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ORIGINAL ARTICLE

Pomegranate extract alleviates disease activity and some blood biomarkers of inflammation and oxidative stress in Rheumatoid Arthritis patients

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BACKGROUND/OBJECTIVES: Since the main characteristics of Rheumatoid Arthritis (RA) are joint dysfunction caused by inflammation and serious pain, anti-inflammatory agents may alleviate the clinical symptoms in RA. Pomegranate juice is rich in polyphenolic compounds that possess antioxidant and anti-inflammatory activities. This study aimed to determine the beneficial effects of pomegranate extract (POMx) in RA patients.

SUBJECTS/METHODS: A total of 55 RA patients were enrolled and randomly allocated to an intervention group ($n = 30$) or a control group ($n = 25$). The intervention group received 2 capsules of 250 mg POMx and the control group 2 capsules of 250 mg cellulose per day for 8 weeks. At the beginning of the study and after 8 weeks, Health Assessment Questionnaire (HAQ) and Disease Activity Score (DAS) 28 were completed and serum concentrations of C-reactive protein (CRP), matrix metalloproteinases 3 (MMP3), malondialdehyde (MDA), glutathione peroxidase (GPx) and erythrocyte sedimentation rate (ESR) were analyzed using standard methods and compared between the two groups.

RESULTS: Compared with the placebo group, POMx supplement significantly reduced the score of DAS28 ($P < 0.001$) which could be related to the decrease in swollen ($P < 0.001$) and tender joints ($P = 0.001$) count, pain intensity ($P = 0.003$) and ESR levels ($P = 0.03$). POMx consumption also decreased HAQ score ($P = 0.007$) and morning stiffness ($P = 0.04$) and increased GPx concentrations ($P < 0.001$). There were no differences in the change in mean MMP3, CRP and MDA levels between two groups.

CONCLUSIONS: POMx alleviates disease activity and improves some blood biomarkers of inflammation and oxidative stress in RA patients.

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INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic systemic autoimmune inflammatory disease, characterized by a symmetrical persistent synovitis of the joints of the hands, wrist, feet and knee, resulting in tender swelling of the joints, pain, limitation in motion and morning stiffness.[1] In the early phase of the disease, the synovial tissue is infiltrated by immune cells including lymphocytes, plasma cells, macrophages and other cells. These cells contribute to the inflammatory process via the production of matrix metalloproteinases (MMPs), cytokines and chemokines, followed by the influx and activation of more immune cells into the synovial tissue.[2] MMP3 is one of MMPs produced by fibroblasts in response to increased levels of inflammatory cytokines and causes joint degradation.[3,4] Inflammatory cytokines activate multiple signaling pathways such as mitogen-activated protein kinase (MAPK) which finally activate nuclear factor-κB (NF-κB).[5] It is a nuclear transcription factor that is a crucial mediator of the inflammatory and immune response.[6] Activated NF-κB then enters a feedback loop in the inflammatory process by upregulating the transcriptional levels of multiple genes, including inducible nitric oxide and cyclo-oxygenase-2[7] which have important roles in the development of certain inflammatory diseases.[8,9] On the other hand, many signal transduction pathways are activated in the RA synovial tissue,[5] but one of the most important signaling pathways involved in the pathogenesis of RA is NF-κB pathway.[10] Since the main characteristics of RA are joint dysfunction caused by inflammation and pain, agents with activity of anti-inflammation and analgesia may improve the RA condition and prevent further progression of the disease.

Pomegranate is a fruit native to tropical and subtropical regions, originating from the Middle East and India. It contains flavonoids, vitamins (A, B and C), tannins and immune-boosting antioxidants.[11] The potent antioxidant activity of pomegranate is attributed to its polyphenols,[11] which has been shown to be more and stronger than many potent antioxidants such as green tea.[12] Anti-inflammatory effects of pomegranate and its products (extract and juice) happen via inhibition of cell signaling pathways including suppression of cyclo-oxygenase-2 and inducible nitric oxide expression, inhibition of activation of NF-κB and inhibition of phosphorylation of MAPKs proteins.[13–15] As standardized extracts of pomegranate prepared from the red peel have been shown to possess antioxidative, anti-inflammatory and cardio-vascular disease-preventing properties,[16] we used pomegranate extract (POMx) in the capsule forms in this study.

