Anti-inflammatory and antioxidant effects of resveratrol in healthy smokers a randomized, double-blind, placebo-controlled, cross-over trial

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26 **Anti-inflammatory and Antioxidant Effects of Resveratrol in Healthy Smokers.**

27 A randomized, double-blind, placebo-controlled, cross-over trial.

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29 **Running header**: resveratrol effects in healthy smokers

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Abstract

Objective: Smokers are characterized by a low-grade systemic inflammatory state and an oxidant-antioxidant imbalance. Few human studies were conducted on the effects of resveratrol, a natural compound with anti-inflammatory and antioxidant properties, and no trial on smokers has been performed to date. We evaluated whether resveratrol has beneficial effects on markers of inflammation and oxidative stress in smokers.

Methods and Results: A randomized, double-blind, cross-over trial was performed in 50 healthy adult smokers: 25 were randomly allocated to "resveratrol-first" (30-days: 500mg resveratrol/day, 30-days wash-out, 30-days placebo) and 25 to "placebo-first" (30-days placebo, 30-days wash-out, 30-days 500mg resveratrol/day).

Resveratrol significantly reduced C-reactive protein (CRP) and triglyceride concentrations, and increased Total Antioxidant Status (TAS) values. After analyzing data with general linear models to assess period and carry-over effects, the ratios of the values after resveratrol to those after placebo were respectively: 0.47 (95%CI 0.38-0.59) –CRP- and 0.71 (95%CI 0.65-0.78) –triglycerides-, while TAS increased by 74.2 μmol/L (95%CI 60.8-87.6). Uric acid, glucose, insulin, cholesterol, liver enzyme concentrations, and weight, waist circumference, and blood pressure values did not significantly change after resveratrol supplementation.

Conclusions: Because resveratrol has anti-inflammatory, anti-oxidant, and hypotriglyceridemic effects, its supplementation may beneficially affect the increased cardiovascular risk of healthy smokers.

Key words: Adult, C-reactive protein, healthy, human, placebo, inflammation, oxidative stress, randomized controlled trial, resveratrol, smokers, Total Antioxidant Status, triglycerides

Introduction

Tobacco smoking is one of the most prevalent addictive habits, and it continues to be the second major cause of death in the world [1]. The consequences of long-term tobacco exposure, which predisposes individuals to chronic systemic diseases, such as cardiovascular diseases, are: an oxidant-antioxidant imbalance with increased products of lipid peroxidation and the depletion of antioxidants, a low-grade systemic inflammatory state with elevated concentrations of C-reactive protein (CRP), fibrinogen, and interleukin-6, and greater total numbers of circulating T-lymphocytes, and endothelial dysfunction with higher values of circulating adhesion molecules (intracellular adhesion molecule-1, selectins), and plasminogen activator inhibitor type I [1-3]. The potential benefits of dietary phenolics for smokers have been previously demonstrated [4]. A high concentration of flavonoids and other polyphenols was measured in red wine; furthermore, the reduction in cardiovascular risk by grapes and grape products is well known (a phenomenon known as the "French Paradox"). Resveratrol is a polyphenolic compound composed of two phenolic rings connected by a double bond and is found in several plants, particularly in grapes [5]. It exists in two isoforms, *trans*-resveratrol and *cis*-resveratrol, and the trans-isomer is the more stable form [6]. A growing number of in vitro and animal studies have evaluated the beneficial properties of resveratrol [7-9]. The following activities have been identified for resveratrol: antioxidant, anti-inflammatory, anti-carcinogenic, anti-platelet aggregation, cardio-protective, neuro-protective, cartilage-protective, anti-aging activities. In addition, this compound has been shown to increase lifespan, act as an insulin sensitizer, reduce body weight, improve endothelial function, and mimic calorie restriction [7-10]. However, the number of published human clinical trials that have evaluated the in vivo effects of resveratrol is limited [10-11], although several ongoing trials at different stages are available in the clinical trials database [12]. The anti-inflammatory and antioxidant properties of resveratrol are particularly interesting, because these effects might account for many of the health benefits reported in laboratory models [10]. Ten individuals randomized to receive six weeks of an extract containing 40 mg resveratrol exhibited suppressed nuclear factor kappa B (NFκB) binding, decreased reactive oxygen species (ROS) generation, and reduced concentrations of tumor necrosis factor alpha, interleukin-6, and CRP with respect to the individuals receiving the placebo [13]. Similarly, a nutritional supplement containing resveratrol was found to have an acute anti-inflammatory and antioxidant effect after the ingestion of a high-fat, high-carbohydrate meal in 10 healthy females [14]. Resveratrol inhibits both the basal and stimulated release of inflammatory cytokines by alveolar macrophages in smokers [15]. To the best of our knowledge, no clinical trial on smokers has been performed to date.

