Article:
Shi, Y and Williamson, G (2016) Quercetin lowers plasma uric acid in pre-hyperuricaemic males: a randomised, double-blinded, placebo-controlled, cross-over trial. British Journal of Nutrition, 115 (5). pp. 800-806. ISSN 0007-1145

https://doi.org/10.1017/S0007114515005310

Quercetin lowers plasma uric acid in pre-hyperuricemic males: a randomized, double-blinded, placebo-controlled, cross-over trial

Yuanlu Shi[1], Gary Williamson[1*]

[1] *School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK*

Running Head: Quercetin lowers uric acid in humans

Key words: quercetin, bioequivalence, dietary supplement, human

*** Corresponding Author:** Gary Williamson, Tel: +44(0)113-343-8380; Email: g.williamson@leeds.ac.uk

Clinical trial registration: The study was registered on ClinicalTrials.gov (identifier number NCT01881919).

Pubmed indexing: Shi, Williamson

Key words: quercetin, hyperuricemia, uric acid, fasting glucose, blood pressure, clinical trial

Abstract

Background: Elevated plasma uric acid is a risk factor for gout, insulin resistance and type 2 diabetes. Quercetin, a flavonoid found at high levels in onions, tea and apples, inhibits xanthine oxidoreductase *in vitro*, the final step in intracellular uric acid production, indicating that quercetin might be able to lower blood uric acid in humans.

Objective: We determined the effects of 4 wk oral supplementation of quercetin on plasma uric acid, blood pressure and fasting glucose.

Design: This randomized, double-blinded, placebo-controlled, cross-over trial recruited 22 healthy males (19-60 y) with baseline plasma uric acid concentration in the higher, but still considered healthy, range (339±51 µmol/L). Intervention was one tablet containing 500 mg quercetin daily for 4 wk, compared to placebo, with a 4-wk washout period between treatments. Primary outcome was change in concentration of plasma uric acid after 2 and 4 wk. Secondary outcome measures were changes in fasting plasma glucose, 24-hour urinary excretion of uric acid and resting blood pressure.

Results: After quercetin treatment, plasma uric acid concentrations were significantly lowered by -26.5 µmol/L (95% confidence interval [CI], -7.6 to -45.5; P=0.008), without affecting fasting glucose, urinary excretion of uric acid or blood pressure.

Conclusions: Daily supplementation of 500 mg quercetin, containing the bioavailable amount of quercetin as present in ~100 g red onions, for 4 wk, significantly reduces elevated plasma uric acid concentrations in healthy males.

Introduction

High blood uric acid (hyperuricemia) is the strongest determinant risk factor for gout, an inflammatory arthritis caused by uric acid crystals, and is higher in males compared to females [1]. Hyperuricemia is also common in patients who develop diabetes [2], obesity [3], hyperglycaemia [4; 5], hypertension [6], and stroke [7], although it is often unattended until their first, if any, gout attack. Gout prevalence increased from ~0.5 to ~3% between 1960 and 2010 in the US [8] and other areas [9] accompanied by a parallel increase in the number of individuals with hyperuricemia [10; 11]. The fact that 25-34 is the age group with the highest blood uric acid level [12] may suggest that hyperuricemia precedes the development of metabolic syndromes [13]. Interestingly, allopurinol, a uric acid lowering agent used in gout therapy, has a protective effect on hypertension, which suggests that excess uric acid synthesis is a causal factor in developing hypertension [14].

Some dietary factors, including purines, alcohol and fructose [15; 16; 17; 18], also elevate blood uric acid. For example, chronic exposure to fructose can lead to development of hyperuricemia [19]. Fructose phosphorylation by fructokinase causes intracellular phosphate depletion leading to the activation of deaminase, which converts adenosine monophosphate to inosine monophosphate. The consumption of ATP activates transformation of inosine monophosphate to inosine, the precursor of uric acid metabolism. Chronic hyperuricemia may also up-regulate fructokinase expression, leading to the amplification of the lipogenic effects of fructose in human hepatocytes [20]. Xanthine oxidoreductase (also called xanthine oxidase or xanthine dehydrogenase depending on proteolytic processing) catalyses the final step in uric acid production. Inhibition of this enzyme has been a target for uric acid-lowering drugs, such as allopurinol [21]. Studies in both healthy humans [22; 23] and in animal

63 models [24] substantiate the importance of increased insulin resistance to
64 hyperuricemia, and *vice versa*, providing a link to excess fructose intake.

65 Quercetin is a dietary flavonoid which is particularly abundant in onion, black tea and
66 apples, and occurs predominantly as quercetin 4′-*O*-glucoside or quercetin-3,4'-*O*-
67 diglucoside in onions and quercetin 3-*O*-rutinoside in tea [25]. The bioavailability of
68 quercetin in humans has been extensively studied, and in plasma, multiple
69 conjugates of quercetin appear post-prandially. In healthy subjects, using urine as a
70 biomarker, we have previously demonstrated that 500 mg quercetin aglycone, as
71 provided in supplements used here, is comparable to the quercetin present in ~100 g
72 of fresh red onion [26]. Quercetin, and its metabolites, inhibit xanthine oxidoreductase
73 *in vitro* [27] and regulate blood uric acid level *in vivo* in animal studies [28; 29; 30], yet
74 whether uric acid metabolism could be similarly affected in humans is still highly
75 debatable [31; 32; 33; 34; 35; 36].

76 Therefore, we performed this randomized, double-blinded, placebo-controlled, cross-
77 over trial to test the hypothesis that 4 wk of quercetin supplementation might result in
78 a reduction in plasma uric acid in male subjects with non-optimal blood uric acid.