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Antioxidant as well as anti-inflammatory effects of POMx on the joint have also been confirmed both in animal models[17] and *in vitro*[18] studies, but there are limited human studies in this area in which POMx has shown anti-inflammatory effects in human osteoarthritis chondrocytes[19] as well as antioxidant and ameliorative effects in patients with active RA.[20] Therefore, the aim of the present study was to investigate the effect of POMx on the disease activity and biomarkers of inflammation and oxidative stress in the RA patients.

MATERIALS AND METHODS

Study population

This study was conducted on RA patients from August to December 2011. The subjects attended two medical centers of Shiraz and Ahvaz cities of Iran. They met the American College of Rheumatology 1987 revised classification criteria for RA.[21] Inclusion criteria were ages 40 years and higher, active RA according to the diagnosis of an experienced rheumatologist with symptoms including suffering from joint pain and swelling in spite of receiving the standard medications (Methotrexate, Hydroxychloroquine, Sulfasalazine and Prednisolone), morning stiffness lasting for more than 1 h, and intake of stable doses of non-steroidal anti-inflammatory drugs and corticosteroids during the past 4 weeks and during the study. Exclusion criteria were comorbidities such as diabetes, hyperlipidemia, hypertension, liver as well as kidney diseases, severe infections, food intolerance or allergies, alcohol abuse, and daily intake of any other drugs or vitamin/mineral supplements. The Ethics Committee on Human Experiments of Shiraz University of Medical Sciences approved the research protocol. The study was registered on the Iranian Registry of Clinical Trials website (http://www.irct.ir/, IRCT 201202183236N2). Written informed consent was obtained from all the patients. Sample size of at least 30 in each group with $\alpha = 0.05$ and power of 80% was determined according to Disease Activity Score 28 (DAS28).[22]

Study design

This double-blind randomized clinical trial was conducted on RA patients who were randomly assigned into two groups using a random numbers table by a statistician. The intervention allocation was blinded for the investigator and participants. The patients received 2 capsules of POMx or cellulose as placebo daily for 8 weeks. POMx capsules were supplied by Puritan's Pride Co, USA and contained 40% ellagic acid. Placebo capsules were supplied by School of Pharmacy Shiraz University of Medical Sciences, Shiraz, Iran. Each capsule contained 250 mg POMx or cellulose. Placebo capsules were quite similar to POMx capsules as to of shape, size and color.

The subjects were asked to consume each capsule with lunch and dinner. Patients were advised to maintain their usual diet and daily activity and not to change the dosage or type of their drugs without consultation with the researcher. All capsules were placed in the same packages and were given to the patients every 4 weeks. The subjects' adherence to the study was maintained by using telephone calls every week.

Laboratory assessment

At the beginning and after 8 weeks of the study, 8 ml fasting venous blood were collected from the patients to measure erythrocyte sedimentation rate (ESR), C- reactive protein (CRP), MMP3, malondialdehyde (MDA) and glutathione peroxidase (GPx). Blood samples were centrifuged at 3000 *g* for 10 min at 4 °C. Serum was separated and frozen at − 70 °C in an ultra-freezer (Sanyo) until analysis. Serum CRP and MMP3 were measured by enzyme linked immunosorbent assay Kits (Diagnostics Biochem Co, Dorchester, ON, Canada for CRP & Glory Science Co, Hangzhou, China for MMP3). Serum GPx was measured by commercial kit (Cayman co, Ellsworth Ann Arbor, MI, USA). MDA concentration was determined using the spectrophotometric method and based on the reaction of MDA and thiobarbituric acid and ESR was measured according to Westergren method.[23]

Clinical and dietary assessments

Patients were assessed at baseline and after 8 weeks. At each visit, the height and weight were measured (Seca) and BMI was calculated by dividing weight (kg) by height^2 (m^2). The DAS (DAS28) was calculated based on swollen and tender joint count, pain intensity, and high ESR or CRP.[24] Pain intensity was measured by a visual analog scale (100 mm). Length of morning stiffness was also evaluated and health assessment questionnaire was completed for each patient to evaluate their function.