115 This study tested the hypothesis that resveratrol when given orally to healthy adult smokers, induces a
116 decrease in the levels of the inflammatory and oxidative mediators that characterize the low-grade systemic
117 inflammatory state and the oxidant-antioxidant imbalance in smokers.
118
119 **Methods**
120 *Recruitment of participants*
121 Fifty eligible healthy volunteers aged 20-50 years were recruited among individuals living in Piedmont
122 (Northern Italy) in July 2011 - March 2012. The inclusion criteria were as follows: aged 20-50 years, current
123 smoking (≥5 cigarettes/day and a smoking history of >20 packs/year), and mean alcohol consumption <30g/day.
124 The exclusion criteria were as follows: current pregnancy, known hyperglycemia, hypertension, cardiovascular
125 disease, impaired renal function, liver disease, or any other systemic chronic or acute conditions, the use of any
126 drug except estrogen, being on a particular diet, the use of vitamins, other nutrients or dietary supplements
127 during the previous six months, a body mass index (BMI)>30 kg/m^2, and an inability to give informed consent.
128 *Design*
129 This study was a randomized, double-blind, placebo-controlled, cross-over trial.
130 *Outcomes*
131 The primary outcome was the change in the circulating concentrations of CRP after resveratrol supplementation
132 relative to the change in the CRP concentrations after treatment with placebo. The secondary outcomes were the
133 differences after resveratrol relative to the change after placebo supplementation in the circulating fasting
134 concentrations of the following: total antioxidant status (TAS), uric acid, glucose, insulin, insulin resistance
135 [evaluated by the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index], total cholesterol,
136 high-density-lipoprotein (HDL)-cholesterol, triglycerides, aspartate aminotransferase (AST), alanine
137 aminotranferase (ALT), and γ-glutamyl transferase (GGT). In addition, differences in weight, waist
138 circumference, and arterial blood pressure were also explored.
139 *Intervention*
140 The subjects were randomly allocated into the "resveratrol-first" group or the "placebo-first" group. Subjects in
141 the "resveratrol-first" group received 30 days of treatment with Transmax® (resveratrol, 500 mg, Biotivia
142 Bioceuticals LLC, one tablet/day in the morning after fasting overnight); 30 days of wash-out (no
143 supplementation), and 30 days of treatment with placebo (one tablet/day in the morning after fasting overnight).
144 Subjects in the "placebo-first" group received 30 days of treatment with placebo (one tablet/day in the morning

after fasting overnight); 30 days of wash-out (no supplementation), and then 30 days of treatment with Transmax® (resveratrol, 500 mg, one tablet/day in the morning after fasting overnight). The researches who administered the tablets to the subjects were blinded to the patient treatment and treatment group.

Time schedule

Fasting blood samples were collected from all subjects in both groups at baseline, after 30-days, after 60-days, and at the end of the study, as detailed in **Figure 1.** After each blood sample was collected, the levels of the following were measured: CRP, TAS, uric acid, glucose, insulin, total cholesterol, HDL-cholesterol, triglycerides, AST, ALT, and GGT. Data related to health status, the use of drugs or supplements, usual dietary habits and exercise levels, weight, waist circumference and arterial blood pressure were collected from all subjects by trained researchers. A food-frequency questionnaire adapted from the EPIC (European Prospective Investigation into Cancer and Nutrition) questionnaire [16] and focused on dietary polyphenol intake was distributed to all subjects. Alcohol intake was assessed by multiplying the mean daily consumption for each beverage by the ethanol content, to give grams of alcohol/day (one can/bottle/glass of beer =13 g, one glass of wine =12 g, one standard drink of spirit =14 g). Each nutrient was adjusted for total energy using the residual method [17]. The exercise level was evaluated in all individuals using the Minnesota-Leisure-Time-Physical-Activity questionnaire [18].

Compliance with the study protocol and adverse events were monitored by phone calls and questionnaire recalls.

