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Original article

Resveratrol reduces albuminuria in diabetic nephropathy: A randomized double-blind placebo-controlled clinical trial

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ABSTRACT

Aim. – Albuminuria is the most important indicator of diabetic nephropathy (DN). Resveratrol, a natural compound found in grape skins and red wine, has antioxidant effects. This study aimed to evaluate the effects of resveratrol on DN.

Methods. – In this randomized, double-blind, placebo-controlled clinical trial, 60 patients with type 2 diabetes and albuminuria were randomly assigned to receive either resveratrol (500 mg/day) or placebo for 90 days. Losartan (12.5 mg/day) was also administered to all participants. Primary outcomes were urinary albumin/creatinine ratio, estimated glomerular filtration rate (eGFR) and serum creatinine levels. Secondary outcomes were oxidative stress markers, and anthropometric and biochemical measures.

Results. – Mean urine albumin/creatinine ratio was significantly reduced in the resveratrol group *vs* placebo (−46.4 mg/g, 95% CI: −64.5 to −28.3 *vs* 29.9 mg/g, 95% CI: 4.9 to 54.9; $P < 0.001$), whereas eGFR (1.7 mL/min/1.73 m^2, 95% CI: −3.4 to 6.8 *vs* −4.0, 95% CI: −8.2 to 0.2; $P = 0.08$) and serum creatinine (−0.3 mg/dL, 95% CI: −0.1 to 0.1 *vs* 0.1 mg/dL, 95% CI: −0.0 to 0.1; $P = 0.13$) were unchanged. Serum antioxidant enzymes were significantly increased with resveratrol. After adjusting for confounding variables, the effect of resveratrol in reducing urinary albumin excretion was still significant ($P < 0.001$). Regression analysis revealed that every 1-cm decrease in waist circumference and 1-μmol/L increase in nitric oxide (NO) was associated with 9.4 mg/g and 4.0 mg/g reductions, respectively, of urine albumin/creatinine ratio.

Conclusion. – This clinical trial has shown that resveratrol may be an effective adjunct to angiotensin receptor blockers (ARBs) for reducing urinary albumin excretion in patients with DN (ClinicalTrials.gov: NCT02704494).

Introduction

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease (ESRD). Approximately 25–40% of patients with type 1 diabetes mellitus (T1DM) and 5–40% with type 2 (T2DM) ultimately develop DN [1,2]. The life expectancy of patients with ESRD due to diabetes is only 3 or 4 years [3], and the increased cardiovascular mortality in diabetes patients aged < 50 years is mostly related to DN and microalbuminuria [4]. The recommended approach to screening for DN is calculation of the urine albumin/creatinine ratio from a first-void morning spot urine specimen [5]. Given the biological variability of urinary albumin excretion, two out of every three specimens of urine albumin/creatinine ratio collected over a 3- to 6-month period should be abnormal to raise a diagnosis of albuminuria. While the currently available therapeutic interventions, including optimization of glycaemic and blood pressure (BP) control, are important, more innovative strategies are necessary for the prevention and treatment of DN.

Despite advances in our knowledge of the molecular and cellular signalling pathways involved in DN, very few new drugs are coming onto the market for its treatment [6]. A variety of agents, such as protein kinase C inhibitors, advanced glycation end-product (AGE) inhibitors and transforming growth factor (TGF)-β inhibitors, have been evaluated in small clinical trials, but have demonstrated high failure rates [6,7]. In light of this, it is now necessary to perform more clinical trials to evaluate the effects of new agents in controlling diabetic kidney disease.

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Resveratrol (3,5,4'-trihydroxystilbene) is a naturally occurring, plant-derived, polyphenolic compound characterized in 1940 after its isolation from the roots of *Veratrum grandiflorum* [8]. It can also be found in a number of plants and dietary products, such as peanuts, grapes and red wine. Resveratrol is one of the most studied polyphenol molecules, and there are more than 1000 reports of its properties in the literature [9,10]. It has been found to exert several pharmacological actions, mainly anti-inflammatory, antioxidant, anti-apoptotic and, in general, cyto-protective effects. These effects have mostly been observed in preclinical studies. However, while several studies have evaluated the antidiabetes effects of resveratrol, none has evaluated its effects on albuminuria. The aim of the present study was therefore to evaluate the effects of resveratrol on DN.

Materials and methods

Trial design

The study was designed as a two-arm randomized, double-blind, placebo-controlled clinical trial with a parallel design using a 1:1 ratio of allocation. There was no change in methods after the trial began.