Subjects and methods

Subjects

22 healthy males were eligibly assigned and successfully compliant to the complete study. Selection criteria included being apparently healthy, age between 19 and 65, BMI between 18.5 and 29.9 kg/m^2, non-smoking and not a heavy drinker (less than 3 units of alcohol regularly per day). Volunteers with diagnosed gout and/or kidney stone, who were experiencing intestinal disorders, or whose plasma uric acid concentration was lower than 300 µmol/L, were excluded. All data were collected from February 2013 to April 2014 and analysed in the School of Food Science and Nutrition at the University of Leeds, UK. The study was conducted according to the guidelines laid down in the declaration of Helsinki of 1975 as revised in 1983 and all procedures involving human subjects were approved by the University of Leeds, MaPS and Engineering joint Faculty Research Ethics Committee (MEEC12-019), UK. Written informed consent was obtained from each of the subjects before commencement of the study.

Study design

The main goal and primary objective of the present study was to examine the chronic effect of quercetin on plasma uric acid concentration. For this purpose, the study was a randomized, double-blinded, placebo-controlled, cross-over, 4-wk intervention trial with 2 treatment groups, with daily consumption of either quercetin dihydrate in a tablet form (500 mg stated on the label, actual measured 544±45 mg quercetin dihydrate aglycone, purchased from Nature's Best, Kent, UK, and containing small amounts of calcium carbonate, cellulose, methylcellulose, glycerine, stearic acid, silicon dioxide, crosslinked cellulose gum, magnesium stearate) [26] or placebo (the

placebo formulation was a white oval tablet and contained lactose monohydrate, magnesium stearate and cellulose, purchased from Fagron, Barsbuttel, Germany). There was a 4-wk washout period between each treatment. Blood and urine samples were taken before, during and at the end of each study phase. Each participant was independently and randomly assigned into one of two groups, receiving both treatments in one order or another.

During the protocol, volunteers made 6 visits to the research unit at day 0, 14 and 28 of each experimental period for measurement and sample collection. In practice, with 24-hour urine collected at home during the day and night before the visit, overnight-fasted subjects arrived at the research unit between 7-10 am. A fasting blood sample was collected, followed by questionnaires and measurements of weight, height and blood pressure. Subjects received a light meal and the study tablets before leaving the research unit. Subjects were asked to maintain their lifestyle and normal dietary habits from 4 wk before the first visit until the end of the entire study. Compliance was assessed at the end of each 4-wk period by call back questionnaires recording date of missing dose (if any), changes of physical activity and intensity, use of exotic diet or non-routine medications, and the occurrence of any side effects. Subjects were also asked to return the unconsumed tablets at each follow-up visit.

Intervention was randomized independently by a coin toss for each volunteer who received a random 3-digit code. A decode list (participant identification and subject code) was kept by a third person in order to blind the researcher assessing outcomes. The size and shape of study tablets were the same but of different colour, and participants were not aware of the identification of the two types of study tablets. The quercetin-containing tablet was light green and the placebo was off-white. Since quercetin is light yellow, it is not immediately obvious which tablet is the active, and

subjects were not informed which tablets were placebo or active. Analysis of the blood and urine samples was also blinded to the researcher using codes held by a third party.

Sample collection and assay

Blood pressure was measured on the upper left arm in a quiet room at normal room temperature, with the use of a cuff-less upper arm blood pressure monitor (Panasonic Co., Japan). Before blood pressure recordings were made, participants rested for 15 min in a seated position. At each assessment, 3 consecutive blood pressure readings were recorded at 5 min intervals. The average of these measurements was used for analysis.

Venous blood was collected following a standard venepuncture protocol into a sodium fluoride/potassium oxalate blood collection tube (GreinerBioOne, Austria). Blood samples were immediately centrifuged at 3 000 g, 4℃ for 10 min and aliquots were stored at -80℃ until analysis. 24-hour urine samples were collected by volunteers in 3 L sterile urine container (Simport, Canada) which contained 3 g of L-ascorbic acid (MP Biomedicals, France). The urine samples were weighed before centrifugation at 2 000 g, 4℃ for 10 min before storage at -20℃. Urine samples for uric acid assay were diluted 10-fold before storage at -80℃.

Analytical methods

Assessment of uric acid in plasma and urine samples was by a specific coupled enzyme reaction, followed by colorimetric determination at 520 nm [37]. The protocol was modified for use in a 96-well plate reader (BMGlabtech, Germany) for high-throughput and improved accuracy. Within-run variation was 1.99±1.20%, and

between-run variation was 2.17±0.52%. Recovery was 92.8±1.6% for plasma and 80.4±3.8% for 10-fold diluted urine. Calibration curves were prepared every time for each plate, with a slope of 0.550±0.003 per mmol/L uric acid, with $R^2 \geq 0.999$ up to a maximum concentration of 1.0 mmol/L.

Plasma glucose was measured with a commercial hexokinase-based assay kit for D-glucose (Sigma-Aldrich, USA). The protocol was modified for use in a 96-well plate reader. Within-run variation was 4.29±2.21% and between-run variation was 3.33±2.51%. Recovery was 104±8%. Calibration curves were prepared every time for each plate, with a slope of 0.923±0.006 per g/L D-glucose, with $R^2 \geq 0.999$ up to a maximum concentration of 1.50 g/L.

Urinary quercetin was quantified by HPLC-ESI/MS as previously described [26].