Sample size

At least a 30% reduction in CRP values should be detected, with a power of 80% and a two-tailed 0.05 α-value. Because the distribution of CRP was highly skewed, the log-transformed value of the CRP concentrations was used to estimate the sample size by the *t*-test for paired-data. Given that a 30%-reduction in the non-transformed CRP level corresponded to an absolute value reduction of –0.36 for log-CRP and that the standard deviation of log-CRP was 0.9 [13], the effect size to be tested was 0.4. A sample size of 50 subjects (25 in the "resveratrol-first" group and 25 in the "placebo-first" group) was required to obtain an 80% power and a two-tailed α-value of 0.05.

Randomization and allocation concealment

The random sequence of treatment (resveratrol/placebo or placebo/resveratrol) was computer-generated in the Epidemiology Unit, using blocks of different lengths (2 and 4) in random order. All subjects involved in the study had no access to the allocation sequence until the end of the statistical analyses.

Randomization implementation and blinding

In accordance with the random sequence, a person who did not take part in the study prepared the bottles for the participants, by putting the tablets of resveratrol and placebo into identical bottles and then applying labels to identify the participants, and a number (1 or 2) according to the sequence in which the subject should consume the tablets in each bottle. The participants and the researchers who interviewed and visited the subjects were blinded to the contents of the bottles. All laboratory measurements were centralized and performed in a blinded manner.

Ethical considerations

All procedures were in compliance with the principles of the Helsinki Declaration. The study protocol was approved by the local ethics committee. All participants provided written informed consent to participate in the study.

Measurements

Serum CRP values were determined using a high-sensitivity latex agglutination assay on HITACHI 911 Analyzer (Sentinel Ch., Milan). The intra-assay and inter-assay coefficients of variation (CVs) were 0.8-1.3% and 1.0-1.5%, respectively. The TAS measurements were performed with a colorimetric assay (ImAnOx TAS Kit, Immundiagnostik AG Bensheim, Germany). The serum glucose level was measured by the glucose oxidase method, and the uric acid, plasma total and HDL-cholesterol, triglyceride, and GGT values by enzymatic colorimetric assay (HITACHI 911 Analyzer, Sentinel Ch., Milan). The serum insulin level was determined using a solid phase enzyme-linked immunosorbent assay kit (LDN, Germany; intra-assay CV: 1.8-2.6%, inter-assay CV: 3.0-6.0%). The AST and ALT values were evaluated with a kinetic determination (HITACHI 911 Analyzer). The HOMA-IR was calculated according to the published algorithm [19].

Statistical analyses

The baseline clinical and laboratory variables are reported using mean and standard deviation (SD) or, for skewed distributions, median and inter-quartile range. The CRP, insulin, triglyceride, HOMA-IR, AST, ALT, and GGT values were logarithmically transformed to approximate normal distributions.

The supplementation effect (Δ) on each variable was defined as the within subject difference between the variable value at the end of resveratrol supplementation and the variable value at the end of placebo administration. In particular, the difference between variable values at blood collection 2 and at blood collection 4 for resveratrol-first group, and the difference between variable values at blood collection 4 and at blood

collection 2 for placebo-first group (Figure 1) were evaluated. The standardized distributions of the supplementation effects (Δ/(standard deviation (Δ)), were represented using box-plots.

General linear models (GLM) with patients as random effects were performed to assess possible period and carry-over effects and to estimate crude and adjusted supplementation effects and 95% confidence intervals (CI). To facilitate the interpretation of log-transformed variables, only those variables were expressed as the ratio of the variable value at the end of the resveratrol supplementation period to the variable value at the end of the placebo treatment period, calculated as exponential of the difference in the logarithmic values.

To assess the baseline imbalance, a sensitivity analysis was performed: the effect of resveratrol supplementation was estimated by using the covariance analysis, adjusting by the baseline within-subject differences.

Statistical analyses were performed using Stata 11.2 (StataCorp LP, College Station, Texas).

Results

Participant flow

Of the 25 participants in the "resveratrol-first" group, 1 was lost during follow-up (he moved away). No participant discontinued supplementation or was lost during follow-up in the "placebo-first" group. Data from 49 participants were thus analyzed. The flow diagram of the trial is presented in **Figure 2**.

Baseline data

The baseline clinical and laboratory characteristics of all the enrolled participants by group are shown in **Table 1**. No meaningful difference was evident between the two groups. The habitual nutrient intake patterns, particularly the estimated resveratrol intakes, were very similar between the two groups.

