

Effects of Allopurinol on Endothelial Dysfunction: A Meta-Analysis

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Key Words

Uric acid · Allopurinol · Endothelial dysfunction · Cardiovascular disease

Abstract

Objective: Several studies have assessed the effect of allopurinol on endothelial function, but these studies were relatively small in size and used different methods of evaluating endothelial function. We conducted a meta-analysis to investigate the effect of allopurinol on both endothelial-dependent and -independent vasodilatation. **Methods:** Electronic databases, Medline, PubMed, EMBASE, SCOPUS, EBSCO and the Cochrane Library Central Register of Clinical Trials were searched from January 1985 to July 2013 on clinical trials (randomized and non-randomized) which assessed the effect of allopurinol on endothelial function. We conducted a sensitivity analysis to assess the contribution of each study to the pooled treatment effect by excluding each study one at a time and recalculating the pooled treatment effect for the remaining studies. Treatment effect was significant if $p < 0.05$. We assessed for heterogeneity in treatment estimates

using the Cochran Q test and the χ^2 statistic (with substantial heterogeneity defined as values $>50\%$). **Results:** The final analysis consisted of 11 studies (2 observational and 9 randomized). For the endothelial-dependent vasodilatation there were 6 studies, including 257 patients, that evaluated flow-mediated dilatation and 5 studies with 87 patients that reported data on forearm blood flow response to acetylcholine or flow-dependent vasodilatation. Overall, there was a significant increase in the endothelium-dependent vasodilatation with allopurinol treatment (MD 2.69%, 95% CI 2.49, 2.89%, $p < 0.001$; heterogeneity $\chi^2 = 319.1$, $I^2 = 96\%$, $p < 0.001$). There was only 1 study (100 patients) assessing nitrate-mediated dilatation and 4 studies (73 patients) evaluating forearm blood flow response to sodium nitroprusside as measures of endothelial-independent vasodilatation. The overall analysis (MD -0.08 , 95% CI -0.50 , 0.34 , $p = 0.70$; heterogeneity $\chi^2 = 9.0$, $I^2 = 44\%$, $p = 0.11$) showed no effect of allopurinol treatment on endothelium-independent vasodilatation. **Conclusions:** We found that treatment of hyperuricemia with allopurinol is associated with an improvement in the endothelial-dependent, but not with the endothelial-independent vasodilatation.

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Introduction

Uric acid, the circulating end product of purine metabolism, is associated with a wide variety of cardiovascular conditions including hypertension [1], coronary artery disease [2], cerebrovascular disease [3], vascular dementia [4], preeclampsia [5] and chronic kidney disease [6]. While it was originally posited that uric acid was elevated secondary to these conditions, there is now strong evidence that elevated uric acid levels independently predict the development of hypertension [7–9], kidney disease [10] and diabetes [11, 12].

Endothelial dysfunction (ED) represents a preliminary phase in the atherosclerotic process. It has also been implicated in the pathophysiology of chronic heart failure, diabetes, hypertension, coronary heart disease and chronic kidney disease [13, 14]. The etiology of ED is complex and involves dysregulation of multiple pathways [14], and the association between high serum uric acid concentrations and ED has been repeatedly shown [15–17]. Experimental studies also show that uric acid can directly alter nitric oxide (NO) bioavailability [18–23] and mediate ED in the animal models [18]. Most importantly, allopurinol, a xanthine oxidase inhibitor, has been shown to improve endothelial function in different studies that included a relative low number of individuals with a variety of pathologies [24–35]. Therefore, the aim of this meta-analysis was to establish more clearly the benefits of uric acid-lowering therapy with allopurinol on endothelial function.

Materials and Methods

Study Protocol

We have conducted a systematic review and meta-analysis according to a previously published protocol (CRD42014006978 at <http://www.crd.york.ac.uk/PROSPERO>).