Participants

Patients (aged $\geq$ 18 years) with proven T2DM and referred to a diabetes clinic (Shahid Motahari Clinic, Shiraz University of Medical Sciences, Iran) with newly diagnosed confirmed albuminuria were evaluated for inclusion in the study. Inclusion criteria were controlled blood sugar [fasting plasma glucose (FPG) $\leq$ 130 mg/dL, glycosylated haemoglobin (HbA_{1c}) $\leq$ 7% (53 mmol/mol)], fasting urine albumin/creatinine ratio $\geq$ 30 mg/g on two separate occasions within the past 3 months and serum creatinine levels $\leq$ 2 mg/dL. Exclusion criteria were pregnancy, lactation, alcoholism, liver failure (acute or chronic), uncontrolled hypertension (BP $\geq$ 140/90 mmHg), use of angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs) within the past 3 months, congestive heart failure, prostate disease, history of any malignancy, bilateral renal artery stenosis, any systemic disease other than diabetes, any infection or rheumatological disorder and the use of warfarin.

Sample size was calculated by a statistician using PASS 11 (2011) software (NCSS LLC, Kaysville, UT, USA) and based on data from a previous study [11]. Given a one-sided significance level of 0.05, a power of 0.80, and applying the expected difference and variance for proteinuria as the primary outcome of the study, the required sample size was calculated to be 29 participants per group.

The study protocol was in compliance with the Declaration of Helsinki, and was also approved by the local ethics committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1394.81). The study was registered on ClinicalTrials.gov (ID: NCT02704494). All patients gave their written informed consent to participate.

Interventions

Resveratrol (Merck KGaA, Darmstadt, Germany) and carboxymethylcellulose (CMC; Merck KGaA) as placebo were dispensed as identical 250-mg hard gelatine capsules by the Faculty of Pharmacy at Nemazee Teaching Hospital in Shiraz, Iran. Patients received either resveratrol (500 mg/day) or placebo (500 mg/day) for 90 days. This resveratrol regime was selected on the basis of the results of previous studies and for safety reasons. Losartan (25-mg tablets; Dr. ABIDI Pharmaceutical Laboratory, Tehran, Iran) at a dose of 12.5 mg/day was also administered to all participants. Patients then continued taking their antidiabetes medications with no modification of either dosages or type of drug throughout the course of the study. They were also recommended to avoid taking any antioxidant or vitamin supplements during the study.

Assessment

Demographic data including age, gender, height and weight, body mass index (BMI), waist and hip circumferences, and BP were measured and recorded in a file maintained for each patient. After 12 h of fasting, 15-mL blood and spot urine samples from each patient were collected at baseline and after 90 days of intervention. All blood samples were immediately centrifuged at 4000 rpm for 10 min, and the sera placed in a –80 °C freezer until needed for biochemical analyses. Fresh morning first-void urine samples were also analyzed for measurement of urine albumin and creatinine.

Primary outcomes

Primary study endpoints were changes in urine albumin/creatinine ratio, estimated glomerular filtration rate (eGFR) and serum creatinine levels. Fasting first-void morning spot urine samples were used to measure urine albumin and creatinine (Selectra XL autoanalyzer, ELITechGroup, Puteaux, France), while urinary albumin/creatinine ratios were calculated to estimate daily urinary albumin excretion. Serum creatinine (mg/dL) was measured with standard kits using an enzymatic colorimetric method (Pars Azmun Co, Alborz Province, Iran), and the eGFR was calculated using the Chronic Kidney Disease Epidemiology (CKD-EPI) Collaboration equation, developed and validated by Levey et al. in 2009.

Secondary outcomes

Secondary endpoints were BMI, waist and hip circumferences, BP, serum oxidative stress biomarkers and antioxidant enzymes [nitric oxide (NO), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), malondialdehyde (MDA) and myeloperoxidase (MPO)], HbA_{1c}, FPG, insulin levels, insulin resistance, lipid profiles, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase and gamma-glutamyl transferase (GGT).

Height was measured with a stadiometer, and weight with a standard balance weighing scale. BMI was calculated as weight divided by the square of height (kg/m^2). Waist and hip circumferences were measured with a standard flexible tape with subjects in standing position. BP was measured two times on the right arm with a standard mercury sphygmomanometer (in mmHg) after a 15-min rest in sitting position.

The method of measuring serum levels of oxidative stress biomarkers and antioxidant enzymes (total NO, SOD, GSH-Px, CAT, MDA, MPO) was by enzyme-linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory; Shanghai Korain Biotech Co, Shanghai, China). HbA_{1c} was measured in whole-blood samples from all patients using the high-performance liquid chromatography (HPLC) method. FPG (mg/dL) was assessed by the enzymatic (glucose oxidase) colorimetric method using standard kits (Pars Azmun Co); insulin levels (μU/mL) were estimated by immunoradiometric assay (IRMA) using standard kits (IZOTOP, Budapest, Hungary); and insulin resistance was assessed by homoeostasis model assessment (HOMA-IR) according to the following equation: fasting insulin (μIU/mL) × FPG (mg/dL)/405. Lipid profiles, including total cholesterol, triglycerides, and low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, as well other biochemical markers were determined by the enzymatic colorimetric method using standard kits (Bionic Pars Co., Tehran, Iran).

