The effect of resveratrol supplementation on serum levels of asymmetric de-methyl-arginine and paraoxonase 1 activity in patients with type 2 diabetes: A randomized, double-blind controlled trial

Tabatabaie, M., Abdollahi, S., Salehi-Abargouei, A., Clark, C. C. T., Karimi-Nazari, E., Fallahzadeh, H., Rahmanian, M. & Mozaffari-Khosravi, H.

Original citation & hyperlink:
Tabatabaie, M, Abdollahi, S, Salehi-Abargouei, A, Clark, CCT, Karimi-Nazari, E, Fallahzadeh, H, Rahmanian, M & Mozaffari-Khosravi, H 2020, 'The effect of resveratrol supplementation on serum levels of asymmetric de-methyl-arginine and paraoxonase 1 activity in patients with type 2 diabetes: A randomized, double-blind controlled trial', Phytotherapy Research, vol. 34, no. 8, pp. 2023-2031.
https://dx.doi.org/10.1002/ptr.6655

DOI 10.1002/ptr.6655
ISSN 0951-418X
ESSN 1099-1573

Publisher: Wiley

The Effect of Resveratrol Supplementation on Serum Levels of Asymmetric De-Methyl-Arginine and Paraoxonase 1 Activity in Patients with Type 2 Diabetes: A Randomized, Double-Blind Controlled Trial

Mahtab Tabatabaie[1,2†], Shima Abdollahi[3†], Amin Salehi-Abargouei[1,2], Cain C. T. Clark[4], Elham Karimi-Nazari[2], Hossein Fallahzadeh[5], Mohammad Hasan Sheikhha[6,7], Masoud Rahmanian[8], Hassan Mozaffari-Khosravi[1,8]

[1]Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

[2]Nutrition and Food Security Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

[3]Department of Nutrition and Public Health, School of Public Health, North Khorasan University, Bojnurd, Iran.

[4] Centre for Sport, Exercise, and Life Sciences, Coventry University, Coventry, CV1 5FB, U.K.

[5]Department of Biostatistics and Epidemiology, Research Center of Prevention and Epidemiology of Non-Communicable Disease, School of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

[6]Department of Genetics, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

[7]Yazd Clinical and Research Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

[8]Yazd Diabetic Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Address all correspondence and requests for reprints to: Hassan Mozaffari-khosravi, Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran, and Yazd Diabetic Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

E-mail: mozaffari.kh@gmail.com

Tel: (9835)38209143

[†]Mahtab Tabatabaie and Shima Abdollahi contributed equally to this work.

ABSTRACT

Objective: The present study sought to investigate the effect of micronized resveratrol supplementation on serum levels of asymmetric de-methyl-arginine (ADMA) and paraoxonase-1 (PON1) activity in patients with type 2 diabetes (T2D).

Methods: In this double-blinded randomized trial, 76 patients with T2D were recruited. Participants were randomly assigned to consume 1000 mg resveratrol or placebo capsules (methylcellulose) per day, for 8 weeks. Serum levels of ADMA and PON1 enzyme activity were measured at the beginning and end of the intervention using the ELISA method. In total, 71 participants completed the study.

Results: Our results showed that resveratrol significantly decreased serum levels of ADMA (-0.16±0.11, P<0.001) and improved PON1 enzyme activity (15.39±13.99, P<0.001) compared with placebo, after adjusting for confounding factors (age, sex and baseline body mass index).

Conclusion: Our findings suggest that 8-week resveratrol supplementation may produce beneficial effects on serum levels of ADMA and PON1 enzyme activity in patients with T2DM. However, further research is needed to confirm the veracity of these results.

Keywords: *Resveratrol, ADMA, PON1 protein, Type 2 diabetes mellitus*

INTRODUCTION

Type 2 diabetes (T2D), the most prevalent endocrine disease, represents one of the most important health issues affecting people globally (1, 2). Empirical evidence indicates that cardiovascular disease is a major cause of mortality and morbidity in patients with diabetes (3). Obesity, dysglycemia, dyslipidemia, and hypertension represent the most important risk factors for cardiovascular diseases, which are especially common in diabetic patients (4). The vascular endothelium plays a pivotal role in maintaining the vascular tone and mediates production (5). One of the mediators is nitric oxide (NO), which is produced in response to stress, and has an important function in vasodilatation and increases circulation (6). Asymmetric dimethylarginine (ADMA) is a competitive endogenous inhibitor for nitric oxide synthase (NOS) and inhibits the production of NO in pathological concentrations (7). Increased serum levels of ADMA have been reported in patients with diabetes, renal failure, hypercholesterolemia, cardiovascular diseases, and hypertension (8).

Chronic hyperglycemia in T2D induces oxidative stress in various pathways, such as; glucose auto-oxidation, glycosylation of operational proteins, activation of the polyol pathway, endothelial NOS (eNOS) uncoupling and oxidative phosphorylation (9-12). Paraoxonases (aryl dialkyl phosphatase) as antioxidant factors, also initially identified as hydrolyzing enzymes of organophosphorus compounds such as, paraoxon, or diazoxone insecticides (13, 14). Paraoxonase-1 (PON1) is an esterase which is produced in the liver and is transported with circulating high-density lipoprotein (HDL) (15, 16). It seems that PON1 is partly responsible for the antioxidant property of HDL (17). Some studies have shown that the PON1 activity is independent of the amount of Apo-lipoprotein HDL (18); PON1 also inhibits LDL peroxidation and oxidized LDL synthesis (19), and hydrolyzes homo-cysteine, which is an important risk factor for cardiovascular

disease (20). PON1 activity is important in the prevention of atherosclerosis progression by inhibition of MCP-1 production (Monocyte Chemoattractant Peptide 1), which is stimulated by oxidized LDL in the endothelial cells (21). Some previous studies have reported that PON1 enzyme activity may be decreased in diabetic patients (22-24), whilst high serum levels of glucose can lead to PON1 separation from HDL (25). Furthermore, it seems that serum levels of ADMA and PON1 activity are affected by antioxidants (26).