Search STRATEGY

Electronic databases, Medline, PubMed, EMBASE, SCOPUS, EBSCO and the Cochrane Library Central Register of Clinical Trials were searched using the MESH terms ‘allopurinol’, ‘endothelial dysfunction’, ‘endothelial function’ with the key words ‘xanthine oxidase inhibitor’, ‘uric acid’, ‘hyperuricemia’, and ‘oxypurinol’. Our search was limited to studies in humans and in peer-reviewed journals from January 1985 to July 2013 without language restriction. In addition, we searched other potentially relevant studies by using a manual search of references from all eligible studies, review articles and Science Citation Index Expanded on the Web of Science, and searched the top 25 citations for each paper through the ‘related articles’ feature of PubMed. We also reviewed congress proceedings of the American Society of Nephrology, International Society of Nephrology, European Renal Association, American College of Cardiology, American Heart Association, and European Society of Cardiology.

Study Selection

Eligible studies included (1) prospective (randomized or non-randomized) or retrospective study designs assessing the effect of allopurinol on endothelial function, (2) parallel or cross-over study design, (3) and documentation of change in ED. There was no restriction criteria imposed on the type of patients studied. Exclusion criteria included (1) no description of primary or secondary outcomes, (2) absence of adequate and reproducible results, and (3) articles that are not designed as clinical trials (review, etc.) and (4) studies with duplicated data, including same group of patients.

Data Extraction, Validity Assessment and Quality Assessment

Data extraction was done independently by two authors (D.S. and O.C.E.) using standard data extraction forms. Where more than one publication of one study exists, reports were grouped together and only the publication with the most complete data was included. Any unclear or missing information was requested from the original author by written correspondence and any relevant information obtained in this manner was included in the review. Disagreements were resolved by consultation with all authors.

Two reviewers (O.T. and O.C.E.) evaluated the quality of the selected studies independently. The scale used three categories to evaluate: selection, comparability and outcome.

The study quality was determined with a Jadad composite score [36], which is a 5-point quality scale with 1 lowest and 5 highest. A study has been defined a high-quality study if it has a Jadad score ≥ 3 . Publication bias was assessed using the funnel plot technique [37].

Outcome Assessment

Primary outcome of this analysis was change in endothelial-dependent and -independent vasodilatation with allopurinol treatment from the beginning of the treatment to follow-up. Secondary outcomes were change in different subgroups of endothelial function measurements (for endothelial-dependent vasodilatation: flow-mediated dilatation (FMD) and forearm blood flow (FBF) response to acetylcholine or flow-dependent flow assessment and for endothelial-independent vasodilatation: nitrate-mediated dilatation (NMD) and FBF response to sodium nitroprusside). Data extracted included identifying information, focus of the study, details of the study protocol and demographic data. We extracted characteristics of each study including baseline and follow-up endothelial function, baseline clinical characteristics of the study population, known diagnosis of hypertension, hyperuricemia, or chronic kidney disease, type of study design, and use of agents that might affect endothelial function and total duration of follow-up.

Statistical Analysis

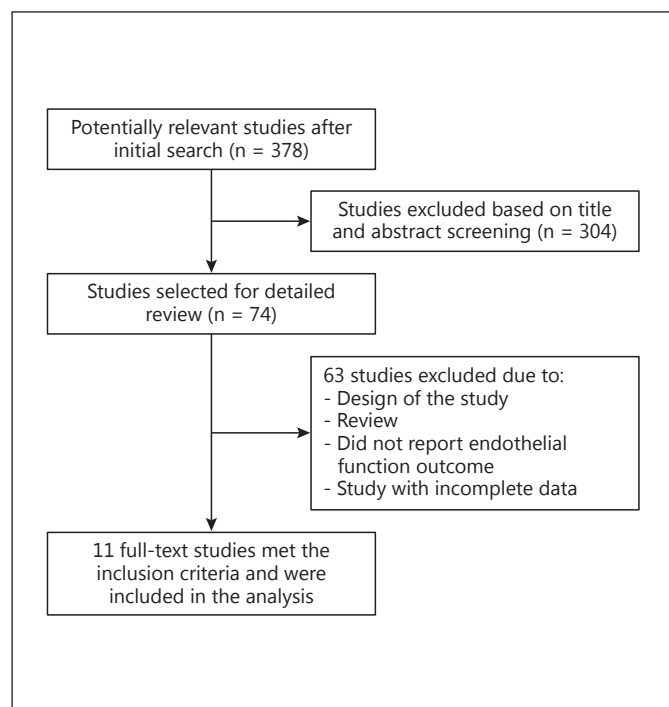
In this meta-analysis to investigate the impact of allopurinol use on endothelial function, two-tailed variance analysis was performed in the paired samples with known arithmetic means and standard deviations Effect size method and adjusted arithmetic mean differences were used in the studies detected with sampling method. Analyses were performed with the packet program Comprehensive Meta-Analysis (Biostat, Englewood, N.J., USA) and with Review Manager Version 5.2 (The Cochrane Collaboration 2012) [38].