Dietary intake of polyphenols was estimated using food frequency questionnaire according to the consumption of 100 richest dietary sources of polyphenols.[25]

Statistical analysis

All the analyses were performed using Statistical Package for Social Sciences (SPSS), version 16 (SPSS Inc., Chicago, IL, USA). The normality of the distribution of data was first checked by one-sample Kolmogorov-Smirnov test. Student's *t*-test and Mann–Whitney test were applied to compare the groups when they were normally or non-normally distributed, respectively. Analysis of covariance was used to identify any differences between the two groups after adjustment for the baseline measurements and covariates. Differences with a $P < 0.05$ were considered to be statistically significant.

RESULTS

Fifty-five participants completed the study (intervention group: 30; control group: 25). Five subjects in the control group were excluded from analysis, due to the unwillingness to continue the study.

Patients' baseline characteristics, including age, BMI, duration of disease, current drugs intake and sex distribution are described in Table 1. There was no significant difference in baseline characteristics between the two groups. Dietary intakes of polyphenols were similar between the two groups at baseline and after 56 days (data has not shown).

Compared with the placebo group, POMx consumption significantly reduced the score of DAS28 ($P < 0.001$) which could be related to the significant decrease in swollen ($P < 0.001$) and tender joints ($P = 0.001$) count, pain intensity ($P = 0.003$) and ESR levels ($P = 0.03$). POMx consumption also decreased the health assessment questionnaire score ($P = 0.007$) and morning stiffness ($P = 0.04$) and increased GPx concentrations ($P < 0.001$) compared with the placebo group after 56 days. There were no differences in the changs in mean MMP3, CRP and MDA levels between the control and intervention groups (see Table 2).

Table 1. Baseline characteristics of patients

Variables	*POMx (n = 30) Mean ± s.d.*	*Placebo (n = 25) Mean ± s.d.*	P-*value*
Age (years)	48.4 ± 11.4	49.1 ± 12.2	0.8
BMI (kg/m^2)	28.2 ± 4.7	29.6 ± 6.1	0.3
Duration of disease (years)	10.9 ± 5.8	12.3 ± 5.8	0.3
Current drugs intake			
Methotrexate (mg/week)	11.3 ± 5.0	11.0 ± 4.7	0.8
No. of patients	30	30	–
Prednisolone (mg/day)	5.5 ± 2.7	5.6 ± 1.6	0.8
No. of patients	27	25	–
Hydroxychloroquine (mg/day)	73.3 ± 122.9	72.0 ± 97.9	0.9
No. of patients	30	30	–
Sulfasalazine (mg/day)	200.0 ± 249.1	160.0 ± 238.0	0.5
No. of patients	28	30	–
No of patients taking NSAIDs & corticosteroids	30	30	–
Sex			
Men	10	10	–
Women	20	20	–

Abbreviations: BMI, body mass index; NSAIDS, non-steroidal anti-inflammatory drugs; POMx, pomegranate extract.