Resveratrol is a polyphenol found mostly in grapes and nuts and has been shown to elicit beneficial effects on diabetes and cardiovascular diseases (27, 28). The cardiovascular protective effects of resveratrol have been widely investigated; however, the exact mechanisms are far from consensual. The results of some meta-analytical studies have shown that resveratrol supplementation can elicit improvements in endothelial function (29), and reductions in inflammatory markers (30-32); however, a previous meta-analysis concluded that resveratrol supplementation has no significant effects on cardiovascular risk factors (33). In the present study, we investigated the effects of resveratrol supplementation on serum levels of ADMA and PON1 activity in patients with type 2 diabetes.

MATERIALS AND METHODS

Study Design and Participants

Patients with T2D were selected from a diabetes center (Yazd, Iran), and the diagnosis of diabetes was confirmed by an endocrinologist (34). The protocol of the present double-blind randomized controlled trial was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences in Yazd (IR.SSU.SPH.REC.1397.073) and registered in the

Iranian Registry of Clinical Trials (www.irct.ir) asIRCT20171118037528N1). Informed consent was provided by all participants prior to study commencement.

Inclusion and Exclusion Criteria

Detailed information about the study design has been previously described in detail (35). Briefly, men and women with T2D aged 30-60 years old, body mass index (BMI) of 25-30 kg/m^2, and glycated hemoglobin (HbA1c) lower than 8% were enrolled in the study. Exclusion criteria included; diagnosed kidney or liver disease, cancer, Alzheimer's, gastrointestinal ulcer, inflammatory and autoimmune diseases, and/or history of myocardial infarction, treatment with any supplement containing antioxidants, insulin, fibrates, warfarin, aspirin or any drugs that inhibit platelet aggregation in the 6 months preceding the study. Patients who consumed alcoholic beverages habitually, and pregnant or lactating women were also excluded.

Setting

A stratified randomized method, using a computer random generated number based on sex and age (30-45, 45-60 years old), was used to assign participants into the intervention or control group, respectively. Patients in the intervention group received two capsules per day, which provided 1000 mg/day purified resveratrol (Mega-Resveratrol, Danbury, USA) for 8 weeks. Two capsules containing methyl cellulose (Barij essence, Kashan, Iran) were taken by patients in the control group for the same duration. The placebo was similar in appearance and taste with the resveratrol supplement. Patients were not deprived of their usual treatment for diabetes.

A person outside the research team performed the packing and labeling (A or B) of the bottles containing resveratrol and placebo. The researchers and participants were not aware of the contents until the end of the intervention. Patients were asked to report any suspected adverse events. The compliance rate of the participants was evaluated using the remaining capsule counts at the end of

the study, and participants were asked to maintain their habitual diet and physical activity throughout the study.

Nutritional and Physical Activity Assessment

To assess nutrient intake, two, 3-day dietary food records (one weekend day and two weekdays) were completed by the participants in the first and last week of the intervention. Data were analyzed using Nutritionist IV software (The Hearst Corporation, San Bruno, California, USA).

To assess the physical activity level, metabolic equivalent (MET) was calculated using a validated questionnaire at the beginning and end of the study (36). In this questionnaire, information on physical activity is classified based on the intensity of each activity in nine different categories (ranging from inactivity to severe sports activities). The duration of each activity was multiplied by the coefficient for each activity, and the values obtained in the nine different classes were summed in order to provide MET/h per day.

Anthropometric and Biochemical Measurements

Anthropometric measures, including height, body weight, waist and hip circumferences, BMI, fat and, fat-free masses, were assessed before and after the intervention using a segmental body composition analyzer (Tanita BC-418, Tokyo, Japan). The results of the anthropometric measures, as well as cardio-metabolic biochemical factors (glycemic indices and lipid profile), have been reported elsewhere (37).

Blood samples for biochemical parameters were collected at the beginning and end of the study after 12h nocturnal fasting. Blood samples were centrifuged for 10 minutes at room temperature (3000 g; Eppendorf AG, Hamburg), and then the serum samples were frozen at -70 ° C until analyses. Serum levels of ADMA were measured applying enzyme-linked immunosorbent assay (ELISA) method using a commercially available kit (Zellbio, Germany) with inter-and intra-

assay<12% and <10%, respectively. The PON1 activity also determined by the ELISA method using a commercially available kit (Zellbio, Germany, inter-and intra-assay: CVs were 4.8% and 4.1%, respectively).

Sample Size and Statistical Analysis

This report is part of a previous study that calculated the sample size based on the *PPARα* gene expression in peripheral blood mononuclear cells (35). Although, a retrospective power analysis was performed to assess the quantity of the sample size for our interested outcomes. The results showed adequate power for ADMA levels (observed power= 1.0).

SPSS software for windows version 23.0 (SPSS, Chicago, IL, USA) was used for all data entry and statistical analyses. The values were expressed as mean ± standard deviation for continuous and proportions for categorical data. The Kolmogorov-Smirnov test was used to evaluate the distribution of variables. To compare the quantitative values between the two groups, an independent samples t-test and within groups paired t-test were used, respectively. Analysis of covariance (ANCOVA) was used to modify possible confounding factors including age, gender, and baseline BMI. Statistical significance was accepted, *a* priori, at P <0.05.

RESULTS

Of the 76 participants enrolled in the study, five patients did not complete the intervention due to pregnancy (n=1), traveling (n=1) and withdrawal of consent (n=3). Finally, data from 71 participants (35 patients in resveratrol and 36 patients in placebo groups) were included in the analysis (**Figure 1**). More than 90% compliance (92.6% in placebo and 93.1% in resveratrol) was detected through capsule counting, and no adverse side effects were reported.