Randomization and masking

A total of 64 eligible patients met our inclusion criteria and were randomly allocated to either of two parallel groups (resveratrol and placebo) by the clinic secretary, who had been instructed to use a randomized list generated by Microsoft Excel software with a block randomization method. All relevant physicians, researchers and statisticians were blind to the patients' allocations. Moreover, because of the identical shapes, sizes and colours of the resveratrol and placebo containers, all patients were also blind to their drug allocation. To ensure patients' compliance with taking their medications correctly, the research team kept in close contact with all patients through regular follow-ups.

Statistical analyses

All data were analyzed using IBM SPSS Statistics version 23 software (IBM Corp., Armonk, NY, USA), and data normality was checked by Shapiro–Wilk test. All data are presented as means ± SD, and probability values $(P) \leq 0.05$ were considered statistically significant. Analyses of baseline anthropometric, clinical and biochemical parameters were performed by independent-samples *t* test, and Chi^2 tests were used for analyses of qualitative variables. For comparisons of variables before and after the intervention in each group, paired-samples *t* tests were performed. Independent-samples *t* tests were also used to compare the two groups (resveratrol and control) for differences in variables (after–before). Analysis of covariance (ANCOVA) was performed to control for confounding variables such as weight, BMI, waist and hip circumferences, FPG, serum insulin and HbA_{1c}. Regression analyses were used to determine the effects of anthropometric measures, drugs (antidiabetics, antihypertensives, lipid-lowering), serum creatinine, eGFR, FPG, serum insulin, HbA_{1c} and antioxidant markers on urine albumin/creatinine ratio.

Results

Between March 2016 and February 2017, 72 patients were screened, of whom 64 were randomized into the present study. Four patients (two in resveratrol group, two in placebo group) failed to return for their outcome measurements and were therefore excluded from the analyses. Thus, 60 patients completed the study: 30 patients each in the resveratrol and placebo groups. Fig. 1 is a flowchart of the study participants: there were no statistically significant differences between the resveratrol and placebo groups in terms of baseline anthropometric, clinical and biochemical parameters (Table 1).

Comparisons of the anthropometric, clinical and biochemical characteristics of patients in both groups are presented in Table II. The mean urine albumin/creatinine ratio was significantly reduced in the resveratrol compared with placebo group (−46.4 mg/g, 95% CI: −64.5 to −28.3 *vs* 29.9 mg/g, 95% CI: 4.9 to 54.9, respectively; $P < 0.001$), while mean eGFR ($P = 0.08$) and mean serum creatinine levels ($P = 0.13$) remained unchanged (Fig. 2).

As shown in Table 2, comparison of the intervention groups for changes in variables (after–before) revealed that urinary albumin