Parallel-group and cross-over design trials were combined in the same meta-analysis based on recommendations by Elbourne et al. [39]. Generic inverse variance based on calculating absolute differences of mean changes in endothelial function (assessed by FMD, NMD, FBF response to acetylcholine or sodium nitroprusside or

Table 1. Demographic and characteristics of studies included in the meta-analysis

Reference (first author)	Country	Age		Gender				BMI		Study population	Jadad score
		allopurinol	control	allopurinol		control		allopurinol	control		
				M	F	M	F				
Yelken 2012 [24]	Turkey	34±11	-	13	6			27.38±3.77	-	non-diabetic CKD patients	0
Meléndez-Ramírez 2012 [27]	Mexico	49.1±6.7	-	9	0			pre-treatment 27.3±1.1	-	normotensive male patients without DM, CKD and HL	0
Kanbay 2011 [25]	Turkey	54.4±8.0	50.4±11.2	16	14	18	19	28.4±2.9	29.7±3.4	subjects with asymptomatic hyperuricemia	1
Dogan 2011 [28]	Turkey	50.5±5.0	50.0±6.0	25	25	26	24	25.5±4.5	25.8±5.0	normotensive subjects with DM	1
Yiginer 2008 [29]	Turkey	63.1±11.7	59.7±9.7	7	21	7	15	25.0±5.3	26.2±4.2	subjects with metabolic syndrome	2
George 2006 [30]	UK	69.7±8	-	25	5	-	-	29.7±4.7	-	CHF (NYHA class II-III) with documented LV systolic dysfunction	4
El Solh 2006 [31]	USA	47.3±8.7 (29-60)	-	7	5	-	-	39.1±13.6 (23-67)	-	moderate-to-severe OSA patients	4
Farquharson 2002 [32]	UK	67.5±5.5	-	10	1	-	-	-	-	compensated mild-to-moderate CHF	4
Doehner 2002 [33]	UK	68±2	69±3	-	-	-	-	26.7±2.1	27.2±1.6	<70 years with CHF caused by dilated CMP or IHD	1
Butler 2000 [34]	UK	65±6.7	58±8	10	1	12	0	26.2±3.5	27.4±3	type 2 DM and coexisting mild HT	4
O'Driscoll 1999 [35]	Australia	48±2	-	7	2	-	-	-	-	hypercholesterolemic volunteers	4

BMI = Body mass index; CHF = congestive heart failure; CKD = chronic kidney disease; CMP = cardiomyopathy; DM = diabetes mellitus; HL = hyperlipidemia; HT = hypertension; IHD = ischemic heart disease; LV = left ventricular; OSA = obstructive sleep apnea.

**Fig. 1.** Selection and description of studies.

flow-dependent flow) between the experimental and control groups and standard errors for each comparison within each study was used. We converted standard deviation and 95% confidence interval (CI) to standard error by using a standard formula [37]. When necessary, we estimated mean and standard deviation from median, range and sample size using the method proposed by Hozo et al. [40]. We conducted a sensitivity analysis to assess the contribution of each study to the pooled treatment effect by excluding each study one at a time and recalculating the pooled treatment effect for the remaining studies. Treatment effect was significant if $p < 0.05$. We assessed for heterogeneity in treatment estimates using the Cochran Q test and the χ^2 statistic (with substantial heterogeneity defined as values $>50\%$).