Table 1 details the general characteristics of the participants before the intervention, and there were no significant differences in baseline variables between the two groups. The mean age of

participants in resveratrol and placebo groups was 50.14 ± 7.38 and 50.06 ± 7.69 years, respectively. No significant between-group differences for dietary intake and physical activity were observed at the baseline and they also did not change following the 8-week intervention (**Table 2**).

Resveratrol significantly reduced ADMA levels compared with baseline and the placebo group (-0.16±0.11 (ng/ml); all P-values<0.001). PON1 activity was also significantly increased after supplementation in the resveratrol group (15.39±13.99 (U/L); P<0.001) and compared with the placebo group (P=0.04). These findings remained significant after adjusting for confounding variables (all P-values<0.001) (**Table 3**).

DISCUSSION

The results of the current study showed a significant reduction in serum levels of ADMA, and significant increase in PON1 activity, following 8-week resveratrol supplementation. In line with our findings, previous studies have reported a significant increase in PON1 activity after resveratrol (38), pomegranate juice (39), eicosapentaenoic acid (40), barberry juice (41), and vitamin E supplementation (42) in patients with type 2 diabetes. Furthermore, one study reported a higher intake of fruit and vegetable leads to an increase in PON1 activity (43). The findings of some in-vitro studies have also showed that resveratrol increases PON1 gene expression and activity in different human cells (44-46).

PON1 is a HDL-associated enzyme that hydrolyzes oxidized LDL-cholesterol, and is known for its atheroprotective capabilities (47). Furthermore, this enzyme plays a critical role in the protection against oxidative stress-related diseases (48, 49); including cardiovascular diseases, the

major cause of mortality among patients with diabetes (50). Moreover, the activity and concentration of PON1 are reported to decrease in these patients (23).

Resveratrol is an antioxidant that appears to affect PON1 activity through several pathways. Resveratrol can result in an increase in carnitine palmitoyl transferase-1, decrease in acetyl-CoA carboxylase and fatty acid synthase genes expression, and, consequently, an elevation in HDL levels (38). The results of the present study also support the beneficial effect of resveratrol on HDL levels (37). Furthermore, it seems that resveratrol might regulate gene expression by binding to the estrogen response element-2 sequences (51). There are similar sequences in the promoter region of PON1 gene, suggesting that PON1 gene expression upregulation induced by resveratrol may be related to the presented sequences (52). Moreover, resveratrol is known as a ligand for aryl-hydro carbon receptors (AhR) and can increase PON1 gene expression and activity through AhR-dependent mechanisms (53).

In the present study, we also observed a significant reduction in serum levels of ADMA following resveratrol supplementation. Previous reports have identified that increased ADMA levels are associated with oxidative stress related diseases, such as diabetes (54-56). ADMA is produced via protein arginine methyl transferase and breaks down to citrulline and dimethyl amine by dimethyl arginine dimethyl amino hydrolase (DDAH). Oxidative stress reduces the gene expression and activity of DDAH resulting in endothelial dysfunction (57, 58), whilst there is substantive evidence that increased ADMA levels contribute to injuries induced by oxidative stress (59). There are numerous reports in the literature asserting that resveratrol can stimulate eNO synthesis and inhibit its degradation in several mechanisms (60-62). However, one study suggested that the levels of eNOS did not significantly change following resveratrol supplementation (63); conceivably due to the small sample size (n=48). Resveratrol activates sirtuin-1 (SIRT1) through AMP-activated

protein kinase (AMPK) pathway (64), and SIRT-1 increases eNOS gene expression by deacetylating Forkhead box O (FOXO) transcription factors (65). It has been shown that elevated NO levels can upregulate DDAH by cyclic GMP induction and subsequently decreased ADMA levels (66). There is also some evidence that resveratrol can independently upregulate DDAH gene expression (67); however, the molecular mechanisms are not well identified. Moreover, DDAH upregulation causes decreases ADMA levels and increases NO production and bioavailability (58, 68).

A number of in-vitro studies in endothelial cells have reported significant decreases in ADMA levels after red wine consumption as a source of resveratrol (58). Some RCTs have also shown that ADMA levels are reduced after coenzyme Q10 (69), alpha-lipoic acid (70, 71), eicosapentaenoic acid (72), and DHA-enriched fish oil consumption (73) in patients with type 2 diabetes, respectively, and also vitamin E supplementation in chronic kidney disease patients (74). Moreover, one animal study indicated DDAH activity increased after intervention with *trans*-3, 5, 4'-trihydroxystilbene as an analog of resveratrol on gastric mucosal injury (67). However, the results of some studies are inconsistent with our results. For instance, one study indicated that vitamin C and E did not affect ADMA levels in children with hyperlipidemia (75), whilst another study reported no significant differences in PON1 activity when omega-3 was administered (76). However, the small number of participants may justify the aforementioned findings.

To the authors' knowledge, this is the first clinical trial study to investigate the effect of resveratrol supplementation on serum levels of ADMA. Although there is one RCT investigating the effect of resveratrol on PON1 activity in patients with diabetes (38), we utilized micronized resveratrol to increase bioavailability, and included patients with overweight exclusively, to adjust oxidative stress induced by obesity. Stratification by gender and age also permitted us to control confounders

related to these factors. Despite the novelty of the present study, there are some limitations that must be considered. The present study was designed for short-term assessment of resveratrol supplementation effects; thus, we have no information as to the longer-term effects, or dose-response relationship beyond this time. Finally, we did not investigate the cellular pathways related to the beneficial effects of resveratrol on our interested outcomes; which clearly represents an avenue for future research.

Conclusion

The findings of the present study demonstrated that 8-week resveratrol supplementation can significantly improve ADMA levels and enhance PON1 activity in patients with diabetes. These findings may support the beneficial, atheroprotective, effects of resveratrol; although, more research is needed to confirm the veracity of our findings.