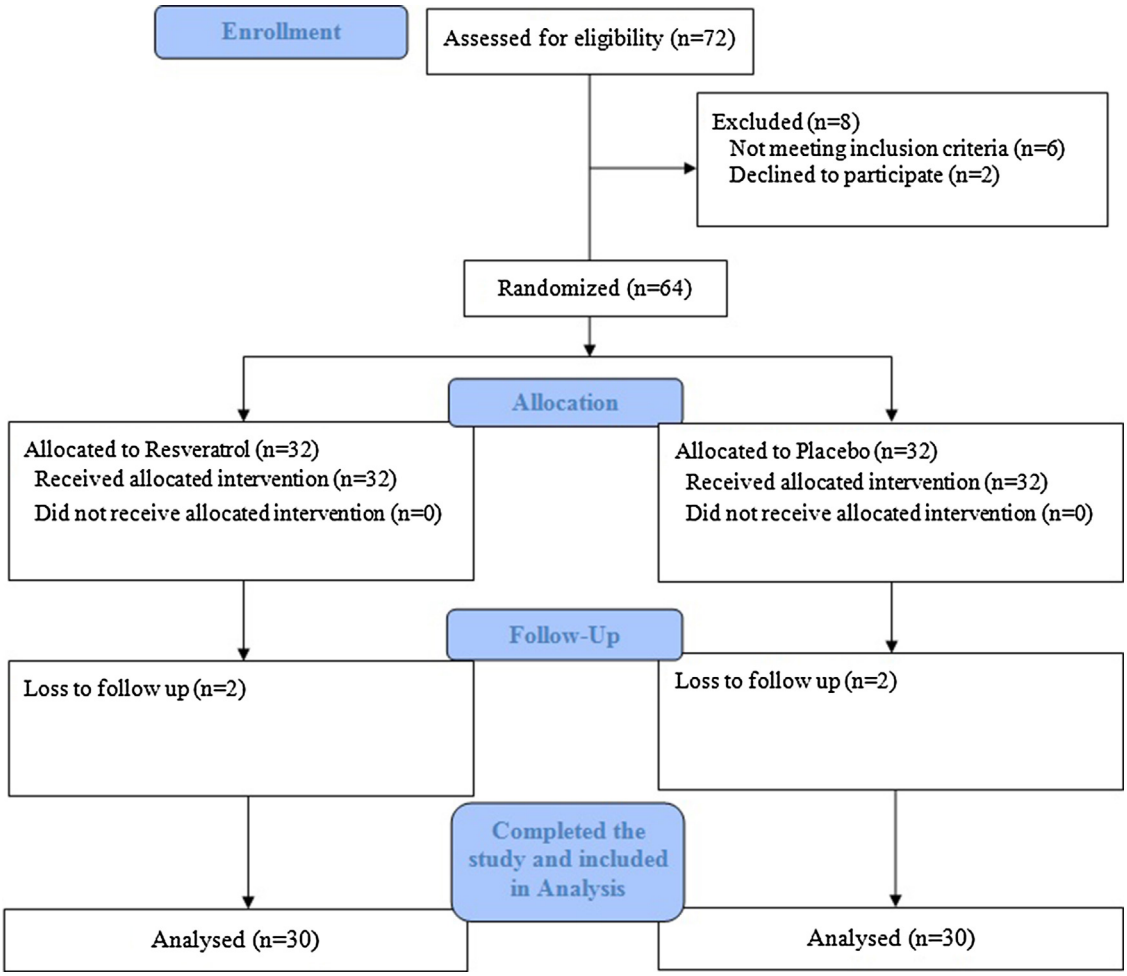

Fig. 1. Flowchart of study participant recruitment, allocation and follow-up.

Table 1
Baseline anthropometric, clinical and biochemical characteristics of patients in the two study arms.

Variables	Resveratrol + losartan ($n = 30$)	Placebo + losartan ($n = 30$)
Age (years)	56.8 ± 9.7	55.7 ± 10.8
Gender (male/female, *n*)	14/16	13/17
Duration of diabetes (years)	16.1 ± 6.6	14.4 ± 6.3
Weight (kg)	73.9 ± 9.6	72.2 ± 12.4
Body mass index (kg/m^2)	28.2 ± 3.8	27.3 ± 4.4
Waist circumference (cm)	100.6 ± 9.3	99.7 ± 10.9
Hip circumference (cm)	107.9 ± 7.9	106.9 ± 13.1
Systolic BP (mmHg)	131.3 ± 10.5	130.5 ± 16.5
Diastolic BP (mmHg)	80.0 ± 10.5	82.1 ± 9.4
Urine albumin/creatinine ratio (mg/g)	123.6 ± 78.6	141.1 ± 100.8
Serum creatinine (mg/dL)	1.1 ± 0.3	1.1 ± 0.4
eGFR (mL/min/1.73 m^2)[a]	68.2 ± 20.2	71.6 ± 21.6
HbA_{1c} (mmol/mol)	49	49
HbA_{1c} (%)	6.6 ± 0.4	6.6 ± 0.4
Fasting plasma glucose (mg/dL)	116.4 ± 20.7	110.1 ± 20.4
Serum insulin (μIU/mL)	14.1 ± 7.2	14.1 ± 4.8
HOMA-IR	4.2 ± 2.7	3.9 ± 1.5
ALT (U/L)	20.9 ± 7.43	22.1 ± 9.52
AST (U/L)	20.7 ± 8.78	20.6 ± 5.07
Alkaline phosphatase (U/L)	229.4 ± 73.2	216.8 ± 67.4
Gamma-glutamyl transferase (U/L)	22.4 ± 11.4	22.1 ± 12.2

Data are means ± SD *n*; BP: blood pressure; eGFR: estimated glomerular filtration rate; HOMA-IR: homoeostasis model assessment of insulin resistance; ALT/AST: alanine/aspartate aminotransferase.
[a] By Chronic Kidney Disease Epidemiology (CKD-EPI) Collaboration equation.