Table 2. Clinical and laboratory parameters at baseline and change in clinical and laboratory parameters between baseline and after 8 weeks, in the POMx and placebo groups

Variables	POMx *(n = 30)*		*Placebo (n = 25)*		P-*value*[a]
	Baseline Mean ± s.e.m	*Change at day 56 Mean ± s.e.m*	*Baseline Mean ± s.e.m*	*Change at day 56 Mean ± s.e.m*	
Swollen joints (no.)	5.7 ± 3.1	− 2.6 ± 2.7	4.4 ± 2.7	0.08 ± 1.6	< 0.001
Tender joints (no.)	5.8 ± 3.87	− 2.1 ± 3.1	7.0 ± 5.4	0.9 ± 3.3	0.001
Pain intensity	59.3 ± 26.1	− 17.6 ± 24.9	51.0 ± 24.9	− 1.6 ± 10.2	0.003
DAS28	4.9 ± 0.8	− 0.9 ± 0.8	4.7 ± 1.1	0.1 ± 0.5	< 0.001
HAQ	1.2 ± 0.6	− 0.4 ± 0.4	1.3 ± 0.7	− 0.1 ± 0.3	0.007
Morning stiffness (min)	37.4 ± 43.6	− 36.1 ± 45.5	71.0 ± 95.6	0.0 ± 83.7	0.04
ESR (mm/h)	29.0 ± 15.6	− 4.3 ± 11.0	30.6 ± 19.6	3.5 ± 15.9	0.03
CRP (mg/dl)	8.0 ± 4.2	− 0.8 ± 3.1	6.6 ± 4.5	0.4 ± 4.7	0.6
MMP3 (ng/ml)	1.4 ± 1.2	− 0.07 ± 0.2	2.2 ± 2.1	0.02 ± 0.9	0.6
MDA (mol/l)	4.1 ± 0.8	0.08 ± 0.4	3.8 ± 1.1	− 0.2 ± 0.8	0.1
GPx (nmol/ml/min)	147.4 ± 24.7	18.3 ± 11.9	152.7 ± 17.0	− 1.6 ± 7.2	< 0.001

Abbreviations: CRP, C-reactive protein; DAS28, Disease Activity Score; ESR, erythrocyte sedimentation rate; GPx, glutathione peroxidase; HAQ, Health Assessment Questionnaire; MDA, malondialdehyde; MMP3, matrix metalloproteinase-3; POMx, pomegranate extract. [a]Compare of mean change between the two groups.

DISCUSSION

This is one of the few randomized clinical trials evaluating the effects of POMx on the disease activity and some biomarkers of inflammation and oxidative stress in RA patients. Our results showed that an 8-week POMx supplementation is able to reduce disease activity including swollen and tender joints count, pain intensity and blood ESR levels in these patients. In addition, health assessment questionnaire score, morning stiffness and GPx levels were improved in the POMx group compared with the control group. However, the changes in serum MMP3, CRP and MDA levels were not statistically significant.

The findings of the present study are in agreement with those of the study conducted by Rasheed *et al.*[19] which indicated that POMx inhibits pro-inflammatory cytokines production via inhibiting the gene expression. This is achieved by blocking MAPK and NF-κB activation in cells. Shukla *et al.*[17] in a study on mice showed that POMx delayed the onset of collagen-induced arthritis and severity of disease. The result of a pilot study by Balbir-Gurman *et al.* showed a significant decrease of the number of tender joints, DAS28, free radical-induced lipid peroxidation and a significant increase in the activity of serum high-density lipoprotein-associated paraoxonase 1 in RA patients following 12 weeks of POMx consumption.[20] Guo *et al.*[26] also showed that daily pomegranate juice intake for 4 weeks resulted in a significant increase in ferric reducing/antioxidant power and the activity of GPx enzyme as well as catalase and decrease of plasma MDA. Consumption of antioxidant-enriched margarine containing: alpha-tocopherol, lycopene, palm oil carotenoids and lutein with intake of vitamin C supplement reduced the number of swollen and painful joints and DAS28 in RA patients.[27]

We used ESR and CRP among the inflammatory markers according to a study by Salamon *et al.*[28] that showed the level of these markers in RA patients is high. MMP3 increases in the synovial fluid of RA patients and especially in active RA disease; hence, it may be a useful biomarker of response to treatment.[4] Among the oxidative stress markers we also used MDA[29] and GPx[30] because it is known to be elevated in RA. Moreover, GSH-Px is the most abundant antioxidant marker in serum and appears to have a major role in reactive oxygen species defenses based on *in vitro* studies.[31]