Acknowledgments

This study was supported by a grant from the Shahid Sadoughi University of Medical Sciences (grant number: 5403). We thank the staff of Yazd Diabetes research for helping in recruiting the patients.

Conflict of interest

There is no conflict of interest in the present study.

REFERENCES

1. Freeman JS. The increasing epidemiology of diabetes and review of current treatment algorithms. The Journal of the American Osteopathic Association. 2010;110(7 Suppl 7):eS2-6.
2. Adeghate E, Schattner P, Dunn E. An update on the etiology and epidemiology of diabetes mellitus. Annals of the New York Academy of Sciences. 2006;1084:1-29.
3. Matheus AS, Tannus LR, Cobas RA, Palma CC, Negrato CA, Gomes MB. Impact of diabetes on cardiovascular disease: an update. International journal of hypertension. 2013;2013:653789.
4. Leon BM, Maddox TM. Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. World journal of diabetes. 2015;6(13):1246-58.
5. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980;288(5789):373-6.
6. Cooke JP, Rossitch E, Jr., Andon NA, Loscalzo J, Dzau VJ. Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator. J Clin Invest. 1991;88(5):1663-71.
7. Boger RH. Asymmetric dimethylarginine (ADMA) and cardiovascular disease: insights from prospective clinical trials. Vascular medicine (London, England). 2005;10 Suppl 1:S19-25.
8. Boger RH. The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor. Cardiovascular research. 2003;59(4):824-33.
9. Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, et al. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation. 2002;105(14):1656-62.
10. Abbasi F, Asagmi T, Cooke JP, Lamendola C, McLaughlin T, Reaven GM, et al. Plasma concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus. The American journal of cardiology. 2001;88(10):1201-3.
11. Cooke JP. Asymmetrical dimethylarginine: the Uber marker? Circulation. 2004;109(15):1813-8.
12. Scalera F, Kielstein JT, Martens-Lobenhoffer J, Postel SC, Tager M, Bode-Boger SM. Erythropoietin increases asymmetric dimethylarginine in endothelial cells: role of dimethylarginine dimethylaminohydrolase. Journal of the American Society of Nephrology : JASN. 2005;16(4):892-8.
13. Costa LG, Cole TB, Vitalone A, Furlong CE. Measurement of paraoxonase (PON1) status as a potential biomarker of susceptibility to organophosphate toxicity. Clinica chimica acta; international journal of clinical chemistry. 2005;352(1-2):37-47.
14. Cole TB, Walter BJ, Shih DM, Tward AD, Lusis AJ, Timchalk C, et al. Toxicity of chlorpyrifos and chlorpyrifos oxon in a transgenic mouse model of the human paraoxonase (PON1) Q192R polymorphism. Pharmacogenetics and genomics. 2005;15(8):589-98.
15. Gaidukov L, Tawfik DS. High affinity, stability, and lactonase activity of serum paraoxonase PON1 anchored on HDL with ApoA-I. Biochemistry. 2005;44(35):11843-54.
16. Gaidukov L, Viji RI, Yacobson S, Rosenblat M, Aviram M, Tawfik DS. ApoE induces serum paraoxonase PON1 activity and stability similar to ApoA-I. Biochemistry. 2010;49(3):532-8.
17. Solati M, Etemadi A, Pezeshk P, Rahbar KH, Azizi F. Lipids, apolipoproteins, lipid oxidation and paraoxonase enzyme activity in diabetic and non-diabetic end stage renal disease patients. IJEM. 2003;5(1):27-32.
18. C Sorenson R, L Primo-Parmo S, L Kuo C, Adkins S, Lockridge O, N La Du B. Reconsideration of the catalytic center and mechanism of mammalian paraoxonase/arylesterase1995. 7187-91 p.
19. Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest. 1998;101(8):1581-90.

20. Kerkeni M, Addad F, Chauffert M, Chuniaud L, Miled A, Trivin F, et al. Hyperhomocysteinemia, paraoxonase activity and risk of coronary artery disease. Clinical biochemistry. 2006;39(8):821-5.
21. Mackness B, Hine D, Liu Y, Mastorikou M, Mackness M. Paraoxonase-1 inhibits oxidised LDL-induced MCP-1 production by endothelial cells. Biochemical and biophysical research communications. 2004;318(3):680-3.
22. Nowak M, Wielkoszynski T, Marek B, Kos-Kudla B, Swietochowska E, Sieminska L, et al. Antioxidant potential, paraoxonase 1, ceruloplasmin activity and C-reactive protein concentration in diabetic retinopathy. Clinical and experimental medicine. 2010;10(3):185-92.
23. Mackness B, Durrington PN, Abuashia B, Boulton AJ, Mackness MI. Low paraoxonase activity in type II diabetes mellitus complicated by retinopathy. Clinical science (London, England : 1979). 2000;98(3):355-63.
24. Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M, et al. Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. Atherosclerosis. 1991;86(2-3):193-9.
25. Gil MI, Tomas-Barberan FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. Journal of agricultural and food chemistry. 2000;48(10):4581-9.
26. Mackness B, Mackness MI, Arrol S, Turkie W, Julier K, Abuasha B, et al. Serum paraoxonase (PON1) 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin dependent diabetes mellitus. Atherosclerosis. 1998;139(2):341-9.
27. Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the in vivo evidence. Nature reviews Drug discovery. 2006;5(6):493-506.
28. Opie LH, Lecour S. The red wine hypothesis: from concepts to protective signalling molecules. European heart journal. 2007;28(14):1683-93.
29. Akbari M, Tamtaji OR, Lankarani KB, Tabrizi R, Dadgostar E, Kolahdooz F, et al. The Effects of Resveratrol Supplementation on Endothelial Function and Blood Pressures Among Patients with Metabolic Syndrome and Related Disorders: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. High blood pressure & cardiovascular prevention : the official journal of the Italian Society of Hypertension. 2019;26(4):305-19.
30. Tabrizi R, Tamtaji OR, Lankarani KB, Mirhosseini N, Akbari M, Dadgostar E, et al. The effects of resveratrol supplementation on biomarkers of inflammation and oxidative stress among patients with metabolic syndrome and related disorders: a systematic review and meta-analysis of randomized controlled trials. Food & function. 2018;9(12):6116-28.
31. Koushki M, Dashatan NA, Meshkani R. Effect of Resveratrol Supplementation on Inflammatory Markers: A Systematic Review and Meta-analysis of Randomized Controlled Trials. Clinical therapeutics. 2018;40(7):1180-92.e5.
32. Haghighatdoost F, Hariri M. Can resveratrol supplement change inflammatory mediators? A systematic review and meta-analysis on randomized clinical trials. European journal of clinical nutrition. 2019;73(3):345-55.
33. Sahebkar A, Serban C, Ursoniu S, Wong ND, Muntner P, Graham IM, et al. Lack of efficacy of resveratrol on C-reactive protein and selected cardiovascular risk factors--Results from a systematic review and meta-analysis of randomized controlled trials. International journal of cardiology. 2015;189:47-55.
34. Standards of Medical Care in Diabetes—2012. Diabetes care. 2012;35(Supplement 1):S11-S63.
35. Abdollahi S, Salehi-Abargouei A, Tabatabaie M, Sheikhha MH, Fallahzadeh H, Rahmanian M, et al. The effect of resveratrol supplementation on the expression levels of factors associated with cellular senescence and sCD163/sTWEAK ratio in patients with type 2 diabetes mellitus: study protocol for a double-blind controlled randomised clinical trial. BMJ open. 2019;9(7):e026337.