Outcomes and estimation

The standardized differences between the value of each variable at the end of the resveratrol supplementation period and the value of the variable at the end of the placebo treatment period are reported in the box-plot in **Figure 3**. The CRP and triglyceride concentrations decreased, whereas the TAS values increased after resveratrol supplementation; the other variables exhibited minor changes.

Period and carry-over effects were tested for all variables using GLM and the results were not statistically significant. The crude and adjusted effects of resveratrol supplementation did not differ; therefore, only the adjusted effects are reported. The CRP and triglyceride concentrations were significantly reduced, and the TAS values increased after resveratrol supplementation (**Table.2**). The estimates did not change after performing a covariance analysis adjusted for the baseline values of the variables.

Adverse events

No adverse events were reported in either groups after supplementation.

Discussion

In healthy smokers, a short period of supplementation with resveratrol exerted anti-oxidant effects and induced a significant reduction in the CRP and triglyceride concentrations, but there were no changes in weight, waist circumference, blood pressure, or other metabolic variables. Intriguingly, the beneficial changes occurred in healthy individuals with baseline laboratory variables within the reference range. It is worth testing this hypothesis in smokers with a chronic inflammatory condition, such as chronic obstructive pulmonary disease.

Anti-inflammatory effects

Both the acute and chronic anti-inflammatory effects of resveratrol have been demonstrated in 10 healthy subjects [13-14]. However, a phenolic compound containing resveratrol plus vitamin D3, quercetin, and rice bran phytate did not significantly affect the levels of inflammatory markers in 34 dysmetabolic patients [20], and 150 mg/day of resveratrol did not reduce the CRP level (although it did reduce tumor-necrosis-factor α) in 11 obese men [21]. The results were difficult to compare because different preparations were used with different resveratrol concentrations: 40mg [13], 100mg [14, 20], and 150mg [21]. Indeed, a mixture containing resveratrol plus green tea extract, polyunsaturated fatty acids, vitamins, and tomato extract [22], a polyphenol-rich grape preparation [23], and a grape extract [24] have been shown to exert significant anti-inflammatory effects in overweight or high-risk patients. These compounds contained a low resveratrol concentration (<10mg), but also other bioactive substances.

We found a reduction in the CRP concentrations of approximately 50% after one month of resveratrol supplementation. This effect was superior to the 26% decrease in the CRP values found after one year of supplementation with a grape nutraceutical containing 8 mg resveratrol [24]. Therefore, it could be hypothesized that resveratrol has a dose-dependent ability to decrease the levels of stimulatory cytokines which affect the release of CRP from the liver.

Long-term cigarette smoking determines a persistent inflammatory response in the lung that leads to tissue damage and dysfunction [25]. CRP, a marker of low grade chronic systemic inflammation, is highly predictive of the subsequent risk of cardiovascular events, diabetes and the metabolic syndrome in apparently healthy men and women, and it is increasingly integrated into cardiovascular risk assessment strategies [26-27]. Given the role of CRP, the identification of strategies that lead to risk reduction in smokers is worth attention. The release of

inflammatory cytokines by bronchoalveolar lavage fluid macrophages isolated from smokers and patients with chronic obstructive pulmonary disease was significantly inhibited by resveratrol, thus potentially leading to the inhibition of neutrophilia and reduced inflammatory cytokine levels in the airways of these patients [15]. Intriguingly, resveratrol proved more effective than corticosteroids under the same experimental conditions [15].

The cellular effects of resveratrol are quite complex [28]. It interacts with multiple receptors and enzymes, and in particular, it stimulates the activities of sirtuin 1 (SIRT1) and adenosine monophosphate-activated protein kinase, both of which regulate metabolism in many tissues. Resveratrol also inhibits cyclooxygenases, adhesion molecules, inducible NO synthase, and activated immune cells, as extensively reviewed elsewhere [10,29-31]. The mechanisms by which resveratrol exerts its anti-inflammatory effects in humans may include the following: the increased expression of SIRT1, with a subsequent reduction in the expression of phosphotyrosine phosphatase-1B, which is induced by inflammation [13]; the suppression of the intranuclear binding of NFκB, the major pro-inflammatory transcription factor [13, 22] or the activator protein-1; the suppression of the expression of two major pro-inflammatory kinases (jun-N-terminal kinase-1 and inhibitor of κB-kinase) [13]; the suppression of cytokine signaling 3 [13-14], and of pro-inflammatory cytokines by mononuclear cells [13-14]; the increase in the level of anti-inflammatory eicosanoid production [22]; the up-regulation of anti-inflammatory genes; and the decreased expression of pro-inflammatory genes [21,23]. Therefore, the activity of resveratrol cannot be ascribed to a single mechanism of action.