RA is a chronic inflammatory and autoimmune disease that leads to cartilage damage, bone erosion and joint deformation. A large number of studies have shown that excessive production of inflammatory cytokines[32–34] as well as reactive oxygen species[35] in joints play a critical role in the pathogenesis of the disease. In fact, reactive oxygen species lead to cascade stimulation of the phenomena through the activity of MAPK and NF-κB pathways and increase the inflammatory cytokines' gene expression which finally creates immune responses and causes inflammation.[36]

Various studies suggested that due to their vitamins, minerals and phytochemical content, fruits and vegetables have protective effects on the progression of RA.[37–40] However, only a few studies have been performed on the antioxidant and anti-inflammatory effects of fruits, vegetables and their phytochemicals in RA disease.[41] With respect to the improvement of disease activity and some blood biomarkers in the present study, our results may show the beneficial antioxidant and anti-inflammatory effects of pomegranate in RA patients.

One of the main compounds in pomegranate fruit is phenolic compounds which are responsible for most of the functional properties of this fruit.[42] Phenolic compounds can reduce the oxidative stress in two ways. In the direct way, these compounds sweep the reactive oxygen species. In fact, due to having several hydroxyl groups, polyphenols neutralize the free radicals by giving them a hydrogen atom. In the indirect way, on the other hand, polyphenols strengthen the endogenous antioxidant defense system. For instance, flavonoid, which is one of the natural polyphenols, stimulate the expression of γ-glutamylcysteine synthetase and consequently increase the glutathione concentration in the cell. This enzyme is a rate-limiting enzyme in the synthesis of a large number of intracellular endogenous antioxidants, such as glutathione.[43,44]

Ellagic acid is one of the phenolic acids present in pomegranate.[42] The anti-inflammatory[45] and antioxidant effects[46] of ellagic acid are proven in some studies. The content of ellagic acid in the pomegranate juice is significantly affected by the processing method.[47] Therefore, different results might be obtained based on the ellagic acid content of pomegranate products.

The other mechanism which might explain our finding is the effect of POMx on microbiota. Some evidence suggest that gut microbiota play a role in the pathogenesis of RA.[48] An altered microbiota is a factor in the initiation and perpetuation of inflammatory diseases, including RA.[49] The results of some studies have shown POMx may change the gut microbiota and lead to

health benefits.[50] Pomegranate peel extract supplementation, which is rich in polyphenols, increased the cecal content of bifidobacteria in obese mice.[51] It was shown that the number of bifidobacteria is inversely correlated with the adipose tissue inflammation.[52]

Our study may have some limitations. First, the sample size was small. Second, the duration of the study was short. Third, a few blood biomarkers of inflammation and oxidative stress were measured. However, this study is one of the few randomized clinical trials evaluating the effects of POMx on RA patients. So, further clinical studies with larger sample size or more biomarkers measurements are needed in this area. Furthermore, assessing the effect of POMx consumption on other inflammatory diseases needs further investigation.

The results of the present study showed the beneficial effects of POMx on the RA patients' disease activity and some blood biomarkers of inflammation and oxidative stress.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

We advise that all authors listed have contributed to the work. MG analyzed the data and drafted the manuscript. ET recruited the patients and collected the samples and performed laboratory tests. KM supervised the biochemical experiments. JH and GS contributed to the data analysis and the interpretation of results. ZM contributed to the study design, supervised the study and reviewed the manuscript.