Sample size

A minimum sample size of 17 was estimated to be required to detect a 10% difference for the primary efficacy variable, plasma concentration of uric acid, and to achieve 80% power to meet the two-tailed equality criteria between quercetin and placebo. A significance level of 0.05 from paired 2-sample *t* test was set for this two-sequence, two period cross-over design [38]. Coefficient of variation of the blood uric acid level among the population was ~20% according to previous cohort reports [39; 40; 41] and 10% of coefficient of variation among study population was estimated since we pre-screened and selected the upper 50% of the volunteers for plasma uric acid.

Statistics

Normality of data distribution was tested by Shapiro-Wilk tests. The paired 2-sample *t* test was used for comparison of normally distributed data. Data that were not

174 normally distributed were compared using the *Wilcoxon signed-rank* test.
175 Relationships between variables were evaluated using Pearson's correlation
176 coefficient. In all cases, a value for $P<0.05$ (2-tailed) was taken to indicate a
177 significant effect. Unless otherwise indicated, results are expressed as mean values
178 and standard deviations (SD). All statistical analyses were performed using the
179 SPSS statistics software (version 21; International Business Machines Corp., New
180 York, USA).

Results

54 male volunteers made contact through advertisements (Figure 1). 52 of them donated blood at the screening stage, with a mean±SD plasma uric acid concentration of 316±56 μmol/L (range 194-472 μmol/L, n=52). 23 subjects were selected and 22 of them completed the study with the following characteristics at baseline: healthy adult males, 29.9±12.9 years, mean BMI of 24.8±3.0 kg/m^2, blood pressure of normal to (pre-) hypertensive (systolic 122.9±8.1 mm Hg and diastolic 74.3±9.0 mm Hg), fasting blood glucose of normal to impaired fasting glycemia with mean of 5.04±0.56 mmol/L, plasma uric acid of 339±51 μmol/L). No significant change of lifestyle or medication occurred during the study based on the lifestyle maintenance questionnaire, and no adverse events for receiving quercetin or placebo were reported. 24-h urinary excretion of quercetin was 0.810±0.704 μmol during quercetin treatment and 0.200±0.366 μmol during placebo treatment. According to the returned unconsumed tablets, participant self-reports and urinary quercetin, none of the participants was classified as non-compliant.

Plasma uric acid was progressively lowered over time among participants during the quercetin supplementation. From baseline to 2 wk, the mean plasma uric acid showed a downward trend (-15.9 μmol/L, 95% CI, 0.9 to -32.8; *P*=0.06). From baseline to 4 weeks, the mean plasma uric acid was decreased significantly by -26.5 μmol/L (95% CI, -7.6 to -45.5, P=0.008). Plasma uric acid remained unchanged throughout the placebo period: 95% CI, -8.9 to 30.0; *P*=0.27 at the 2-week interval and 95% CI, -15.1 to 25.5; *P*=0.60 after 4-weeks. No difference was observed between the baselines of each arm (*P*=0.21) (Table 1, Figure 2).

There was a trend for mean diastolic blood pressure to decrease by -2.0 mm Hg (95% CI, 0.1 to -4.1; *P*=0.07) during the quercetin phase, whereas there was no change during the placebo phase. No change was observed in fasting glucose nor in systolic blood pressure in either group by either treatment (Table 1). Renal excretion of uric acid was assessed by total 24-h urinary uric acid and did not significantly vary between the two time points after either treatment: from 2.15±1.80 to 1.61±1.56 mmol after quercetin treatment (*P*=0.11, Wilcoxon signed-rank test) and from 1.42±1.33 to 1.64±1.42 mmol after placebo treatment (*P*=0.35, Wilcoxon signed-rank test).

Discussion

In this randomized controlled trial, supplementation with quercetin at 500 mg/d for 4 wk progressively reduced plasma concentrations of uric acid without inducing changes in BMI, in fasting blood glucose or showing any adverse effects. The reduction in plasma uric acid was equivalent to ~8% with high significance (*p* value of 0.008 after 4 wk). The dose of quercetin was carefully considered based on both realistic food composition and a bioavailability test we which we have previously reported on healthy volunteers. In this comparison, we showed that quercetin (as glycoside conjugates) in 100 g fresh red onion provides a similar amount of bioavailable quercetin to the tablet used here (500 mg of pure quercetin aglycone), as assessed using urinary excretion [26]. This dose was sufficient to produce the observed change after 4 wk, and provided a more reproducible, practical and acceptable form of consuming quercetin. Similar approaches have been reported recently [42; 43].

There are several possible mechanisms for the observed change in plasma uric acid. The most likely is the direct inhibition of xanthine oxidoreductase activity, since, *in vitro*, bovine xanthine oxidoreductase is inhibited strongly by quercetin (K_i = 1.40±0.78 µmol/L) [44]. The drug, allopurinol, is comparable (K_i = 0.34±0.22 µmol/L) [44] and furthermore some conjugates such as quercetin-4′-O-glucuronide also inhibited xanthine oxidoreductase (K_i =0.25±0.03 µmol/L) [27]. Additional mechanisms are also possible, including promoted renal excretion of uric acid, which could be as a result of an increased glomerular filtration of uric acid. Some drugs such as Losartan inhibit directly URAT1, involved in uric acid reabsorption, and thereby decrease plasma uric acid [45], whereas some treatments down regulate mURAT1 and mGLUT9 in mice [46]. Up-regulation of transporters mOAT1 [46], rOAT1 [47] and