36. Aadahl M, Jorgensen T. Validation of a new self-report instrument for measuring physical activity. Medicine and science in sports and exercise. 2003;35(7):1196-202.
37. Abdollahi S, Salehi-Abargouei A, Toupchian O, Sheikhha MH, Fallahzadeh H, Rahmanian M, et al. The Effect of Resveratrol Supplementation on Cardio-Metabolic Risk Factors in Patients with Type 2 Diabetes: A Randomized, Double-Blind Controlled Trial. Phytotherapy research : PTR. 2019.
38. Gharib M, Ghatreh Samani K, ZarrinÅbadi Z, Mokhtari M, Heydarian E. Effect of Resveratrol Supplementation on Antioxidant Parameters, Lipids Profile and Several Biochemical Indices in Type 2 Diabetic Patients: A Double-Blind Randomized-Controlled Clinical Trial. Iranian Journal of Nutrition Sciences & Food Technology. 2018;12(4):33-42.
39. Parsaeyan N, Mozaffari-Khosravi H, Mozayan MR. Effect of pomegranate juice on paraoxonase enzyme activity in patients with type 2 diabetes. J Diabetes Metab Disord. 2012;11(1):11-.
40. Golzari MH, Hosseini S, Koohdani F, Saboor Yaraghi AA, Javanbakht MH, Mohammadzadeh-Honarvar N, et al. The Effect of Eicosapentaenoic Acid on the Serum Levels and Enzymatic Activity of Paraoxonase 1 in the Patients With Type 2 Diabetes Mellitus. Acta medica Iranica. 2017;55(8):486-95.
41. Lazavi F, Mirmiran P, Sohrab G, Nikpayam O, Angoorani P, Hedayati M. The barberry juice effects on metabolic factors and oxidative stress in patients with type 2 diabetes: A randomized clinical trial. Complementary therapies in clinical practice. 2018;31:170-4.
42. Rafraf M, Bazyun B, Sarabchian MA, Safaeiyan A, Gargari BP. Vitamin E Improves Serum Paraoxonase-1 Activity and Some Metabolic Factors in Patients with Type 2 Diabetes: No Effects on Nitrite/Nitrate Levels. Journal of the American College of Nutrition. 2016;35(6):521-8.
43. Daniels JA, Mulligan C, McCance D, Woodside JV, Patterson C, Young IS, et al. A randomised controlled trial of increasing fruit and vegetable intake and how this influences the carotenoid concentration and activities of PON-1 and LCAT in HDL from subjects with type 2 diabetes. Cardiovascular diabetology. 2014;13:16.
44. Gouedard C, Barouki R, Morel Y. Induction of the paraoxonase-1 gene expression by resveratrol. Arteriosclerosis, thrombosis, and vascular biology. 2004;24(12):2378-83.
45. Gupta N, Kandimalla R, Priyanka K, Singh G, Gill KD, Singh S. Effect of Resveratrol and Nicotine on PON1 Gene Expression: In Vitro Study. Indian J Clin Biochem. 2014;29(1):69-73.
46. Curtin BF, Seetharam KI, Dhoieam P, Gordon RK, Doctor BP, Nambiar MP. Resveratrol induces catalytic bioscavenger paraoxonase 1 expression and protects against chemical warfare nerve agent toxicity in human cell lines. Journal of Cellular Biochemistry. 2008;103(5):1524-35.
47. Getz GS, Reardon CA. Paraoxonase, a cardioprotective enzyme: continuing issues. Curr Opin Lipidol. 2004;15(3):261-7.
48. Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology. 2001;21(4):473-80.
49. Precourt LP, Amre D, Denis MC, Lavoie JC, Delvin E, Seidman E, et al. The three-gene paraoxonase family: physiologic roles, actions and regulation. Atherosclerosis. 2011;214(1):20-36.
50. Mackness B, Hine D, McElduff P, Mackness M. High C-reactive protein and low paraoxonase1 in diabetes as risk factors for coronary heart disease. Atherosclerosis. 2006;186(2):396-401.
51. Bowers JL, Tyulmenkov VV, Jernigan SC, Klinge CM. Resveratrol acts as a mixed agonist/antagonist for estrogen receptors alpha and beta. Endocrinology. 2000;141(10):3657-67.
52. Gupta N, Kandimalla R, Priyanka K, Singh G, Gill KD, Singh S. Effect of Resveratrol and Nicotine on PON1 Gene Expression: In Vitro Study. Indian Journal of Clinical Biochemistry. 2014;29(1):69-73.
53. Gouedard C, Barouki R, Morel Y. Dietary polyphenols increase paraoxonase 1 gene expression by an aryl hydrocarbon receptor-dependent mechanism. Molecular and cellular biology. 2004;24(12):5209-22.