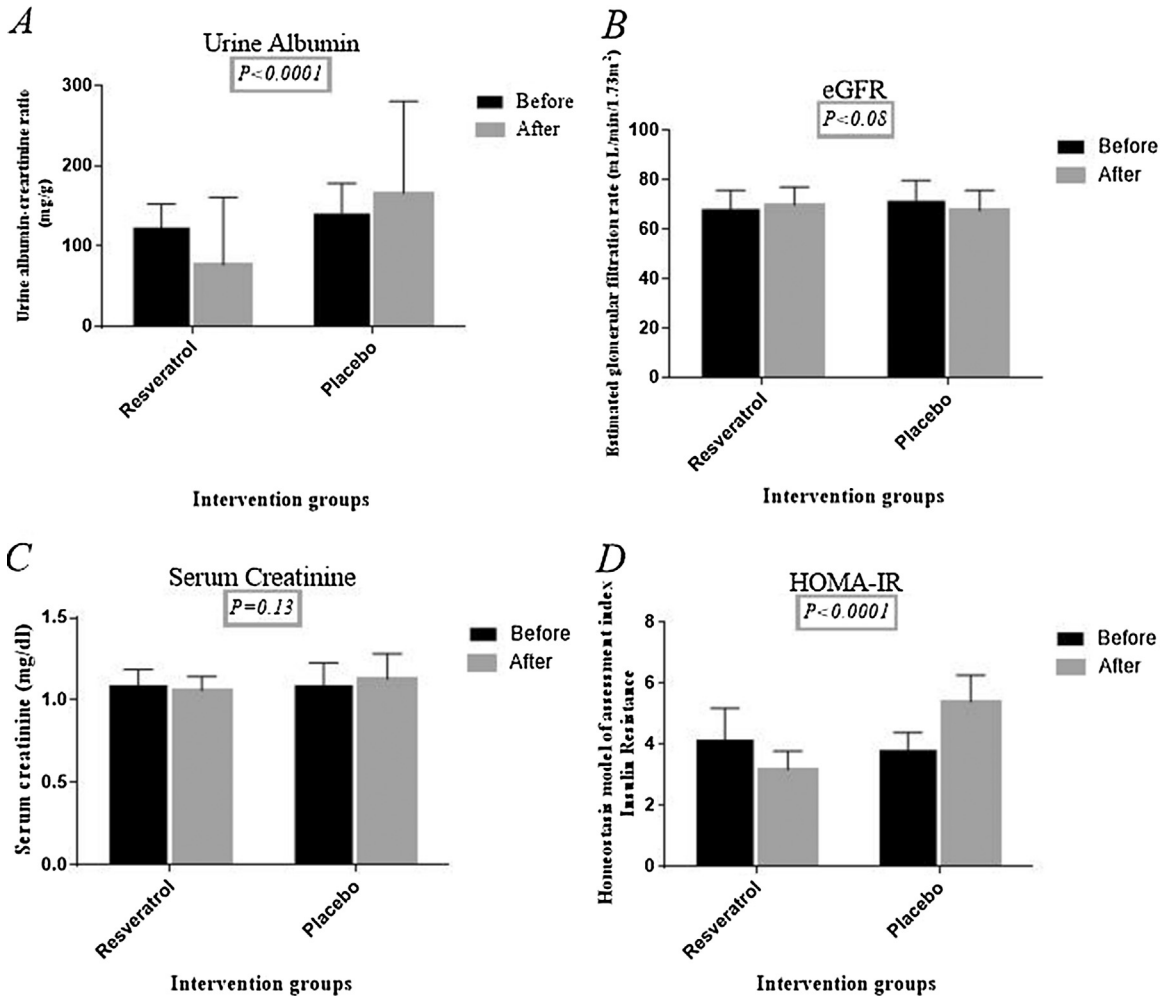

Fig. 2. Mean changes (After–Before) and 95% confidence intervals (CIs) in intervention groups for: urine albumin/creatinine ratio (A); estimated glomerular filtration rate (B); serum creatinine levels (C); and homoeostasis model assessment of insulin resistance (HOMA-IR) (D).

Table 2
Comparison of anthropometric, clinical and biochemical variables before and after resveratrol and placebo interventions.