Results

Selection and Description of Studies

We included in our final analysis 11 studies (2 observational and 9 randomized) involving 344 patients (minimum 9 and maximum 100 patients) (fig. 1). The follow-up was between 1 week and 4 months. We analyzed endothelium-dependent vasodilatation (6 studies evaluated FMD and 5 studies FBF response to acetylcholine or flow-dependent flow assessment) and endothelium-indepen-

Table 2. Baseline characteristics of the studies included in the meta-analysis

Reference (first author)	Design of study	Duration of follow-up	Basal uric acid, mg/dl		Allopurinol dose, mg	Inclusion criteria	Exclusion criteria
			allopurinol	control			
Yelken 2012 [24]	prospective cohort	16 weeks	8.6±1.2	–	150	UA >7 mg/dl in men, UA >6 mg/dl in women in CKD	ACS, CHF, DM, IHD, TIA, VHD, taking ULM
Meléndez-Ramírez 2012 [27]	prospective cohort	30 days	7.8±0.36	–	300	normotensive, >18 age <65 years	history of smoking, DM, HT, CKD, HL
Kanbay 2011 [25]	RCT	4 months	8.3±1.1	7.9±0.7	300	asymptomatic hyperuricemia without DM, HT, CHF	smoking, history of CAD, and patients on RAS blockers, statins
Dogan 2011 [28]	RCT	12 weeks	5.0±0.8	4.8±1.1	900	patients with DM	HT, smoking, CHF, history of CAD
Yiginer 2008 [29]	RCT	1 month	6.6±0.6	6.5±0.6	300	metabolic syndrome	history of gout, CAD, uncontrolled HT, smoking, CHF
George 2006 [30]	RCT	1 month	7.12±1.53	–	300	mild-to-moderate CHF	serum creatinine >2.03 mg/dl, uncontrolled HT, on allopurinol
El Solh 2006 [31]	RCT	4 weeks	4.6±0.9	4.6±0.9	–	moderate-to-severe OSA	smoking, history of CHF, HT, DM, gout
Farquharson 2002 [32]	RCT, cross-over	1 month	4.6±0.9	–	300	mild-to-moderate CHF	history of DM, HL, uncontrolled HT
Doehner 2002 [33]	RCT, cross-over,	1 week + 1 week (cross-over)	9±0.37	9.9±0.62	300	CHF patients with hyperuricemia	history of malignancy, heart and liver disease, stroke
Butler 2000 [34]	RCT, cross-over	1 month	–	–	300	type I DM with mild HT and without end-organ damage	evidence of target organ damage, history of CAD, CKD
O'Driscoll 1999 [35]	RCT, cross-over	1 month	0.25±0.03 mmol/l	0.35±0.02 mmol/l	300	patients with HL not receiving cholesterol-lowering therapy	history of CKD, DM and using RAS blockers

ACS = Acute coronary syndrome; CAD = coronary artery disease; CHF = congestive heart failure; CKD = chronic kidney disease; DM = diabetes mellitus; FDF = flow-dependent flow; HL = hyperlipidemia; HT = hypertension; IHD = ischemic heart disease; OSA = obstructive sleep apnea; RAS = renin angiotensin system; RCT = randomized controlled trial; TIA = transient ischemic attack; UA = uric acid; ULM = urate-lowering medication (allopurinol and probenecid); VHD = valvular heart disease.

dent vasodilatation (1 study evaluated NDM and 4 studies FBF response to sodium nitroprusside). Allopurinol was the intervention agent in all trials. The dose of allopurinol varied between 150 and 900 mg daily.

Quality Assessment

We included both randomized and observational studies in our analyses, therefore the included studies had of variable quality. There were 6 studies with good quality (Jadad score ≥3) with low risk of bias and 5 studies with low quality (Jadad score <3) and high risk of bias.

Endothelium-Dependent and -Independent Measurements

Baseline characteristics of the included studies are stated in tables 1 and 2. In all studies the end-diastolic brachial diameter was used for evaluation. The technique used for assessing FDM and NMD was in agreement with previously described methods [41–43] in all studies, with

the exception of that of El Solh et al. [31]. Transducers with frequencies between 5 and 12 MHz were used, but in one study this detail was not presented [25, 29, 31]. In the majority of the studies [24, 25, 27, 31], measurements were made by a single observer. Reproducibility was only reported in two trials [28, 31]. Dogan et al. [28] reported the intra- and inter-observer coefficients of variation for overall FMD and NMD measurements (3.2, 2.8, 4.1 and 3.3%, respectively). El Solh et al. [31] reported the variability for baseline diameter measurements expressed as intra-session variability of 0.6%.