54. Nakhjavani M, Karimi-Jafari H, Esteghamati A, Khalilzadeh O, Asgarani F, Ghadiri-Anari A. ADMA is a correlate of insulin resistance in early-stage diabetes independent of hs-CRP and body adiposity. Ann Endocrinol (Paris). 2010;71(4):303-8.
55. Borgeraas H, Strand E, Ringdal Pedersen E, Dierkes J, Ueland PM, Seifert R, et al. Omega-3 Status and the Relationship between Plasma Asymmetric Dimethylarginine and Risk of Myocardial Infarction in Patients with Suspected Coronary Artery Disease. Cardiol Res Pract. 2012;2012:201742-.
56. Sydow K, Münzel T. ADMA and oxidative stress. Atherosclerosis Supplements. 2003;4(4):41-51.
57. Pope AJ, Karuppiah K, Cardounel AJ. Role of the PRMT-DDAH-ADMA axis in the regulation of endothelial nitric oxide production. Pharmacological research. 2009;60(6):461-5.
58. Scalera F, Fulge B, Martens-Lobenhoffer J, Heimburg A, Bode-Böger SM. Red wine decreases asymmetric dimethylarginine via SIRT1 induction in human endothelial cells. Biochem Biophys Res Commun. 2009;390(3):703-9.
59. Zoccali C, Maas R, Cutrupi S, Pizzini P, Finocchiaro P, Cambareri F, et al. Asymmetric dimethyl-arginine (ADMA) response to inflammation in acute infections. Nephrology Dialysis Transplantation. 2006;22(3):801-6.
60. Xia N, Förstermann U, Li H. Resveratrol and endothelial nitric oxide. Molecules (Basel, Switzerland). 2014;19(10):16102-21.
61. Li H, Xia N, Hasselwander S, Daiber A. Resveratrol and Vascular Function. Int J Mol Sci. 2019;20(9):2155.
62. Liu Z, Song Y, Zhang X, Liu Z, Zhang W, Mao W, et al. Effects of trans-resveratrol on hypertension-induced cardiac hypertrophy using the partially nephrectomized rat model. Clinical and experimental pharmacology & physiology. 2005;32(12):1049-54.
63. Seyyedebrahimi S, Khodabandehloo H, Nasli Esfahani E, Meshkani R. The effects of resveratrol on markers of oxidative stress in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled clinical trial. Acta diabetologica. 2018;55(4):341-53.
64. Shakibaei M, Buhrmann C, Mobasheri A. Resveratrol-mediated SIRT-1 interactions with p300 modulate receptor activator of NF-κB ligand (RANKL) activation of NF-κB signaling and inhibit osteoclastogenesis in bone-derived cells. Journal of Biological Chemistry. 2011;286(13):11492-505.
65. Xia N, Strand S, Schlufter F, Siuda D, Reifenberg G, Kleinert H, et al. Role of SIRT1 and FOXO factors in eNOS transcriptional activation by resveratrol. Nitric Oxide. 2013;32:29-35.
66. Sakurada M, Shichiri M, Imamura M, Azuma H, Hirata Y. Nitric oxide upregulates dimethylarginine dimethylaminohydrolase-2 via cyclic GMP induction in endothelial cells. Hypertension (Dallas, Tex : 1979). 2008;52(5):903-9.
67. Li L, Luo X-J, Liu Y-Z, Zhang Y-S, Yuan Q, Tan N, et al. The role of the DDAH–ADMA pathway in the protective effect of resveratrol analog BTM-0512 on gastric mucosal injury. Canadian journal of physiology and pharmacology. 2010;88(5):562-7.
68. Xia N, Forstermann U, Li H. Resveratrol and endothelial nitric oxide. Molecules (Basel, Switzerland). 2014;19(10):16102-21.
69. Hosseinzadeh Attar MJ, Kolahdouz Mohammadi R, Eshraghian MR, Nakhjavani M, Khorrami E, Ebadi M, et al. Reduction in Asymmetric dimethylarginine plasma levels by Coenzyme Q10 supplementation in patients with type 2 diabetes mellitus. Minerva endocrinologica. 2015;40.
70. Mittermayer F, Pleiner J, Francesconi M, Wolzt M. Treatment with alpha-lipoic acid reduces asymmetric dimethylarginine in patients with type 2 diabetes mellitus. Translational research : the journal of laboratory and clinical medicine. 2010;155(1):6-9.
71. Chang JW, Lee EK, Kim TH, Min WK, Chun S, Lee KU, et al. Effects of alpha-lipoic acid on the plasma levels of asymmetric dimethylarginine in diabetic end-stage renal disease patients on hemodialysis: a pilot study. American journal of nephrology. 2007;27(1):70-4.