Variables	Resveratrol + losartan ($n = 30$)			Placebo + losartan ($n = 30$)			P^b
	Before	After	After–Before	Before	After	After–Before	
Weight (kg)	73.9 ± 9.6	73.1 ± 10.8	−0.8 ± 2.6	72.2 ± 12.4	71.9 ± 12.3	−0.2 ± 1.6	0.32
Body mass index (kg/m^2)	28.2 ± 3.8	27.9 ± 4.0	−0.3 ± 1.0	27.3 ± 4.4	27.2 ± 4.4	−0.1 ± 0.6	0.26
Waist circumference (cm)	100.5 ± 9.3	99.3 ± 9.9[a]	−1.3 ± 2.5	99.7 ± 10.9	99.4 ± 11.2	−0.3 ± 1.7	0.07
Hip circumference (cm)	107.9 ± 7.9	106.4 ± 7.5[a]	−1.4 ± 3.0	106.9 ± 13.1	106.8 ± 12.7	−0.1 ± 2.0	0.05
Systolic BP (mmHg)	131.3 ± 10.5	124.7 ± 16.3	−6.6 ± 2.9	130.5 ± 16.5	126.4 ± 20.3	−4.1 ± 23.2	0.68
Diastolic BP (mmHg)	80.0 ± 10.5	77.5 ± 10.5	−3.2 ± 12.8	82.1 ± 9.4	77.1 ± 9.6	−5.1 ± 13.3	0.59
Urine albumin/creatinine ratio (mg/g)	123.6 ± 78.6	77.2 ± 83.6[a]	−46.4 ± 48.5	141.1 ± 100.8	166.4 ± 114.5	25.3 ± 69.3	*< 0.001*
Serum creatinine (mg/dL)	1.1 ± 0.3	1.1 ± 0.2	−0.0 ± 0.2	1.1 ± 0.4	1.1 ± 0.4	0.1 ± 0.2	0.13
eGFR (mL/min/1.73 m^2)	68.2 ± 20.2	69.9 ± 19.5	1.7 ± 13.6	71.6 ± 21.6	67.6 ± 21.6	−4.0 ± 11.1	0.08
HbA_{1c} (mmol/mol)	49	44[†]	–	49	49	–	*0.04*
HbA_{1c} (%)	6.6 ± 0.4	6.2 ± 0.6[a]	−0.4 ± 0.7	6.6 ± 0.4	6.7 ± 1.1	0.0 ± 1.1	
Fasting plasma glucose (mg/dL)	116.9 ± 20.7	113.2 ± 27.2	−3.7 ± 28.7	110.1 ± 20.4	136.2 ± 33.4[†]	26.1 ± 32.0	*< 0.001*
Serum insulin (μIU/mL)	14.1 ± 7.2	11.1 ± 4.3[a]	−3.0 ± 6.8	14.1 ± 4.8	16.1 ± 5.4[†]	2.0 ± 3.5	*0.001*
HOMA-IR	4.2 ± 2.7	3.2 ± 1.6	−1.0 ± 3.0	3.8 ± 1.5	5.4 ± 2.4[†]	1.6 ± 1.9	*< 0.001*
ALT (U/L)	20.8 ± 7.4	18.6 ± 6.9[a]	−2.5 ± 5.2	22.1 ± 9.5	20.1 ± 7.4	−2.0 ± 8.2	0.78
AST (U/L)	20.7 ± 8.8	18.4 ± 5.0	−2.6 ± 7.6	20.6 ± 5.1	21.1 ± 9.5	0.6 ± 9.0	0.16
Alkaline phosphatase (U/L)	229.4 ± 73.2	221.0 ± 83.0	−10.7 ± 75.2	216.8 ± 67.4	221.8 ± 68.8	4.97 ± 34.1	0.31
Gamma-glutamyl transferase (U/L)	22.4 ± 11.4	23.6 ± 14.8	1.3 ± 6.8	22.1 ± 12.2	26.9 ± 14.4	4.8 ± 13.3	0.21

Data are means ± SD; BP: blood pressure; eGFR: estimated glomerular filtration rate; HOMA-IR: homoeostasis model assessment of insulin resistance; ALT/AST: alanine/aspartate aminotransferase.
[a] Statistically significant change within intervention group.
[b] Significance of changes (After–Before) in variables between period

excretion, FPG, insulin, HOMA-IR (Fig. 2) and HbA_{1c} were all significantly decreased in the resveratrol compared with the placebo group. Moreover, using ANCOVA to adjust for confounding variables such as weight, BMI, waist and hip circumferences, FPG, insulin, HbA_{1c}, serum creatinine, eGFR and antioxidant biomarkers revealed that the decrease in urinary albumin excretion level in the resveratrol group was still significant ($P < 0.001$).

Baseline mean serum levels of oxidative stress biomarkers and antioxidant enzymes did not differ significantly between the two study groups (Table 3). Levels of NO ($t = -4.615$; $P < 0.001$), SOD ($t = -4.886$; $P < 0.001$), GSH-Px ($t = -5.123$; $P < 0.001$) and CAT ($t = -6.679$; $P < 0.001$) were all significantly increased, whereas MDA decreased significantly ($t = 2.269$; $P = 0.03$) with resveratrol. In the placebo group, there were significant decreases in serum levels of SOD ($t = 2.452$; $P < 0.02$) and significant increases in serum levels of MDA ($t = -3.768$; $P = 0.001$) and MPO ($t = -4.0$; $P < 0.001$).

