies are usually short. However, we included studies with a robust design (i.e. randomized placebo-controlled trials) and were able to detect a significant effect, even with this number of included trials. Next, the dose of allopurinol used in the selected studies was not comparable across the different studies. Moreover, exposure to CV risk factors varied among the subjects enrolled in the selected trials; these risk factors were managed in a variety of ways; and studies were not originally designed to recruit subjects with impaired FMD at baseline. In particular, statins, which can mildly but significantly improve FMD [49], were not systematically prescribed, and details on low-density lipoprotein cholesterol (LDL-C) target reached and treatment duration were not reported. It could be interesting to evaluate the eventual synergistic effect on FMD of allopurinol and statins and antihypertensive drugs. However, overall, the observed treatment effect was homogeneous and supported a significant effect of allopurinol on FMD [50]. In addition, the results showed that the significance of the estimated pooled effect size was not biased by any single study, and—overall—the quality of the selected studies was estimated as high. Thus, we did not conduct a sensitivity analysis. Finally, we did not formally register a priori the meta-analysis protocol.

5 Conclusion

This meta-analysis of available RCTs suggests a significant beneficial effect of allopurinol treatment on FMD in humans, independent of its effect on SUA and on baseline SUA level. The effect seems to be particularly relevant when baseline function is not severely compromised. Given the low cost and the good overall tolerability of allopurinol at standard doses of 300 mg/day, additional trials exploring its impact on CVD outcomes in hyperuricemic patients are warranted to clarify whether allopurinol treatment may represent a novel and sustainable strategy for CVD risk prevention.

Author Contributions AFGC, MP, MB and AS conceived and designed the work and contributed to the drafting of the manuscript. Arrigo F.G. Cicero and Matteo Pirro performed the searches and extracted data. Amirhossein Sahebkar performed the statistical analysis. GFWa, DPM and MB contributed to the interpretation of the work and revised the manuscript critically for important intellectual content. All authors approved the final manuscript and agree to be held accountable for all aspects of the work.

Compliance with Ethical Standards

Conflicts of interest Prof. Watts has received honoraria for advisory boards and lectures from Amgen, Sanofi, Regeneron, Kowa and MSD. Prof. Banach has served on the speaker's bureau and as an advisory board member for Amgen, Sanofi, Aventis and Lilly. Prof. Mikhailidis has given talks and attended conferences sponsored by MSD, Libytec and AstraZeneca. No professional writer was involved in the preparation of this meta-analysis.

Funding This meta-analysis was written independently; no company or institution supported it financially.

References

1. Hyndman D, Liu S, Miner JN. Urate handling in the human body. Curr Rheumatol Rep. 2016;18(6):34.
2. Maiuolo J, Oppedisano F, Gratteri S, Muscoli C, Mollace V. Regulation of uric acid metabolism and excretion. Int J Cardiol. 2016;213:8–14.
3. Katsiki N, Karagiannis A, Athyros VG, Mikhailidis DP. Hyperuricaemia: more than just a cause of gout? J Cardiovasc Med. 2013;14:397–402.
4. Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. N Engl J Med. 2008;359:1811–21.
5. Borghi C. Uric acid as a cross-over between rheumatology and cardiovascular disease. Curr Med Res Opin. 2013;29(Suppl 3):1–2.
6. Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. Nature. 2003;425:516–21.