Forearm venous occlusion plethysmography was used in all studies to evaluate FBF. Three studies used a Medasonics Vasculab SPG-16 plethysmograph [32, 34, 35], one study used an EC4, Hokanson plethysmograph [33], while one study did not report what type of device was used [30]. For the assessment of endothelial-dependent vasodilatation, four studies used acetylcholine infusions (maximum dose 100 nmol/min [30, 32, 34] and 40 µg/

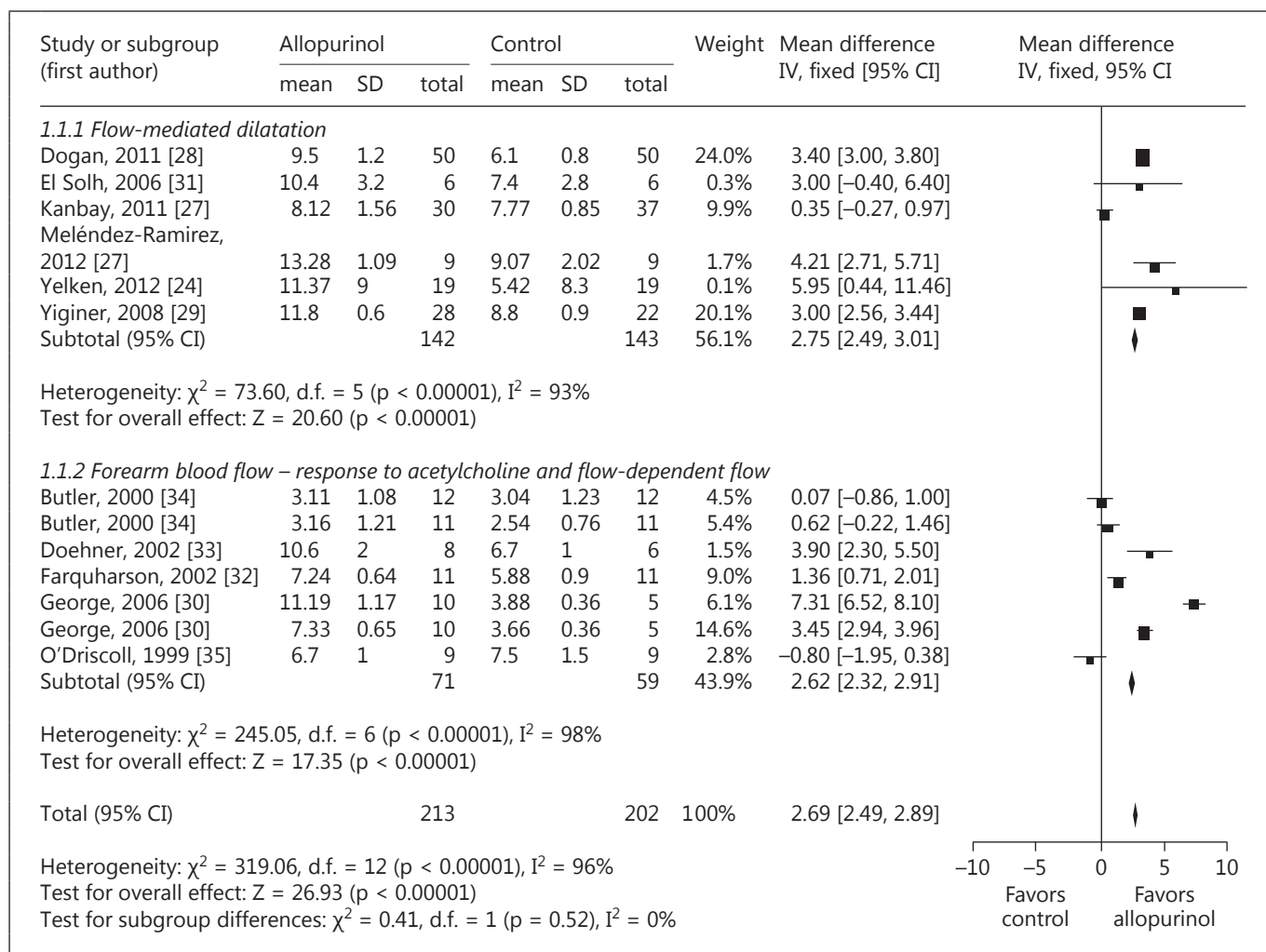


Fig. 2. Forest plot of endothelial-dependent dilatation.