Regression analyses revealed that, among the changes in weight, BMI, waist and hip circumferences, systolic and diastolic BP, serum creatinine, eGFR, FPG, insulin, HbA_{1c}, HOMA-IR and serum oxidative biomarkers, a decrease in waist circumference (WC) and increase in serum NO levels were the significant determining factors for reducing urine albumin/creatinine ratio. A 1-cm decrease in WC was associated with a 9.4-mg/g reduction in urinary albumin excretion (B = 9.389; $t = 2.439$; $P = 0.018$), whereas a 1-μmol/L increase in serum NO levels was associated with a 4.0-mg/g reduction in urinary albumin excretion (B = −4.063; $t = -2.729$; $P = 0.009$).

Throughout the study, only two patients (one in the resveratrol group, the other in the placebo group) complained of gastrointestinal side-effects such as mild dyspepsia, but they nonetheless continued to take their allocated treatments up to the end of the study.

Discussion

This randomized clinical trial has shown that resveratrol can ameliorate DN by decreasing urinary albumin excretion probably through antioxidant mechanisms. There was no significant change in either mean serum creatinine levels or eGFR.

Microalbuminuria is an early marker of progressive diabetic kidney disease, and it is well documented that any intervention to prevent or treat microalbuminuria can delay ESRD. Preclinical studies of DN have demonstrated that some polyphenols are able to reduce albuminuria and other indicators of renal damage by reducing oxidative stress biomarkers. However, reports of clinical trials evaluating the effects of polyphenols on diabetic albuminuria are limited. Borges et al. [12] conducted a randomized double-blind study of 42 diabetes patients receiving the maximum recommended dose for renin–angiotensin inhibition to investigate the effect of green-tea polyphenols (GTP) on residual albuminuria. Treatment with GTP for 12 weeks resulted in a 41% reduction in urinary albumin/creatinine ratio.

Resveratrol has been shown to have renal-protective effects in some animal studies by, for example, decreasing albuminuria [13]. In human studies, Goh et al. [14] used doses of 500 mg/day of resveratrol with 500-mg increments every 3 days to a maximum of 3 g/day for 12 weeks in 10 diabetes patients: there were no significant changes in serum creatinine levels in any patient at the end of the study. Movahed et al. [15] gave either resveratrol at a dose of 1 g/day or placebo to 66 diabetes patients for 45 days: the mean decrease in serum creatinine in the resveratrol group was not significant. In another clinical trial but of longer duration, Tome-Carneiro et al. [16] treated 22 patients with T2DM and coronary artery disease with resveratrol-enriched grape extract (8 mg/day) for 12 months: the result was a non-significant decrease in serum creatinine. In the Kumar et al. [17] study, 57 diabetes patients took 250 mg/day of resveratrol or placebo for 6 months: a significant decrease in serum creatinine levels was observed in the resveratrol-treated group. Hausenblas et al. [18] carried out a systematic review and identified all randomized controlled clinical trials using resveratrol as an adjunct to pharmaceutical interventions, which involved 196 T2DM patients (104 resveratrol, 92 placebo). In general, this meta-analysis found that resveratrol supplementation was more effective than placebo/control for reducing serum creatinine levels.

In the present study, there were no significant changes in serum creatinine and eGFR in either the resveratrol or placebo groups, although this might have been due to the short duration of the intervention, the use of resveratrol at a low dose or the inclusion of patients with serum creatinine levels ≤ 2 mg/dL. Moreover, it

Table 3
Oxidative stress biomarkers before and after resveratrol and placebo interventions.

Variables	Resveratrol + losartan ($n = 30$)			Placebo + losartan ($n = 30$)			P^b
	Before	After	After–Before	Before	After	After–Before	
Nitric oxide (NO), μmol/L	37.5 ± 7.5	42.2 ± 6.0[a]	4.4 ± 5.61	38.5 ± 8.5	30.0 ± 7.6	−0.5 ± 5.0	*< 0.001*
Superoxide dismutase (SOD), U/L	49.7 ± 7.1	54.4 ± 8.4[a]	4.8 ± 5.3	49.9 ± 10.8	45.7 ± 9.7[a]	−4.2 ± 9.3	*< 0.001*
Glutathione peroxidase (GS-HPx), U/L	123.7 ± 17.4	137.1 ± 29.9[†]	13.4 ± 14.3	117.5 ± 26.2	111.8 ± 25.0	−5.7 ± 24.4	*0.001*
Catalase (CAT), U/L	60.4 ± 6.1	66.3 ± 7.8[a]	5.9 ± 4.8	57.7 ± 10.5	54.9 ± 9.7	−2.7 ± 11.7	*0.001*
Malondialdehyde (MDA), nmol/mL	5.1 ± 0.8	4.4 ± 1.0[a]	−0.4 ± 0.9	4.3 ± 1.2	5.2 ± 1.4[a]	0.9 ± 1.3	*< 0.001*
Myeloperoxidase (MPO), ng/mL	1.5 ± 0.2	1.5 ± 0.2	0.0 ± 0.3	1.5 ± 0.4	1.6 ± 0.4[a]	0.1 ± 0.2	*0.03*