72. Hagiwara H, Nishiyama Y, Katayama Y. Effects of eicosapentaenoic acid on asymmetric dimethylarginine in patients in the chronic phase of cerebral infarction: a preliminary study. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2011;20(5):474-8.
73. Toupchian O, Sotoudeh G, Mansoori A, Nasli-Esfahani E, Djalali M, Keshavarz SA, et al. Effects of DHA-enriched fish oil on monocyte/macrophage activation marker sCD163, asymmetric dimethyl arginine, and insulin resistance in type 2 diabetic patients. Journal of clinical lipidology. 2016;10(4):798-807.
74. Saran R, Novak JE, Desai A, Abdulhayoglu E, Warren JS, Bustami R, et al. Impact of vitamin E on plasma asymmetric dimethylarginine (ADMA) in chronic kidney disease (CKD): a pilot study. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2003;18(11):2415-20.
75. Engler MM, Engler MB, Malloy MJ, Chiu EY, Schloetter MC, Paul SM, et al. Antioxidant vitamins C and E improve endothelial function in children with hyperlipidemia: Endothelial Assessment of Risk from Lipids in Youth (EARLY) Trial. Circulation. 2003;108(9):1059-63.
76. Stirban A, Nandrean S, Gotting C, Stratmann B, Tschoepe D. Effects of n-3 polyunsaturated fatty acids (PUFAs) on circulating adiponectin and leptin in subjects with type 2 diabetes mellitus. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2014;46(7):490-2.

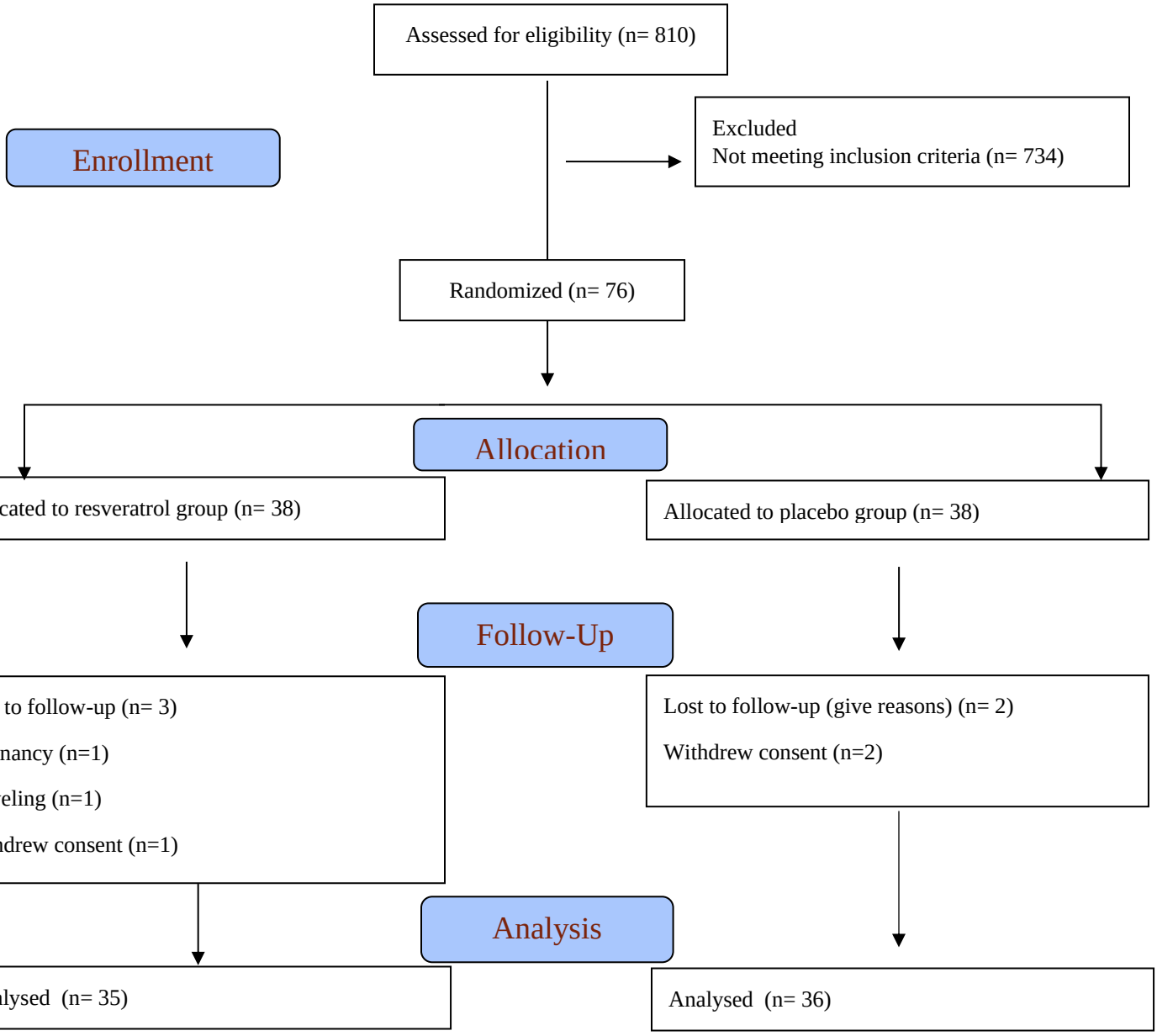

Figure 1. CONSORT diagram outlining the number of subjects involved in enrollment, intervention allocation, follow-up, and data analysis.