hOAT1 [48], which increase kidney urate secretion in the proximal tubules of the renal cortex, is also possible. However, a change in urinary excretion is unlikely since 2-week of quercetin administration did not change renal excretion, as assessed using 24 h urine. This implies an overall effect of quercetin on uric acid production rather than an increase in excretion. Other additional mechanisms could involve an indirect antioxidant effect that reduces microvascular ischemia in glomeruli and leads to increased local blood flow, dilation of afferent arterioles, and competition for reabsorption with ions such as sodium and potassium that exert osmotic effects [49]. A trend for reduction of diastolic blood pressure after quercetin supplementation lends partial support to this hypothesis. The -2.0 mm Hg (95% CI, 0.1 to -4.1; *P*=0.07) trend in reduction is potentially noteworthy, since a decrease of similar magnitude has been calculated to result in a substantial decrease in the prevalence of hypertension in population studies [50; 51]. We found no significant effect on systolic blood pressure in this study. Quercetin has been shown to reduce systolic and diastolic blood pressure in hypertensive subjects [52], but our subjects were chosen for their high blood uric acid levels and not specifically for exhibiting hypertension.

Quercetin has demonstrated some effects on various biomarkers in intervention studies, but the results are dependent on dose, nature of the cohort and length of time of treatment [42,43,53-55]. Some effects of quercetin may only be seen for defined genotypes [56]. A very limited number of studies have examined changes in plasma uric acid as a result of quercetin supplementation or high flavonol-diets, but none as a primary outcome. For example, 150 mg per day for 6 weeks gave no change in plasma uric acid [39], and a diet high in onions and tea for 2 weeks did not change plasma uric acid in patients with type 2 diabetes [33].

The present study was intentionally designed to be on a homogeneous population with higher than average blood uric acid to minimise confounding influences of gender, medication, diet, or other lifestyle factors. Hence, our result may be valid only for male individuals who are mildly or pre-hyperuricemic but otherwise healthy, and we cannot predict if the findings will extend to populations that include lower plasma uric acid level, females, hypertensive, older or younger populations. The role of habitual diet should also be considered. The intervention in the present study was designed to provide proof of principle and only one dose was tested, but there were no adverse events. Quercetin is part of the normal diet and consumed in very different amounts by individuals according to their dietary patterns.

It is noteworthy in our study that the hypo-uricemic effect of quercetin is more significant in subjects with higher uric acid level (Figure 3), which is in accordance with animal models [46]. These findings have served implications. Dietary quercetin could help to maintain a healthy blood uric acid, and help to prevent formation of uric acid crystals (gouty arthritis) [57]. Although hyperuricemia alone is not sufficient to cause gout, a dose-response relationship between serum uric acid and the risk of developing gout is well documented [58]. These findings may also help recovering gout patients where the primary treatment is to achieve an end point of serum uric acid levels less than 360 μmol/L over a period of three months [57]. This includes the use of the drug allopurinol to inhibit xanthine oxidoreductase and uric acid production, or the use of uricosuric drugs which increase renal excretion of uric acid. However, for patients also presenting kidney disease, liver disease, diabetes, congestive heart failure or hypertension, the dosage of allopurinol has to be adjusted in this stage [21]. Once restored, patients are often advised to make comprehensive dietary modifications for prevention against recurrent gout attacks. In the above

287 situations, adoption of one quercetin tablet that has efficacy to reduce blood uric acid
288 in the habitual diet is easier to adhere to compared to making major dietary changes.
289 Therefore quercetin may be a promising approach to lower uric acid in individuals
290 with above-optimal blood uric acid either for those at high risk who have not yet
291 developed any disease, or for patients recovering after therapy.

Acknowledgements

We express our sincere gratitude to all of the subjects who participated in this trial.

Financial support:

This work was supported by the China Scholarship Council and the University of Leeds as a PhD studentship to YS. The funders had no role in designing and conducting of the study, nor collection, management, analysis, and interpretation of the data or preparation, review, nor approval of the manuscript.

Conflict of interest: This work did not receive funding from a commercial organisation, but GW has recently, or currently, received other research funding from Nestle and Florida Department of Citrus, and conducted consultancy for Nutrilite, USA.

The authors' responsibilities: YS: study concept and design, data interpretation, volunteer recruitment, clinical study management, protocol implementation, sample acquisition, data collection and analysis, statistical analysis, writing and revision of the manuscript; GW: supervision of the study, study concept and design, writing and revision of the manuscript. YS had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. GW had primary responsibility for final content. Both authors have read and approved the final manuscript.

References

1. Richette P, Bardin T (2010) Gout. *Lancet* **375**, 318-328.

2. Chien K-L, Chen M-F, Hsu H-C *et al.* (2008) Plasma uric acid and the risk of type 2 diabetes in a Chinese community. *Clin Chem* **54**, 310-316.

3. Remedios C, Shah M, Bhasker AG *et al.* (2012) Hyperuricemia: a reality in the Indian obese. *Obes Surg* **22**, 945-948.

4. Nakanishi N, Okamoto M, Yoshida H *et al.* (2003) Serum uric acid and risk for development of hypertension and impaired fasting glucose or Type II diabetes in Japanese male office workers. *Eur J Epidemiol* **18**, 523-530.

5. Lv Q, Meng XF, He FF *et al.* (2013) High serum uric acid and increased risk of type 2 diabetes: a systemic review and meta-analysis of prospective cohort studies. *PLoS One* **8**, e56864.

6. Grayson PC, Kim SY, LaValley M *et al.* (2011) Hyperuricemia and incident hypertension: a systematic review and meta-analysis. *Arthritis Care Res* **63**, 102-110.

7. Li M, Hou W, Zhang X *et al.* (2014) Hyperuricemia and risk of stroke: a systematic review and meta-analysis of prospective studies. *Atherosclerosis* **232**, 265-270.