min [35]) and one study flow-dependent flow evaluation [33]. For the determination of endothelial-independent vasodilatation the same four studies used sodium nitroprusside infusions (maximum dose 37.8 nmol/min [30, 32, 34] and 8 $\mu\text{g}/\text{min}$ [35]). Only one study reported reproducibility data. Butler et al. [34] reported the baseline variability of the data was $<10\%$, when blood flow was analyzed repeatedly in a steady state and a quiet environment. The variability of repeated analysis of the same raw plethysmographic data was $<5\%$.

Outcome Measures Reporting

Allopurinol Effect on Endothelium-Dependent Vasodilatation

All studies assessed the effect of allopurinol on endothelium-dependent vasodilatation. Six studies, including

257 patients, evaluated FMD and the remaining 5 studies with 87 patients reported data on FBF response to acetylcholine or flow-dependent vasodilatation. Overall, there was a significant increase in the endothelium-dependent vasodilatation with allopurinol treatment (MD 2.69%, 95% CI 2.49, 2.89%, $p < 0.001$; heterogeneity $\chi^2 = 319.1$, $I^2 = 96\%$, $p < 0.001$) (fig. 2).

Analyzed separately, there was also a significant increase in both FMD (MD 2.75%, 95% CI 2.49, 3.01%, $p < 0.001$; heterogeneity $\chi^2 = 73.6$, $I^2 = 93\%$, $p < 0.001$) and FBF (MD 2.62, 95% CI 2.32, 2.91, $p < 0.001$; heterogeneity $\chi^2 = 245.1$, $I^2 = 98\%$, $p < 0.001$). The results of our analysis were not affected if the 2 non-randomized studies from the FMD subgroup were excluded (MD 2.69, 95% CI 2.43, 2.96, $p < 0.001$; heterogeneity $\chi^2 = 68.5$, $I^2 = 96\%$, $p < 0.001$). Similarly, the conclusion was also the