REFERENCES

1 Rudan I, Sidhu S, Papana A, Meng SJ, Xin-Wei Y, Wang W *et al.* Prevalence of rheumatoid arthritis in low- and middle-income countries: a systematic review and analysis. *J Glob Health* 2015; **5**: 010409.
2 Steiner G. Auto-antibodies and autoreactive T-cells in rheumatoid arthritis: pathogenetic players and diagnostic tools. *Clin Rev Allergy Immunol* 2007; **32**: 23–36.
3 Birkedal-Hansen H. Role of matrix metalloproteinases in human periodontal diseases. *J Periodontol* 1993; **64**: 474–484.
4 Stachowska E, Dolegowska B, Dziedziejko V, Rybicka M, Kaczmarczyk M, Bober J *et al.* Prostaglandin E2 (PGE2) and thromboxane A2 (TXA2) synthesis is regulated by conjugated linoleic acids (CLA) in human macrophages. *J Physiol Pharmacol* 2009; **60**: 77–85.
5 Tas SW, Remans PH, Reedquist KA, Tak PP. Signal transduction pathways and transcription factors as therapeutic targets in inflammatory disease: towards innovative antirheumatic therapy. *Curr Pharm Des* 2005; **11**: 581–611.
6 Sehnert B, Burkhardt H, May MJ, Zwerina J, Voll RE. Sneaking-ligand fusion proteins attenuate serum transfer arthritis by endothelium-targeted NF-kappaB inhibition. *Methods Mol Biol* 2015; **1280**: 579–591.
7 Jungel A, Distler O, Schulze-Horsel U, Huber LC, Ha HR, Simmen B *et al.* Microparticles stimulate the synthesis of prostaglandin E(2) via induction of cyclooxygenase 2 and microsomal prostaglandin E synthase 1. *Arthritis Rheum* 2007; **56**: 3564–3574.
8 Turini ME, DuBois RN. Cyclooxygenase-2: a therapeutic target. *Annu Rev Med* 2002; **53**: 35–57.
9 Moncada S. Nitric oxide: discovery and impact on clinical medicine. *J R Soc Med* 1999; **92**: 164–169.
10 Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. *Annu Rev Immunol* 2009; **27**: 693–733.
11 Costantini S, Rusolo F, De Vito V, Moccia S, Picariello G, Capone F *et al.* Potential anti-inflammatory effects of the hydrophilic fraction of pomegranate (Punica granatum L.) seed oil on breast cancer cell lines. *Molecules* 2014; **19**: 8644–8660.
12 Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J Agric Food Chem* 2000; **48**: 4581–4589.
13 Afaq F, Saleem M, Krueger CG, Reed JD, Mukhtar H. Anthocyanin- and hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF-kappaB pathways and inhibits skin tumorigenesis in CD-1 mice. *Int J Cancer* 2005; **113**: 423–433.
14 Adams LS, Seeram NP, Aggarwal BB, Takada Y, Sand D, Heber D. Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. *J Agric Food Chem* 2006; **54**: 980–985.
15 Lansky EP, Newman RA. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *J Ethnopharmacol* 2007; **109**: 177–206.
16 de Nigris F, Williams-Ignarro S, Sica V, Lerman LO, D'Armiento FP, Byrns RE *et al.* Effects of a pomegranate fruit extract rich in punicalagin on oxidation-sensitive genes and eNOS activity at sites of perturbed shear stress and atherogenesis. *Cardiovasc Res* 2007; **73**: 414–423.
17 Shukla M, Gupta K, Rasheed Z, Khan KA, Haqqi TM. Consumption of hydrolyzable tannins-rich pomegranate extract suppresses inflammation and joint damage in rheumatoid arthritis. *Nutrition* 2008; **24**: 733–743.