Table 1. Baseline characteristics of the study participants[1]

Variable	Resveratrol (n=35)	Placebo (n= 36)	P-value[2]
Age (years)	50.14 ± 7.38	50.06± 7.69	0.96
Diabetes duration (years)	9.40 ± 7.07	8.11 ± 6.90	0.44
Gender (female), n (%)	15 (42.9)	16 (44.4)	0.89
Menopause status, n (%)	4 (26.6)	3 (18.8)	0.68
Smoker, n (%)	5 (14.3)	2 (5.6)	0.21
HbA1C (%)	7.33± 0.65	7.33± 0.65	0.92
Complications			
Hypertension, n (%)	11 (31.4)	7 (19.4)	0.24
Kidney stone, n (%)	2 (5.7)	3 (8.3)	0.66
Non-alcoholic fatty liver, n (%)	3 (8.6)	2 (5.6)	0.62
Neuropathy, n (%)	2 (5.7)	2 (5.6)	0.97
Retinopathy, n (%)	5 (14.3)	5 (13.9)	0.96
Family T2DM History, n (%)	25 (71.4)	30 (83.3)	0.23
Medications			
Metformin, n (%)	30 (85.7)	31 (86.1)	0.96
Glibenclamide, n (%)	11 (31.4)	16 (44.4)	0.25
Statins, n (%)	3 (8.6)	4 (11.1)	0.70
Blood pressure lowering drugs, n (%)	6 (17.1)	5 (13.9)	0.72
Anthropometric measures			
Weight (kg)	73.69± 8.24	72.71± 10.52	0.66
Height (cm)	164.94 ± 7.22	162.08 ± 11.29	0.20
BMI (kg m^{-2})	27.10± 2.69	27.66± 2.71	0.39
HC (cm)	101.97± 6.05	103.47± 8.04	0.37
WC (cm)	91.75± 7.4	92.58± 8.53	0.66
WHR	0.9± 0.06	0.89± 0.05	0.53
WHtR	0.55± 0.05	0.57± 0.07	0.25

[1]Data are expressed as mean ± SD for continuous variables or as frequency and percentage for categorical variables.

[2]Differences between the control and intervention groups were evaluated using the Independent sample t-test for continuous variables and chi-square test for categorical variables.BMI, Body mass index; HbA1c, glycated hemoglobin; HC, Hip circumference; WC, Waist circumference; WHR, Waist to hip ratio; WHtR, Waist to height ratio

513 **Table 2**. Dietary intake and physical activity during study in resveratrol and placebo groups (mean ± SD)

514 [1]The presented P-values are associated with within-group comparisons obtained paired t test.

Variable	Resveratrol (n=35)			Placebo (n= 36)			
	Before	After	P-value[1]	Before	After	P-value[1]	P-value[2]
Energy (kcal)	1612.87± 587.87	1544.71± 597.37	0.45	1708.79± 515.39	1674.16±597.07	0.55	0.47
Carbohydrate (%)	59.76 ± 12.71	61.36± 11.2	0.43	60.82 ± 9.96	60.61± 8.76	0.88	0.7
Protein (%)	15.5 ± 4.65	16.28± 5.17	0.47	15.48 ± 3.48	15.84± 4.02	0.56	0.97
Fat (%)	25.34 ± 14.55	24.14± 11.02	0.58	24.61 ± 10.42	24.26± 9.63	0.77	0.81
Fiber (g/d)	9.43± 4.11	9.69± 4.32	0.81	10.44± 5.23	10.86±5.27	0.64	0.2
Cholesterol (mg/d)	219± 29	208± 47	0.77	189± 71	191± 63	0.61	0.75
PUFA (%)	8.22± 4.13	8.28± 4.24	0.81	9.13± 4.35	9.71± 5.12	0.43	0.76
MUFA (%)EPA (%)	6.32± 4.21	6.12± 5.1	0.53	5.67± 3.72	5.82± 3.22	0.62	0.41
Zinc	0.01± 0.69	96.62±571.66	0.32	0.0038± 0.0097	0.0007± 0.0014	0.14	0.17
Vitamin E	6.72± 2.64	6.79± 3.12	0.64	7.03± 2.83	7.73± 4.004	0.71	0.65
Vitamin C	3.45± 2.01	4.44± 5.44	0.25	3.75± 2.96	4.22± 3.82	0.28	0.64
Selenium	57.24± 49.18	53.09± 54.36	0.73	64.36± 44.64	51.31± 41.69	0.23	0.55
Beta-Carotene	0.09± 0.47	0.10± 0.06	0.15	0.08± 0.07	0.109± 0.1005	0.06	0.66
DHA	365.67± 816.21	206.60± 424.54	0.25	289.63± 694± 62	348.61± 640.04	0.45	0.69
PA (MET-h/d)	0.05± 0.18	19.20± 111.93	0.32	0.005± 0.01	0.007± 0.01	0.21	0.12
	35.61 ± 5.22	36.33± 5.7	0.14	37.54 ± 7.82	36.99± 5.87	0.31	0.24

515 [2]The presented P-values are associated with baseline comparisons of the resveratrol and control groups obtained independent
516 sample t test

517 EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; MUFA, Mono-unsaturated fatty acid; PA, physical activity.
518 PUFA, Poly-unsaturated fatty acid.

Table3: Comparison of serum levels of ADMA and PON1 enzyme activity at baseline and after intervention in resveratrol and placebo groups (mean ± SD).

Variable	Resveratrol (n=35)				Placebo (n= 36)							
	Before	After	P-value[1]	Change	Before	After	P-value[1]	Change	P-value[2]	P-value[3]	P-value[4]	P-value[5]
ADMA (ng/ml)	0.61±0.47	0.44±0.38	0.000	-0.16±0.11	0.60±0.45	0.57±0.26	0.06	0.04±0.07	0.527	0.000	0.000	0.000
PON1 (U/L)	97.32±18.68	112.72±24.91	0.000	15.39±13.99	100.12±24.60	101.06±24.14	0.223	0.94±4.95	0.592	0.049	0.000	0.000

[1]The presented P-values are associated with within-group comparisons obtained paired t test.

[2]The presented P-values are associated with baseline comparisons of the resveratrol and control groups obtained independent sample t test

[3]The presented P-values are associated with between groups comparisons after intervention obtained independent sample t test.

[4] The presented P-values are associated with mean changes comparisons obtained from independent-sample t test.

[5] The presented P-values are associated with mean changes comparisons adjusted for age, gender, and BMI obtained from analysis of covariance (ANCOVA).

ADMA, asymmetric de-methyl-arginine; PON1, paraoxonase 1.