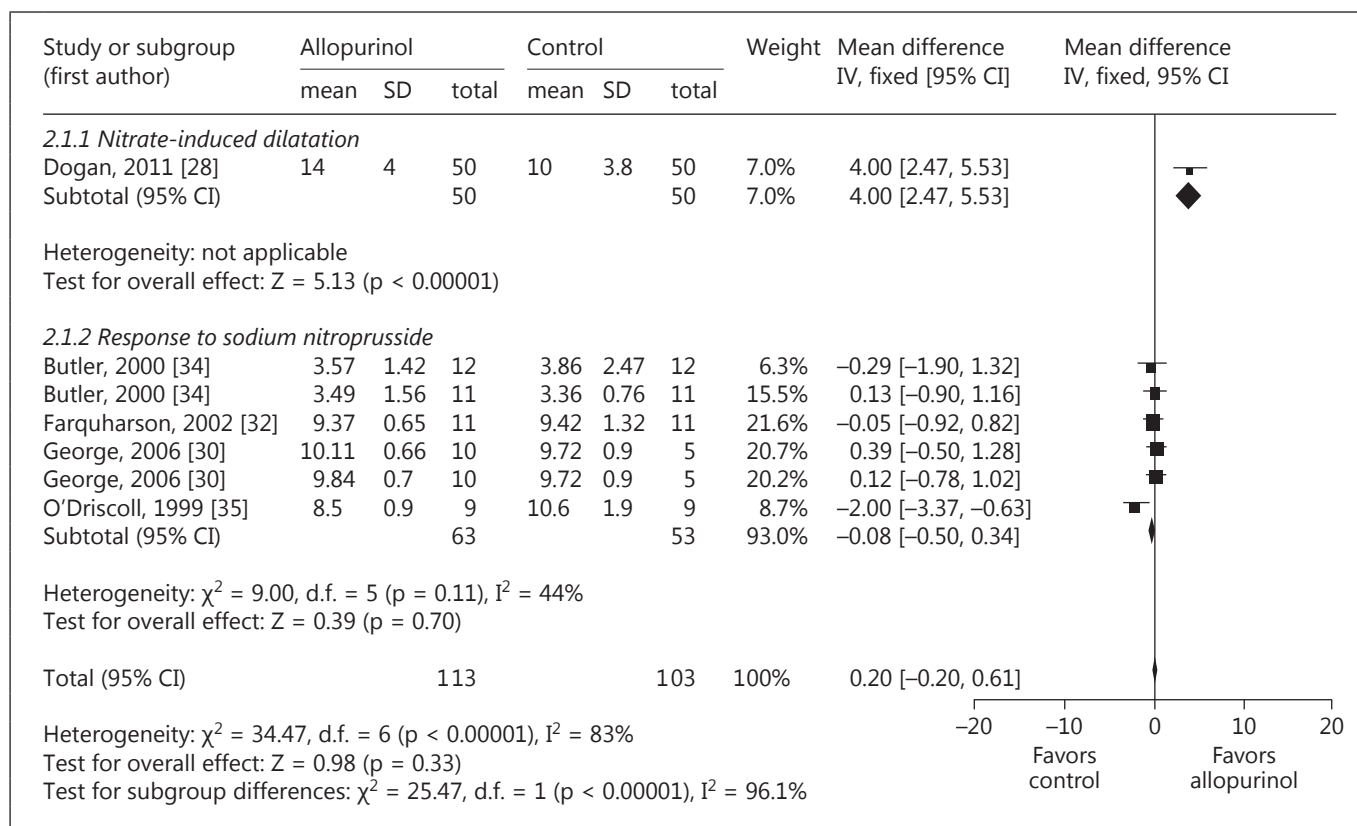


Fig. 3. Forest plot of endothelial-independent dilatation.

same if we excluded from the FBF subgroup the study that evaluated endothelium-dependent vasodilatation using flow-dependent flow [33] (MD 2.57, 95% CI 2.27, 2.87, $p < 0.001$; heterogeneity $\chi^2 = 242.5$, $I^2 = 98\%$, $p < 0.001$).

Allopurinol Effect on Endothelium-Independent Vasodilatation

Only 1 study assessed NMD (100 patients) and 4 studies (73 patients) evaluated FBF response to sodium nitroprusside. Although allopurinol treatment improved NMD (MD 4.00, 95% CI 2.47, 5.53, $p < 0.001$), the FBF response to sodium nitroprusside (MD 0.20, 95% CI -0.20, 0.61, $p = 0.33$; heterogeneity $\chi^2 = 34.5$, $I^2 = 83\%$, $p < 0.001$) and the overall analysis (MD -0.08, 95% CI -0.50, 0.34, $p = 0.70$; heterogeneity $\chi^2 = 9.0$, $I^2 = 44\%$, $p = 0.11$) showed no effect of allopurinol treatment on endothelium-independent vasodilatation (fig. 3). Yiginer et al. [29] also evaluated NMD, but they only reported that it did not change from baseline in both groups (allopurinol and placebo).

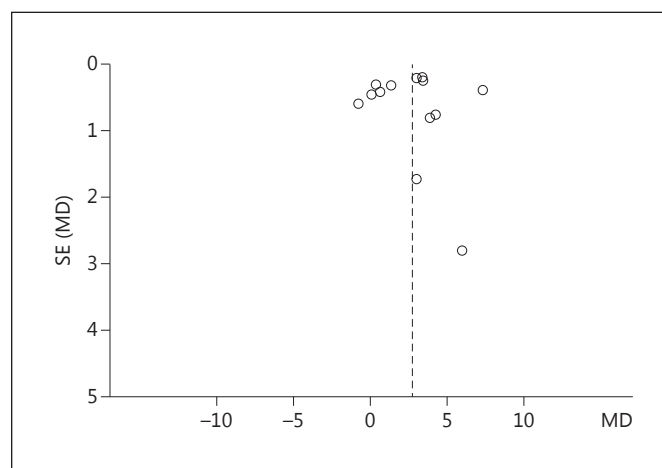


Fig. 4. Funnel plot of the mean differences in endothelial-dependent dilatation versus standard errors of the mean differences of all 11 studies which assessed allopurinol effect on endothelial-dependent dilatation. The x-axis is in m/s.