18 Ahmed S, Wang N, Hafeez BB, Cheruvu VK, Haqqi TM, Punica granatum L. extract inhibits IL-1beta-induced expression of matrix metalloproteinases by inhibiting the activation of MAP kinases and NF-kappaB in human chondrocytes in vitro. *J Nutr* 2005; **135**: 2096–2102.
19 Rasheed Z, Akhtar N, Haqqi TM. Pomegranate extract inhibits the interleukin-1beta-induced activation of MKK-3, p38alpha-MAPK and transcription factor RUNX-2 in human osteoarthritis chondrocytes. *Arthritis Res Ther* 2010; **12**: R195.
20 Balbir-Gurman A, Fuhrman B, Braun-Moscovici Y, Markovits D, Aviram M. Consumption of pomegranate decreases serum oxidative stress and reduces disease activity in patients with active rheumatoid arthritis: a pilot study. *Isr Med Assoc J* 2011; **13**: 474–479.
21 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; **31**: 315–324.
22 Willich SN, Rossnagel K, Roll S, Wagner A, Mune O, Erlendson J *et al.* Rose hip herbal remedy in patients with rheumatoid arthritis - a randomised controlled trial. *Phytomedicine* 2010; **17**: 87–93.
23 Alexy T, Pais E, Meiselman HJ. A rapid method to estimate Westergren sedimentation rates. *Rev Sci Instrum* 2009; **80**: 096102.
24 Fransen J, van Riel PL. The Disease Activity Score and the EULAR response criteria. *Clin Exp Rheumatol* 2005; **23**: S93–S99.
25 Perez-Jimenez J, Neveu V, Vos F, Scalbert A. Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. *Eur J Clin Nutr* 2010; **64**: S112–S120.
26 Guo C, Wei J, Yang J, Xu J, Pang W, Jiang Y. Pomegranate juice is potentially better than apple juice in improving antioxidant function in elderly subjects. *Nutr Res* 2008; **28**: 72–77.
27 van Vugt RM, Rijken PJ, Rietveld AG, van Vugt AC, Dijkmans BA. Antioxidant intervention in rheumatoid arthritis: results of an open pilot study. *Clin Rheumatol* 2008; **27**: 771–775.
28 Salamon L, Morović-Vergles J, Marasović-Krstulović D, Kehler T, Šakić D, Badovinac O *et al.* Differences in the prevalence and characteristics of metabolic syndrome in rheumatoid arthritis and osteoarthritis: a multicentric study. *Rheumatol Int* 2015; **35**: 2047–2057.
29 Darrah E, Andrade F. Editorial: citrullination, and carbamylation, and malondialdehyde-acetaldehyde! Oh my! Entering the forest of autoantigen modifications in rheumatoid arthritis. *Arthritis Rheumatol* 2015; **67**: 604–608.
30 Jacobson GA, Ives SJ, Narkowicz C, Jones G. Plasma glutathione peroxidase (GSH-Px) concentration is elevated in rheumatoid arthritis: a case-control study. *Clin Rheumatol* 2012; **31**: 1543–1547.
31 Raes M, Michiels C, Remacle J. Comparative study of the enzymatic defense systems against oxygen-derived free radicals: the key role of glutathione peroxidase. *Free Radic Biol Med* 1987; **3**: 3–7.
32 Hetland ML, Christensen IJ, Lottenburger T, Johansen JS, Svendsen MN, Hørslev-Petersen K *et al.* Circulating VEGF as a biological marker in patients with rheumatoid arthritis? Preanalytical and biological variability in healthy persons and in patients. *Dis Markers* 2008; **24**: 1–10.
33 Chabaud M, Fossiez F, Taupin JL, Miossec P. Enhancing effect of IL-17 on IL-1-induced IL-6 and leukemia inhibitory factor production by rheumatoid arthritis synoviocytes and its regulation by Th2 cytokines. *J Immunol* 1998; **161**: 409–414.