Anti-oxidant effects

A few human studies [13-14,22-23,32] have confirmed the antioxidant effects of resveratrol that were previously found in experimental or animal studies [33-34]. One of many mechanisms may be responsible: the direct [35] and indirect suppression of lipid oxidation; a direct reaction with ROS and an interaction with the enzymatic pathways involved in ROS generation [13,36]; the induction of the transcription factor -nuclear factor (erythroid-derived 2)-like 2 (Nrf-2)- which activates the transcription of a series of antioxidant genes [14]; or the down-regulation of the expression of pro-oxidant genes [22]. In addition, a pro-oxidant activity has reported for resveratrol, and this activity is cell-type dependent [6]. In airway cells, resveratrol helps counteract the oxidative stress generated by cigarette smoking by inducing Nrf2 activation, leading to greater antioxidant defense [37]. Furthermore, in lungs exposed to smoke, the SIRT1 levels are decreased and undergo post-traslational oxidative/nitrosative modifications [33] and the histone deacetylase activity (which is inhibited by oxidative stress and is responsible for the reduced responsiveness to glucocorticoids in smokers) is decreased [6]. By

activating of SIRT1 and modulating histone deacetylase activity, resveratrol can attenuate smoke-induced damage [6,33].

Change in the triglyceride concentrations

Resveratrol supplementation significantly reduced the triglyceride levels in our study population. Significant changes in the concentrations of medium and long chain triglycerides, decreased apolipoprotein C-III (apo CIII) and hepatic acyl-CoA cholesterol acyl-transferase activity, and the up-regulation of genes involved in lipid metabolism, resulting in a reduction in plasma triglycerides, have been observed after resveratrol supplementation [22-23]. Timmers has hypothesized that fat is liberated from peripheral depots to be metabolized by the muscle after resveratrol supplementation, as suggested by the increased intramyocellular lipid levels, improved muscle fat oxidative capacity, and reduced intrahepatic lipid content and plasma triglyceride concentrations [21]. Thus, resveratrol has been suggested to mimic the effects of endurance training [21].

Other variables

We did not find any effects of resveratrol on other metabolism-related variables. Increased HDL-cholesterol and apolipoprotein A1 (apo A-1) values [22,24], reduced LDL-cholesterol levels [23-24], decreased oxidized-LDL [24] and glucose concentrations [23], improved insulin sensitivity [38], reduced arterial blood pressure and reduced hepatic liver content [21] have reported. However, other authors did not find any effects on body weight [20-21], blood pressure, insulin resistance, the lipid profile [20], or the glucose [24], and insulin values [38]. These differences might be due to the preparations used, with contained different concentrations of resveratrol or other substances (e.g. fish oil, green tea, antioxidant vitamins), the different durations of the follow-up, and, above all, the different populations studied. Other cohorts included overweight [21-24], hypertensive [24], or diabetic [24,37] individuals. Therefore, it could be more difficult to improve values that are already within the reference range at baseline, as in the case of our patients. We observed minor variations in the liver enzyme values, in line with the results of another study [24], suggesting that resveratrol does not harm the liver.

Limitations

We could not evaluate compliance with the study protocol, because plasma resveratrol concentrations were not measured. Nevertheless, the variations in the TAS values, which were measured in a blind manner, were consistent with the use of resveratrol or placebo according to the study protocol. The short follow-up period prevented us from reaching conclusions about the long-term effects and safety of resveratrol. However, a one-year supplementation study with 8 mg resveratrol reported no adverse events [24] and a short-term study with

high doses (2.5-5g/day) found minor gastrointestinal effects [39]. Four-weeks of supplementation with 1g of resveratrol modulated the enzyme systems involved in detoxification, which could potentially lead to adverse reactions or altered efficacy of drugs [40]. The optimal dose of resveratrol has yet to be established in human studies, as recently reported in a systematic review [41].

Conclusions

These results add a small piece to the published evidence about the potential health benefits of resveratrol in humans, but clearly indicate the need for further trials in patients with chronic diseases or conditions, before this substance can be recommended for disease prevention or treatment in smokers.