8. Roddy E, Choi HK (2014) Epidemiology of gout. *Rheum Dis Clin North Am* **40**, 155-175.

9. Mikuls TR, Farrar JT, Bilker WB *et al.* (2005) Gout epidemiology: results from the UK General Practice Research Database, 1990-1999. *Ann Rheum Dis* **64**, 267-272.

10. Luk AJ, Simkin PA (2005) Epidemiology of hyperuricemia and gout. *Am J Manag Care* **11**, S435-442.

11. Grassi D, Ferri L, Desideri G *et al.* (2013) Chronic hyperuricemia, uric acid deposit and cardiovascular risk. *Curr Pharm Des* **19**, 2432-2438.

12. Qiu L, Cheng XQ, Wu J *et al.* (2013) Prevalence of hyperuricemia and its related risk factors in healthy adults from Northern and Northeastern Chinese provinces. *BMC Public Health* **13**, 664.

13. Krishnan E, Kwoh CK, Schumacher HR *et al.* (2007) Hyperuricemia and incidence of hypertension among men without metabolic syndrome. *Hypertension* **49**, 298-303.

14. Feig DI, Soletsky B, Johnson RJ (2008) Effect of allopurinol on blood pressure of adolescents with newly diagnosed essential hypertension: a randomized trial. *JAMA* **300**, 924-932.

15. Dalbeth N, House ME, Gamble GD *et al.* (2013) Population-specific influence of SLC2A9 genotype on the acute hyperuricaemic response to a fructose load. *Ann Rheum Dis* **72**, 1868-1873.

16. Lin WT, Huang HL, Huang MC *et al.* (2013) Effects on uric acid, body mass index and blood pressure in adolescents of consuming beverages sweetened with high-fructose corn syrup. *Int J Obes* **37**, 532-539.

17. Choi HK, Curhan G (2008) Soft drinks, fructose consumption, and the risk of gout in men: prospective cohort study. *Br Med J* **336**, 309-312.

18. Rho YH, Zhu Y, Choi HK (2011) The epidemiology of uric acid and fructose. *Semin Nephrol* **31**, 410-419.

19. Lim JS, Mietus-Snyder M, Valente A *et al.* (2010) The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. *Nature reviews Gastroenterology & hepatology* **7**, 251-264.

20. Lanaspa MA, Sanchez-Lozada LG, Cicerchi C *et al.* (2012) Uric acid stimulates fructokinase and accelerates fructose metabolism in the development of fatty liver. *PLoS One* **7**, e47948.

21. Becker MA, Schumacher HR, Jr., Wortmann RL *et al.* (2005) Febuxostat compared with allopurinol in patients with hyperuricemia and gout. *N Engl J Med* **353**, 2450-2461.

22. Facchini F, Chen Y, Hollenbeck CB *et al.* (1991) Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. *JAMA* **266**, 3008-3011.

23. Vuorinen-Markkola H, Yki-Jarvinen H (1994) Hyperuricemia and insulin resistance. *J Clin Endocrinol Metab* **78**, 25-29.

24. Zhu Y, Hu Y, Huang T *et al.* (2014) High uric acid directly inhibits insulin signalling and induces insulin resistance. *Biochem Biophys Res Commun* **447**, 707-714.

25. Neveu V, Perez-Jimenez J, Vos F *et al.* (2010) Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database : the journal of biological databases and curation* **2010**, bap024.

26. Shi Y, Williamson G (2015) Comparison of the urinary excretion of quercetin glycosides from red onion and aglycone from dietary supplements in healthy subjects: a randomized, single-blinded, cross-over study. *Food & function* **6**, 1443-1448.

27. Day AJ, Bao Y, Morgan MR *et al.* (2000) Conjugation position of quercetin glucuronides and effect on biological activity. *Free Radic Biol Med* **29**, 1234-1243.

28. Haidari F, Rashidi MR, Eshraghian MR *et al.* (2008) Hypouricemic and antioxidant activities of Allium cepa Lilliaceae and quercetin in normal and hyperuricemic rats. *Saudi Med J* **29**, 1573-1579.

29. Mo SF, Zhou F, Lv YZ *et al.* (2007) Hypouricemic action of selected flavonoids in mice: structure-activity relationships. *Biol Pharm Bull* **30**, 1551-1556.

30. Auclair S, Silberberg M, Gueux E *et al.* (2008) Apple polyphenols and fibers attenuate atherosclerosis in apolipoprotein E-deficient mice. *J Agric Food Chem* **56**, 5558-5563.

31. Egert S, Wolffram S, Bosy-Westphal A *et al.* (2008) Daily quercetin supplementation dose-dependently increases plasma quercetin concentrations in healthy humans. *J Nutr* **138**, 1615-1621.

32. Boots AW, Wilms LC, Swennen EL *et al.* (2008) In vitro and ex vivo anti-inflammatory activity of quercetin in healthy volunteers. *Nutrition* **24**, 703-710.

33. Lean ME, Noroozi M, Kelly I *et al.* (1999) Dietary flavonols protect diabetic human lymphocytes against oxidative damage to DNA. *Diabetes* **48**, 176-181.

34. Princen HM, van Duyvenvoorde W, Buytenhek R *et al.* (1998) No effect of consumption of green and black tea on plasma lipid and antioxidant levels and on LDL oxidation in smokers. *Arterioscler Thromb Vasc Biol* **18**, 833-841.