34 Corvaisier M, Delneste Y, Jeanvoine H, Preisser L, Blanchard S, Garo E *et al.* IL-26 is overexpressed in rheumatoid arthritis and induces proinflammatory cytokine production and Th17 cell generation. *PLoS Biol* 2012; **10**: e1001395.
35 Jeong M, Cho J, Shin JI, Jeon YJ, Kim JH, Lee SJ *et al.* Hempseed oil induces reactive oxygen species- and C/EBP homologous protein-mediated apoptosis in MH7A human rheumatoid arthritis fibroblast-like synovial cells. *J Ethnopharmacol* 2014; **154**: 745–752.
36 Li J, Li J, Yue Y, Hu Y, Cheng W, Liu R *et al.* Genistein suppresses tumor necrosis factor alpha-induced inflammation via modulating reactive oxygen species/Akt/nuclear factor kappaB and adenosine monophosphate-activated protein kinase signal pathways in human synoviocyte MH7A cells. *Drug Des Devel Ther* 2014; **8**: 315–323.
37 Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA, Saag KG. Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. *Arthritis Rheum* 2004; **50**: 72–77.
38 Shapiro JA, Koepsell TD, Voigt LF, Dugowson CE, Kestin M, Nelson JL. Diet and rheumatoid arthritis in women: a possible protective effect of fish consumption. *Epidemiology* 1996; **7**: 256–263.
39 Linos A, Kaklamani VG, Kaklamani E, Koumantaki Y, Giziaki E, Papazoglou S *et al.* Dietary factors in relation to rheumatoid arthritis: a role for olive oil and cooked vegetables? *Am J Clin Nutr* 1999; **70**: 1077–1082.
40 Cerhan JR, Saag KG, Merlino LA, Mikuls TR, Criswell LA. Antioxidant micronutrients and risk of rheumatoid arthritis in a cohort of older women. *Am J Epidemiol* 2003; **157**: 345–354.
41 Pattison DJ, Harrison RA, Symmons DP. The role of diet in susceptibility to rheumatoid arthritis: a systematic review. *J Rheumatol* 2004; **31**: 1310–1319.
42 Viuda-Martos M, Fern´andez-L´opez J, P´erez-´Alvarez JA. Pomegranate and its many functional components as related to human health: a review. *Compr Rev Food Sci Food Saf* 2010; **9**: 635–654.
43 Moskaug JO, Carlsen H, Myhrstad MC, Blomhoff R. Polyphenols and glutathione synthesis regulation. *Am J Clin Nutr* 2005; **81**: 277S–283S.
44 Myhrstad MC, Carlsen H, Nordström O, Blomhoff R, Moskaug JØ. Flavonoids increase the intracellular glutathione level by transactivation of the gamma-glutamylcysteine synthetase catalytical subunit promoter. *Free Radic Biol Med* 2002; **32**: 386–393.
45 Cornelio Favarin D, Martins Teixeira M, Lemos de Andrade E, de Freitas Alves C, Lazo Chica JE, Artério Sorgi C *et al.* Anti-inflammatory effects of ellagic acid on acute lung injury induced by acid in mice. *Mediat Inflamm*; e-pub ahead of print 27 February 2013; doi:10.1155/2013/164202.
46 Galano A, Francisco Marquez M, Perez-Gonzalez A. Ellagic acid: an unusually versatile protector against oxidative stress. *Chem Res Toxicol* 2014; **27**: 904–918.
47 Fischer UA, Carle R, Kammerer DR. Identification and quantification of phenolic compounds from pomegranate (Punica granatum L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD-ESI/MS(n). *Food Chem* 2011; **127**: 807–821.
48 Scher JU, Abramson SB. The microbiome and rheumatoid arthritis. *Nat Rev Rheumatol* 2011; **7**: 569–578.
49 Yeoh N, Burton JP, Suppiah P, Reid G, Stebbings S. The role of the microbiome in rheumatic diseases. *Curr Rheumatol Rep* 2013; **15**: 314.
50 Li Z, Henning SM, Lee RP, Lu QY, Summanen PH, Thames G *et al.* Pomegranate extract induces ellagitannin metabolite formation and changes stool microbiota in healthy volunteers. *Food Funct* 2015; **6**: 2487–2495.
51 Neyrinck AM, Van Hée VF, Bindels LB, De Backer F, Cani PD, Delzenne NM. Polyphenol-rich extract of pomegranate peel alleviates tissue inflammation and hypercholesterolaemia in high-fat diet-induced obese mice: potential implication of the gut microbiota. *Br J Nutr* 2013; **109**: 802–809.
52 Delzenne NM, Cani PD. Interaction between obesity and the gut microbiota: relevance in nutrition. *Annu Rev Nutr* 2011; **31**: 15–31.