Abbreviations: alanine aminotranferase (ALT), aspartate aminotransferase (AST), body mass index (BMI), coefficient of variation (CV), confidence intervals (CI), C-reactive protein (CRP), European Prospective Investigation into Cancer and Nutrition(EPIC), γ-glutamyl transferase (GGT), high-density cholesterol (HDL), homeostasis model assessment of insulin resistance (HOMA-IR), general linear models (GLM), nuclear factor kappa B (NFκB), reactive oxygen species (ROS), standard deviations (SD), Total Antioxidant Status (TAS).

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Conflict of interest.

NONE

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Figure.1 Time schedule of the study

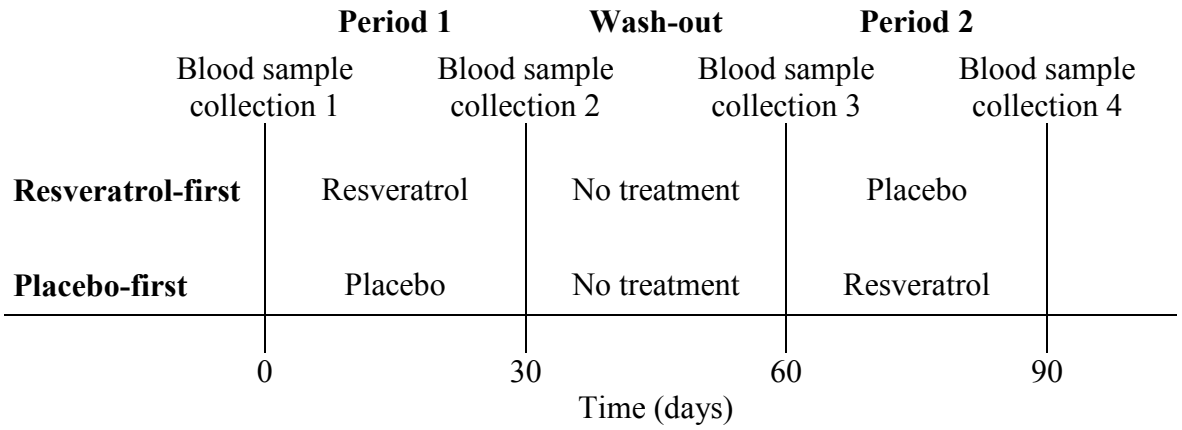


Figure.2 Flow of the participants.

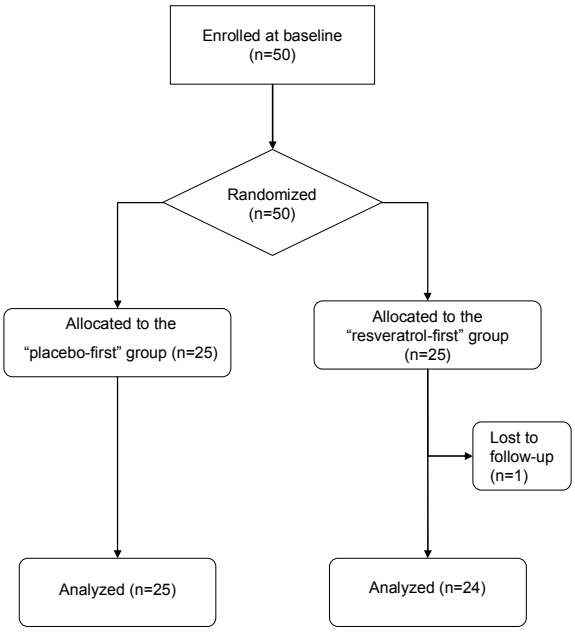


Table 1. Baseline clinical and laboratory characteristics of the enrolled patients.