Data are means ± SD.
[a] Statistically significant change within intervention group.
[b] (After–Before) in variables between intervention groups.

should be borne in mind that none of the above-mentioned studies had evaluated patients with DN who presented with albuminuria, whereas one of the main aims of our present study was to evaluate the effects of resveratrol on urinary albumin excretion as a primary outcome measure. In addition, as it was ethically necessary to prescribe an approved drug for albuminuria along with the resveratrol or placebo during the study, losartan at a dose of 12.5 mg/day was prescribed for all patients. In the end, there was a significant decrease in urinary albumin excretion with resveratrol compared with placebo.

To interpret the mechanisms behind the probable renoprotective effects of resveratrol, another outcome measure of our study was the measurement of serum oxidative stress biomarkers and antioxidant enzymes. Previous studies had demonstrated the kidney-protective effects of resveratrol in various pathological and experimental models, and also suggested that resveratrol had potential antioxidant and anti-inflammatory effects such as reducing free radicals, the excess production of proinflammatory mediators and the altered expression of adhesion molecules while inhibiting neutrophil function [19–22]. Oxidative stress is induced by an imbalance between oxidant and antioxidant status; therefore, exogenous antioxidants and modulation of antioxidant enzymes may be expected to reduce oxidative stress. In our study, the antioxidant potential of resveratrol was evaluated by measuring serum levels of SOD, GSH-Px and CAT: compared with placebo, the resveratrol-treated patients showed significant increases in serum levels of SOD, GSH-Px, CAT and NO.

Previous experimental studies found that NO is generated in kidney tissue, and plays an important role in the regulation of renal blood flow and glomerular filtration. NO has also been identified as a crucial factor for mediating the protective effects of resveratrol, which enhances endothelial NO synthase (eNOS) expression in endothelial cells and improves ventricular function during ischaemia–reperfusion injury [23,24]. Also, our present study found that every 1-μmol/L increase in serum levels of NO was associated with a 4.0-mg/g decrease in urinary albumin excretion. These findings suggest that resveratrol may be able to lower urinary albumin excretion levels through both NO-dependent and NO-independent antioxidant mechanisms.

Waist circumference conferred an incremental risk for the development of microalbuminuria in some studies [25,26], whereas the effect of weight loss in improving proteinuria in obese diabetes patients has been reported in others [27]. A meta-analysis by Elgebaly et al. [28] revealed a linear correlation between baseline urine microalbumin and WC; our study found that a WC decrease of 1 cm was accompanied by a 9.4-mg/g decrease in urinary albumin excretion.

Several studies have indicated that glycaemic indices, such as FPG, HbA_{1c}, insulin levels and insulin resistance, correlate with microalbuminuria [29–31]. However, the effects of resveratrol supplementation on those parameters have differed across several relevant studies. The meta-analysis by Hausenblas et al. [18] revealed a significant decrease only in HbA_{1c} levels, whereas Liu et al. [32] demonstrated beneficial effects with resveratrol on all of those parameters in diabetes patients. In the present study, the ability of resveratrol to lower FPG, HbA_{1c}, insulin levels and HOMA-IR scores was significant compared with placebo. It is therefore logical to conclude that the effect of resveratrol in lowering urinary albumin excretion is probably due to its ability to lower FPG, HbA_{1c} and insulin resistance. In addition, even after adjusting for the effects of such confounding variables, the reduction of urine albumin/creatinine ratio in resveratrol-treated patients was still significant. This suggests that other mechanisms, such as antioxidant and/or anti-inflammatory activities, may be involved in the beneficial effects of resveratrol on albuminuria.

The present study has some limitations: the most important one was the duration of the study, while another was the dose of resveratrol used. It may be that resveratrol would have been effective for concomitantly lowering serum creatinine levels and urinary albumin excretion at higher doses and/or longer durations of intervention and/or larger sample populations. Yet another limitation was that inflammatory markers were not measured to further determine the functional mechanisms of resveratrol due to limited study funds.

In conclusion, the present clinical trial has demonstrated that resveratrol may be effective as an adjunct to ARBs in reducing urinary albumin excretion in patients with DN. More clinical trials of longer duration and with larger samples and different doses of resveratrol are now needed to confirm the effects of resveratrol on DN and to more precisely determine its mechanisms of action.

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Disclosure of interest

The authors declare that they have no relevant financial interests.

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