7. Netea MG, Kullberg BJ, Blok WL, Netea RT, van der Meer JW. The role of hyperuricemia in the increased cytokine production after lipopolysaccharide challenge in neutropenic mice. Blood. 1997;89:577–82.
8. Erdogan D, Gullu H, Caliskan M, Yildirim E, Bilgi M, Yildirim E, et al. Relationship of serum uric acid to measures of endothelial function and atherosclerosis in healthy adults. Int J Clin Pract. 2005;59:1276–82.
9. Cicero AF, Salvi P, D'Addato S, Rosticci M. Borghi C for the Brisighella Heart Study group. Association between serum uric acid, hypertension, vascular stiffness and subclinical atherosclerosis: data from the Brisighella Heart Study. J Hypertens. 2014;32:57–64.
10. Borghi C, Cicero AFG. Serum uric acid and cardiometabolic disease: another brick in the wall? Hypertension. 2017;69(6):1011–3.
11. Borghi C, Cicero AF. Cardiometabolic risks and obesity in the young. N Engl J Med. 2016;374(6):591–2.
12. Wang Y, Hu JW, Lv YB, Chu C, Wang KK, Zheng WL, Cao YM, et al. The role of uric acid in hypertension of adolescents, prehypertension and salt sensitivity of blood pressure. Med Sci Monit. 2017;23:790–5.
13. Kivity S, Kopel E, Maor E, Abu-Bachar F, Segev S, Abu-Bachar F, Segev S, Sidi Y, Olchovsky D. Association of serum uric acid and cardiovascular disease in healthy adults. Am J Cardiol. 2013;111:1146–51.
14. Vlachopoulos C, Xaplanteris P, Aboyans V, Brodmann M, Cífková R, Cosentino F, De Carlo M, et al. The role of vascular biomarkers for primary and secondary prevention. A position paper from the European Society of Cardiology Working Group on peripheral circulation: endorsed by the Association for Research into Arterial Structure and Physiology (ARTERY) Society. Atherosclerosis. 2015;241(2):507–32.
15. Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, Hamburg NM, et al. The assessment of endothelial function: from research into clinical practice. Circulation. 2012;126(6):753–67.
16. Moher D, Liberati A, Tetzlaff J. Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ. 2009;339:b2535.
17. Higgins JPT, Green S. Handbook for systematic reviews of interventions. Version 5.0.2 ed. London: The Cochrane Collaboration; 2009
18. Borenstein M, Higgins JP. Comprehensive Meta-analysis and subgroups. Prev Sci. 2013;14(2):134–43.
19. Sutton AJ, Abrams KR, Jones DR, Sheldon TA, Song F. Methods for meta-analysis in medical research. Chichester: Wiley; 2000.
20. Bucher HC, Guyatt GH, Griffith LE, Walter SD. The results of direct and indirect treatment comparisons in meta-analysis of randomized controlled trials. J Clin Epidemiol. 1997;50(6):683–91.
21. Duval STR. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. Biometrics. 2000;56(2):455–63.
22. El Solh AA, Saliba R, Bosinski T, Grant BJ, Berbary E, Miller N. Allopurinol improves endothelial function in sleep apnoea: a randomised controlled study. Eur Respir J. 2006;27(5):997–1002.
23. Yiginer O, Ozcelik F, Inanc T, Aparci M, Ozmen N, Cingozbay BY, Kardesoglu E, Suleymanoglu S, Sener G, Cebeci BS. Allopurinol improves endothelial function and reduces oxidant-inflammatory enzyme of myeloperoxidase in metabolic syndrome. Clin Res Cardiol. 2008;97(5):334–40.
24. Kao MP, Ang DS, Gandy SJ, Nadir MA, Houston JG, Lang CC, Struthers AD. Allopurinol benefits left ventricular mass and endothelial dysfunction in chronic kidney disease. J Am Soc Nephrol. 2011;22(7):1382–9.
25. Jalal DI, Decker E, Perrenoud L, Nowak KL, Bispham N, Mehta T, Smits G, You Z, Seals D, Chonchol M, Johnson RJ. Vascular function and uric acid-lowering in stage 3 CKD. J Am Soc Nephrol. 2017;28(3):943–52.
26. Dogan A, Yarlioglues M, Kaya MG, Karadag Z, Dogan S, Ardic I, Dogdu O, Kilinc Y, Zencir C, Akpek M, Ozdogru I, Oguzhan A, Kalay N. Effect of long-term and high-dose allopurinol therapy on endothelial function in normotensive diabetic patients. Blood Press. 2011;20(3):182–7.
27. Szwejkowski BR, Gandy SJ, Rekhraj S, Houston JG, Lang CC, Morris AD, George J, Struthers AD. Allopurinol reduces left ventricular mass in patients with type 2 diabetes and left ventricular hypertrophy. J Am Coll Cardiol. 2013;62(24):2284–93.
28. Rajendra NS, Ireland S, George J, Belch JJ, Lang CC, Struthers AD. Mechanistic insights into the therapeutic use of high-dose allopurinol in angina pectoris. J Am Coll Cardiol. 2011;58(8):820–8.
29. Rekhraj S, Gandy SJ, Szwejkowski BR, Nadir MA, Noman A, Houston JG, Lang CC, George J, Struthers AD. High-dose allopurinol reduces left ventricular mass in patients with ischemic heart disease. J Am Coll Cardiol. 2013;61(9):926–32.
30. Tousoulis D, Andreou I, Tsiatas M, Miliou A, Tentolouris C, Siasos G, Papageorgiou N, Papadimitriou CA, Dimopoulos MA, Stefanadis C. Effects of rosuvastatin and allopurinol on circulating endothelial progenitor cells in patients with congestive heart failure: the impact of inflammatory process and oxidative stress. Atherosclerosis. 2011;214(1):151–7.
31. Robertson AJ, Struthers AD. A randomized controlled trial of allopurinol in patients with peripheral arterial disease. Can J Cardiol. 2016;32(2):190–6.
32. Wang Y, Bao X. Effects of uric acid on endothelial dysfunction in early chronic kidney disease and its mechanisms. Eur J Med Res. 2013;18:26.
33. Kanbay M, Segal M, Afsar B, Kang DH, Rodriguez-Iturbe B, Johnson RJ. The role of uric acid in the pathogenesis of human cardiovascular disease. Heart. 2013;99:759–66.
34. Kang DH, Park SK, Lee IK, Johnson RJ. Uric acid-induced C-reactive protein expression: implication on cell proliferation and nitric oxide production of human vascular cells. J Am Soc Nephrol. 2005;16:3553–62.
35. Yu MA, Sánchez-Lozada LG, Johnson RJ, Kang DH. Oxidative stress with an activation of the renin-angiotensin system in human vascular endothelial cells as a novel mechanism of uric acid-induced endothelial dysfunction. J Hypertens. 2010;28:1234–42.
36. Choi HY, Kim SH, Choi AR, Kim SG, Kim H, Lee JE, Kim HJ, Park HC. Hyperuricemia and risk of increased arterial stiffness in healthy women based on health screening in Korean population. PLoS One. 2017;12(6):e0180406.
37. Cicero AF, Rosticci M, Bove M, Fogacci F, Giovannini M, Urso R, D'Addato S. Borghi C; Brisighella Heart Study Group. Serum uric acid change and modification of blood pressure and fasting plasma glucose in an overall healthy population sample: data from the Brisighella heart study. Ann Med. 2017;49(4):275–82.
38. Riesterer L, Jaques R. The anti-inflammatory action of a xanthine oxidase inhibitor (allopurinol). Pharmacology. 1969;2(5):288–94.
39. Muir SW, Harrow C, Dawson J, Lees KR, Weir CJ, Sattar N, Walters MR. Allopurinol use yields potentially beneficial effects on inflammatory indices in those with recent ischemic stroke: a randomized, double-blind, placebo-controlled trial. Stroke. 2008;39(12):3303–7.
40. Daskalopoulou SS, Tzovaras V, Mikhailidis DP, Elisaf M. Effect on serum uric acid levels of drugs prescribed for indications other than treating hyperuricaemia. Curr Pharm Des. 2005;11(32):4161–75.
41. Matsuzawa Y, Kwon TG, Lennon RJ, Lerman LO, Lerman A. Prognostic value of flow-mediated vasodilation in brachial artery