35. Castilla P, Echarri R, Davalos A *et al.* (2006) Concentrated red grape juice exerts antioxidant, hypolipidemic, and antiinflammatory effects in both hemodialysis patients and healthy subjects. *Am J Clin Nutr* **84**, 252-262.

36. Kimira M, Arai Y, Shimoi K *et al.* (1998) Japanese intake of flavonoids and isoflavonoids from foods. *J Epidemiol* **8**, 168-175.

37. Fossati P, Prencipe L, Berti G (1980) Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clin Chem* **26**, 227-231.

38. Machin D, Campbell M, Fayers P *et al.* (1997) *Sample Size Tables for Clinical Studies*. 2th ed. Hoboken: John Wiley & Sons, Ltd.

39. Egert S, Bosy-Westphal A, Seiberl J *et al.* (2009) Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study. *Br J Nutr* **102**, 1065-1074.

40. Kansui Y, Ohtsubo T, Goto K *et al.* (2011) Association of serum uric acid with blood pressure in Japanese men. Cross-sectional study in work-site group. *Circ J* **75**, 2827-2832.

41. Forman JP, Choi H, Curhan GC (2007) Plasma uric acid level and risk for incident hypertension among men. *J Am Soc Nephrol* **18**, 287-292.

42. Dower JI, Geleijnse JM, Gijsbers L *et al.* (2015) Effects of the pure flavonoids epicatechin and quercetin on vascular function and cardiometabolic health: a randomized, double-blind, placebo-controlled, crossover trial. *Am J Clin Nutr* **101**, 914-921.

43. Dower JI, Geleijnse JM, Gijsbers L *et al.* (2015) Supplementation of the Pure Flavonoids Epicatechin and Quercetin Affects Some Biomarkers of Endothelial Dysfunction and Inflammation in (Pre)Hypertensive Adults: A Randomized Double-Blind, Placebo-Controlled, Crossover Trial. *J Nutr* **145**, 1459-1463.

44. Lin CM, Chen CS, Chen CT *et al.* (2002) Molecular modeling of flavonoids that inhibits xanthine oxidase. *Biochem Biophys Res Commun* **294**, 167-172.

45. Hamada T, Ichica K, Hosoyamada M *et al.* (2008) Uricosuric action of losartan via the inhibition of urate transporter 1 (URAT1) in hypertensive patients. *Am J Hypertens* **21**, 1157-1162.

46. Hu QH, Zhang X, Wang X *et al.* (2012) Quercetin regulates organic ion transporter and uromodulin expression and improves renal function in hyperuricemic mice. *Eur J Nutr* **51**, 593-606.

47. Hu QH, Wang C, Li JM *et al.* (2009) Allopurinol, rutin, and quercetin attenuate hyperuricemia and renal dysfunction in rats induced by fructose intake: renal organic ion transporter involvement. *Am J Physiol Renal Physiol* **297**, F1080-1091.

48. Hong SS, Seo K, Lim SC *et al.* (2007) Interaction characteristics of flavonoids with human organic anion transporter 1 (hOAT1) and 3 (hOAT3). *Pharmacol Res* **56**, 468-473.

49. Fabre G, Bayach I, Berka K *et al.* (2015) Synergism of antioxidant action of vitamins E, C and quercetin is related to formation of molecular associations in biomembranes. *Chem Commun* **51**, 7713-7716.

50. Cook NR, Cohen J, Hebert PR *et al.* (1995) IMplications of small reductions in diastolic blood pressure for primary prevention. *Arch Intern Med* **155**, 701-709.

51. Whelton PK, He J, Appel LJ *et al.* (2002) Primary prevention of hypertension: clinical and public health advisory from The National High Blood Pressure Education Program. *JAMA* **288**, 1882-1888.

52. Edwards RL, Lyon T, Litwin SE, Rabovsky A, Symons JD, Jalili T. (2007) Quercetin reduces blood pressure in hypertensive subjects. J Nutr. **137,** 2405-2411.

53. Loke WM, Hodgson JM, Proudfoot JM *et al.* (2008) Pure dietary flavonoids quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. *Am J Clin Nutr* **88**, 1018-1025.

54. Hubbard GP, Wolffram S, Lovegrove JA *et al.* (2004) Ingestion of quercetin inhibits platelet aggregation and essential components of the collagen-stimulated platelet activation pathway in humans. *J Thromb Haemost* **2**, 2138-2145.

55. Conquer JA, Holub BJ (1998) Effect of supplementation with different doses of DHA on the levels of circulating DHA as non-esterified fatty acid in subjects of Asian Indian background. *J Lipid Res* **39**, 286-292.

56. Pfeuffer M, Auinger A, Bley U *et al.* (2013) Effect of quercetin on traits of the metabolic syndrome, endothelial function and inflammation in men with different APOE isoforms. *Nutr Metab Cardiovasc Dis* **23**, 403-409.

57. Schlesinger N (2004) Management of acute and chronic gouty arthritis - Present state-of-the-art. *Drugs* **64**, 2399-2416.

58. Campion EW, Glynn RJ, DeLabry LO (1987) Asymptomatic hyperuricemia. Risks and consequences in the Normative Aging Study. *Am J Med* **82**, 421-426.