	Total	Placebo-first	Resveratrol-first
Age (years)	35.0 [9.8][2]	35.4 [10.4]	34.7 [9.4]
Males (%)	15 [34.0]	8 [36.0]	7 [32.0]
Dietary variables[1]:			
Energy (Kcal/day)	2089.6 [692.1]	2070.5 [497.8]	2108.7 [854.1]
Protein (% energy)	16.3 [2.4]	16.3 [2.3]	16.2 [2.5]
Carbohydrates (% energy)	47.1 [7.6]	46.9 [8.7]	47.2 [6.5]
Fiber (g/day)	25.7 [10.4]	24.8 [7.9]	26.6 [12.5]
Alcohol (g/day)	13.5 [7.4]	13.5 [7.1]	13.6 [7.8]
Resveratrol (mg/day)	0.9 [1.0]	0.9 [0.9]	0.9 [1.1]
Metabolic equivalent task (h/week)	78.5 [50.7]	78.3 [49.8]	78.6 [52.3]
Years of smoking	18.6 [10.4]	18.6 [11.2]	18.5 [9.8]
CRP (mg/L)[3]	0.8 [1.6]	0.8 [1.3]	0.8 [1.8]
TAS (μmol/L)	256.8 [42.5]	253.5 [41.3]	260.2 [44.2]
Uric acid (mg/dL)	3.5 [0.9]	3.4 [1.0]	3.5 [0.9]
Fasting glucose (mg/dL)	84.8 [9.7]	84.6 [8.1]	84.9 [11.2]
Fasting insulin (μU/mL) [3]	7.4 [3.1]	7.8 [4.2]	7.4 [2.4]
HOMA-IR (mmol/L x μU/mL) [3]	1.5 [0.7]	1.6 [0.8]	1.5 [0.8]
Total cholesterol (mg/dL)	199.2 [37.7]	198.5 [35.3]	199.9 [40.7]
HDL cholesterol (mg/dL)	50.2 [10.6]	50.8 [10.4]	49.7 [11.1]
Triglycerides (mg/dL) [3]	78.0 [46.0]	77.0 [28.0]	90.0 [56.0]
AST (U/L) [3]	28.0 [13.0]	27.0 [14.0]	29.0 [9.0]
ALT (U/L) [3]	18.0 [10.0]	18.0 [10.0]	19.0 [10.0]
GGT (U/L) [3]	17.0 [6.0]	18.0 [5.0]	17.0 [8.0]
Weight (kg)	65.4 [11.7]	66.3 [13.8]	64.5 [9.4]
Body mass index (kg/m^2)	23.0 [3.4]	23.1 [3.6]	23.0 [3.2]
Waist circumference (cm)	79.2 [10.6]	80.2 [12.5]	78.2 [8.4]
Systolic pressure (mmHg)	117.8 [10.3]	118.4 [11.2]	117.2 [9.5]
Diastolic pressure (mmHg)	76.4 [7.0]	75.7 [6.6]	77.0 [7.5]

[1]Nutrient dietary intake was energy-adjusted [2] Mean [SD] (all such values, with the exception of variables marked with[3]) [3] Median [inter-quartile range] Alanine aminotranferase (ALT); aspartate aminotransferase (AST); C-reactive protein (CRP); γ-glutamyl transferase (GGT).

Table 2. Adjusted estimated effects of resveratrol supplementation as difference[1] (left) and ratio (right) from effects of placebo administration.

	Effects (difference)	95% CI	p-value	Effects (ratio)	95% CI
CRP [2]	-0.75	[-0.97,-0.54]	<0.001	0.47	[0.38,0.59]
TAS	74.2	[60.8,87.6]	<0.001		
Uric acid	-0.10	[-0.42,0.22]	0.53		
Fasting glucose	-2.6	[-6.3,0.99]	0.15		
Fasting insulin[1]	-0.06	[-0.14,0.02]	0.14	0.94	[0.87,1.0]
HOMA-IR[2]	-0.10	[-0.21,0.01]	0.07	0.91	[0.81,1.0]
Total cholesterol	0.03	[-7.2,7.3]	0.99		
HDL cholesterol	-0.61	[-2.9,1.7]	0.59		
Triglycerides [2]	-0.35	[-0.44,-0.25]	<0.001	0.71	[0.65,0.78]
AST[2]	-0.06	[-0.14,0.02]	0.15	0.94	[0.87,1.0]
ALT[2]	-0.06	[-0.18,0.06]	0.31	0.94	[0.83,1.1]
GGT[2]	0.01	[-0.04,0.06]	0.82	1.0	[0.96,1.1]
Weight	0.23	[-0.29,0.76]	0.39		
BMI	0.07	[-0.10,0.24]	0.44		
Waist circumference	-0.14	[-0.98,0.71]	0.75		
Systolic pressure	1.1	[-2.0,4.1]	0.51		
Diastolic pressure	0.17	[-1.5,1.8]	0.84		