and fingertip artery for cardiovascular events: a systematic review and meta-analysis. J Am Heart Assoc. 2015;4(11):e002270.
42. Singh JA, Cleveland J. Allopurinol and the risk of ventricular arrhythmias in the elderly: a study using US Medicare data. BMC Med. 2017;15(1):59.
43. MacIsaac RL, Salatzki J, Higgins P, Walters MR, Padmanabhan S, Dominiczak AF, Touyz RM, Dawson J. Allopurinol and cardiovascular outcomes in adults with hypertension. Hypertension. 2016;67(3):535–40.
44. de Abajo FJ, Gil MJ, Rodríguez A, García-Poza P, Álvarez A, Bryant V, García-Rodríguez LA. Allopurinol use and risk of non-fatal acute myocardial infarction. Heart. 2015;101(9):679–85.
45. Grimaldi-Bensouda L, Alpérovitch A, Aubrun E, Danchin N, Rossignol M, Abenhaim L, Richette P, PGRx MI Group. Impact of allopurinol on risk of myocardial infarction. Ann Rheum Dis. 2015;74(5):836–42.
46. Bove M, Cicero AF, Veronesi M, Borghi C. An evidence-based review on urate-lowering treatments: implications for optimal treatment of chronic hyperuricemia. Vasc Health Risk Manag. 2017;13:23–8.
47. Tsuruta Y, Kikuchi K, Tsuruta Y, Sasaki Y, Moriyama T, Itabashi M, Takei T, et al. Febuxostat improves endothelial function in hemodialysis patients with hyperuricemia: a randomized controlled study. Hemodial Int. 2015;19(4):514–20.
48. Lytvyn Y, Har R, Locke A, Lai V, Fong D, Advani A, Perkins BA, Cherney DZI. Renal and vascular effects of uric acid lowering in normouricemic patients with uncomplicated type 1 diabetes. Diabetes. 2017;66(7):1939–49.
49. Zhang L, Gong D, Li S, Zhou X. Meta-analysis of the effects of statin therapy on endothelial function in patients with diabetes mellitus. Atherosclerosis. 2012;223(1):78–85.
50. Masoura C, Pitsavos C, Aznaouridis K, Skoumas I, Vlachopoulos C, Stefanadis C. Arterial endothelial function and wall thickness in familial hypercholesterolemia and familial combined hyperlipidemia and the effect of statins. A systematic review and meta-analysis. Atherosclerosis. 2011;214(1):129–38.