Tables

Table 1 Effect of quercetin and placebo treatements on plasma biomarkers and blood pressure (n=22) [a]

		Quercetin			Placebo	
	Measures, mean±SD	Mean difference from baseline (95% CI)	*P* value	Measures, mean±SD	Mean difference from baseline (95% CI)	*P* value
Plasma uric acid, µmol/L						
Baseline	330±56			315±45		0.21
2-wk	314±55	-15.9 (0.9, -32.8)	0.06 *	325±52	10.6 (-8.9, 30.0)	0.27
4-wk	304±48	-26.5 (-7.6, -45.5)	0.008 **	320±47	5.2 (-15.1, 25.5)	0.60
Plasma glucose, mmol/L						
Baseline	5.04±0.60			5.09±0.49		0.35
2-wk	5.01±0.65	-0.03 (0.15, -0.21)	0.73	5.13±0.58	0.03 (-0.12, 0.19)	0.65
4-wk	5.10±0.69	0.06 (-0.13, 0.26)	0.48	5.02±0.77	-0.07 (0.18, -0.33)	0.57
Systolic blood pressure, mm Hg						
Baseline	123.2±7.2			122.5±9.9		0.58
4-wk	122.0±8.9	-1.1 (1.7, -4.0)	0.41	124.6±10.6	2.1 (-0.8, 5.1)	0.14
Diastolic blood pressure, mm Hg [b]						
Baseline	73.8±9.2			73.1±7.8		0.43
4-wk	71.8±8.9	-2.0 (0.1, -4.1)	0.07 *	72.7±9.7	-0.4 (2.0, -2.9)	0.79

* indicates $P<0.1$ and ** indicates $P<0.05$ when compared to baseline.

[a] 2-tailed paired *t* test were used if not stated otherwise.

[b] *Wilcoxon signed-rank* test was used as the data is not normally distributed.

Figure legends

Figure 1 Participant flow diagram of the progress through this double-blinded, placebo-controlled, randomized, cross-over trial

Figure 2 Effect of consumption of quercetin on plasma uric acid

Comparison of plasma uric acid at baseline, 2 and 4 wk after consuming quercetin (containing 500 mg of quercetin) or a placebo daily in 22 healthy subjects. Error bars indicate 95% CI. * indicates a trend (P<0.1) and ** indicates signficance (P<0.05) when compared to baseline by paired t test.

Figure 3 Changes of plasma uric acid from observations in relation to baseline plasma uric

The magnitude of plasma uric acid reduction was higher in individuals with higher baseline plasma uric acid in both treatments. Plasma uric acid in the majority of subjects declined after 4 wk in treatment by quercetin (17/22) but not by placebo (10/22). Correlation coefficient r was calculated by the *Pearson* test.