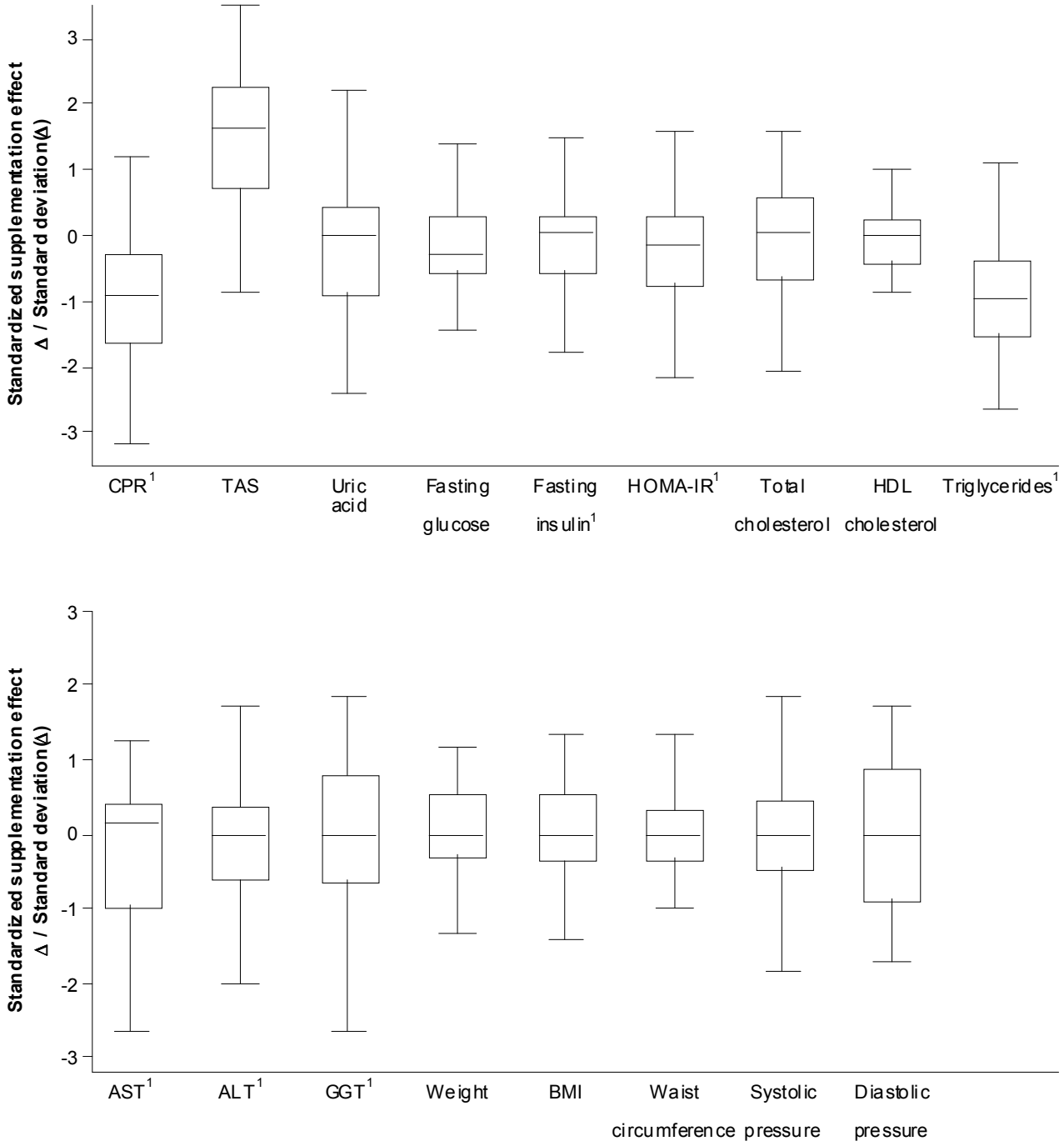
[1]Adjusted estimated effects of resveratrol supplementation as difference (left) and ratio (right) from effects of placebo administration; 95% CI and p-values were estimated by general linear models with patients as random effects, adjusted for period and carry-over effects.

[2] log-transformed variable

Alanine aminotranferase (ALT); aspartate aminotransferase (AST); C-reactive protein (CRP); γ-glutamyl transferase (GGT).

Legend to Figure 3.

Box-plots of standardized supplementation effects (Δ/(standard deviation (Δ)); 5th and 95th percentile (⊥), upper and lower quartile (□); median (—); [1]log-transformed values.



[1] Log-trasformed