Evaluation of Heterogeneity and Publication Bias

The funnel plot (fig. 4) showed an asymmetrical plot in the presence of reporting bias indicating that small studies with negative results have not been published. Homogeneity test for Q statistics was calculated, according to a fixed-effect model, Q statistics was found to be negative in terms of homogeneity test. According to the fixed-effect model, effect sizes were found to be heterogeneous. Because the Q statistics for the homogeneity test was significant, the variability of the effect sizes were considered to be higher than expected from a bias caused by a sampling error. Because the homogeneity test originated from sampling error was found to be higher than expected, the model was switched to a random-effect model by calculating the variance of random-effect components.

Discussion

Endothelial-dependent vasodilatation is primarily a function of endothelial NO. ED is usually reported as an impaired NO release in response to acetylcholine or FMD, which results in impaired endothelium-dependent vasodilatation. Our primary finding was that allopurinol use is associated with an improvement in the endothelial-dependent, but not endothelial-independent vasodilatation. While there was significant heterogeneity between trials included in this meta-analysis, most of it could be explained by differences in the methodological quality of the trials.

Allopurinol is a xanthine oxidase inhibitor. The metabolism of xanthine by xanthine oxidase generates uric acid and oxidants. Thus, by blocking xanthine oxidase, allopurinol may reduce both uric acid and xanthine oxidase-associated oxidants. There is evidence that uric acid per se may inhibit NO bioavailability in endothelial cells. Several mechanisms have been reported, including uric acid-induced oxidative stress with scavenging of NO [20, 44–46], alteration in L-arginine transport or metabolism [23, 47], or by direct scavenging of NO [19].

It is also possible that the beneficial effect of allopurinol on endothelial function involves the blockade of xanthine oxidase-associated oxidants rather than uric acid per se. In line with this assertion, George et al. [30] reported that high-dose allopurinol, but not probenecid (a uricosuric), could improve endothelial function in subjects with congestive heart failure. However, allopurinol will also reduce intracellular uric acid levels more effectively by blocking intracellular uric acid gen-

eration, and the mechanism by which uric acid induces ED is via its intracellular effects [46]. The use of uricase, which degrades uric acid, has also been reported to not improve endothelial function [48], but uricase treatment also generates oxidants when uric acid is degraded. Hence, the exact protective mechanism by which allopurinol protects the endothelial NO levels remains unclear.

In our analysis, there was only one study that found a significant increase in endothelial-independent dilatation [28] following allopurinol treatment. Although it was the only included study that evaluated NMD, these positive findings could be related to initial higher NMD values in the placebo group and also to a longer period of administration.

This meta-analysis has several limitations. Although the authors were contacted to identify the missing data and to clarify areas of concern, we received no responses on our queries. Therefore, this meta-analysis included published data only. This meta-analysis was also limited by the methodological quality of studies included, the large number of studies with small cohorts, non-randomized or cross-over design, variable duration of follow-up and dosage and the lack of multicenter studies.

Taken together, the results of this meta-analysis showing a beneficial effect of lowering serum uric acid level with allopurinol only on endothelial-dependent dilatation imply that this effect is not related to correcting a generally abnormal responsiveness to vasodilator stimuli. Because this review is based on observational studies, and because the number of included studies and the sample size are limited, the application of allopurinol for improvement of endothelial function in patients with hyperuricemia is not yet routinely recommended. Nevertheless, given that there is abundant evidence of an association between hyperuricemia and ED from clinical and animal studies, we recommend adequately powered, high-quality, randomized placebo-controlled trials to definitively evaluate the benefits and risks of treatment of hyperuricemia with allopurinol on endothelial function and cardiovascular disease.

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Disclosure Statement

Dr. R.J. Johnson is listed as an inventor on patents for lowering uric acid as a means for reducing blood pressure and insulin resistance. The patents have been licensed by XORT Therapeutics, Inc.,

for which he has received shares. He has also written lay books on uric acid and sugar (The Fat Switch, Mercola.com and the Sugar Fix, Rodale), and he is also on the Scientific Board of Nutralite. He has received research grants from Danone, Amway, Questcor and the National Institutes of Health.

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