Figures

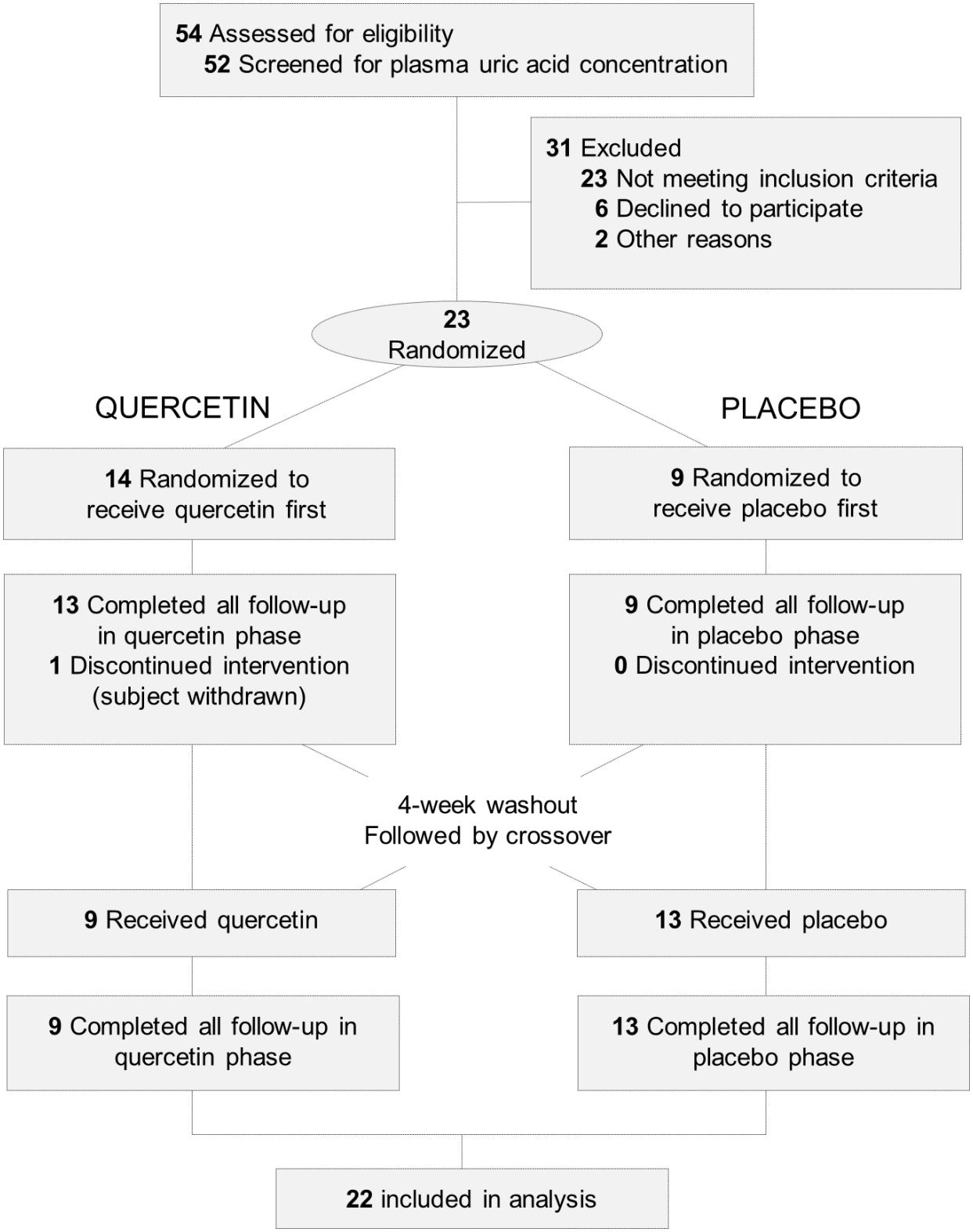

Figure 1

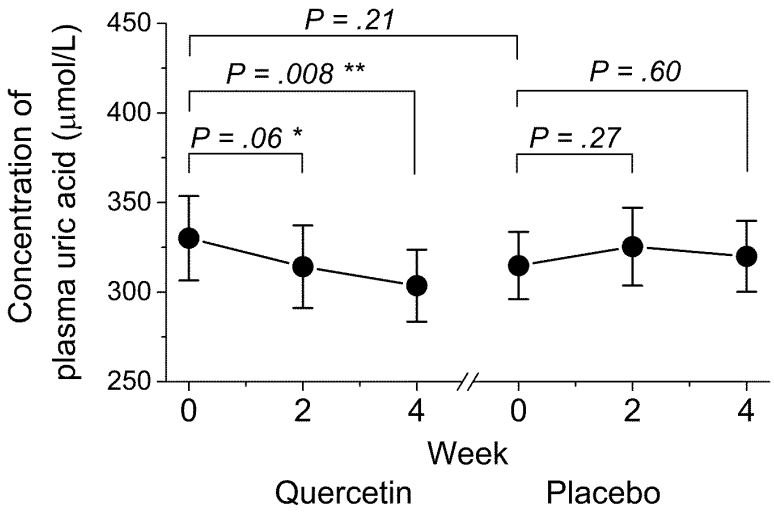
P = .21
P = .008 **
P = .60
P = .06 *
P = .27
Concentration of plasma uric acid (μmol/L)
450
400
350
300
250
0
2
4
0
2
4
Week
Quercetin
Placebo

Figure 2

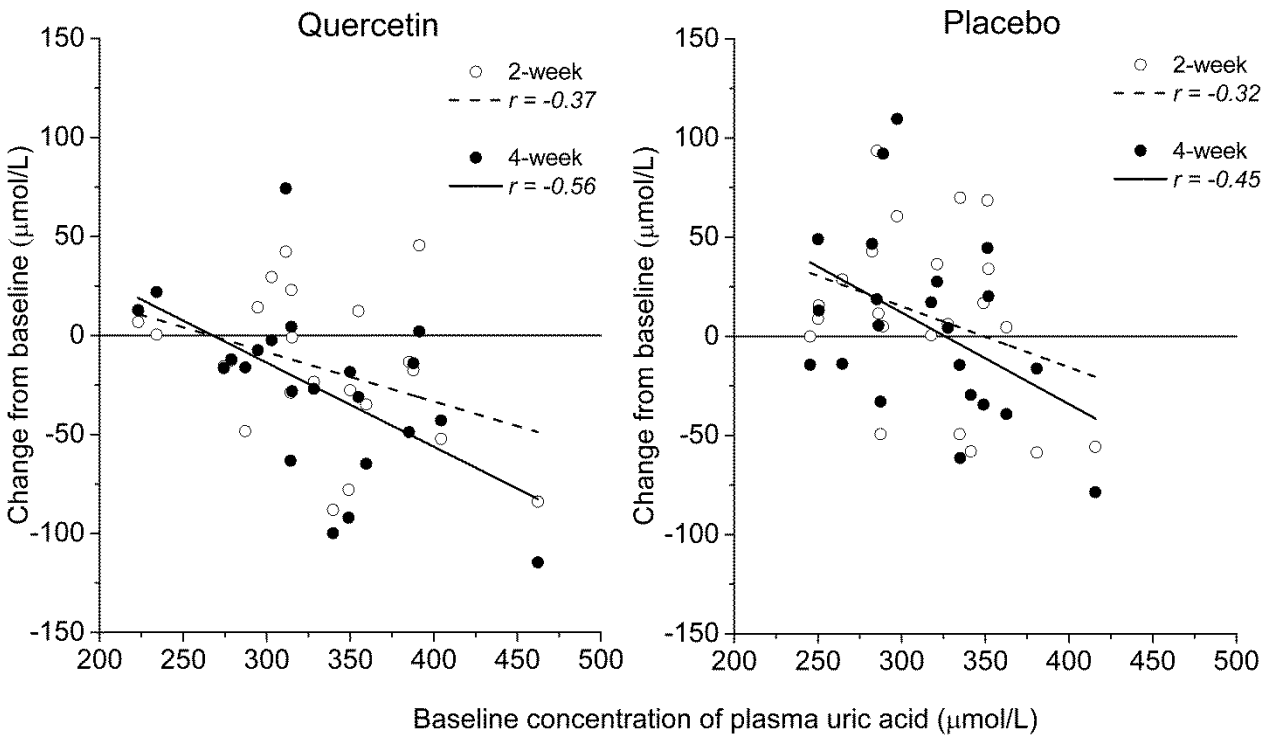
Quercetin
Placebo
2-week
r = -0.37
4-week
r = -0.56
2-week
r = -0.32
4-week
r = -0.45
Change from baseline (μmol/L)
Baseline concentration of plasma uric acid (μmol/L)
150
100
50
0
-50
-100
-150
200
250
300
350
400
450
500

Figure 3