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

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

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

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

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NONE

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58 **Abstract**

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

63 *Methods and Results:* A randomized, double-blind, cross-over trial was performed in 50 healthy adult smokers:  
64 25 were randomly allocated to “resveratrol-first” (30-days: 500mg resveratrol/day, 30-days wash-out, 30-days  
65 placebo) and 25 to “placebo-first” (30-days placebo, 30-days wash-out, 30-days 500mg resveratrol/day).

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

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

74

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

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## 86 Introduction

87 Tobacco smoking is one of the most prevalent addictive habits, and it continues to be the second major cause of  
88 death in the world [1]. The consequences of long-term tobacco exposure, which predisposes individuals to  
89 chronic systemic diseases, such as cardiovascular diseases, are: an oxidant-antioxidant imbalance with increased  
90 products of lipid peroxidation and the depletion of antioxidants, a low-grade systemic inflammatory state with  
91 elevated concentrations of C-reactive protein (CRP), fibrinogen, and interleukin-6, and greater total numbers of  
92 circulating T-lymphocytes, and endothelial dysfunction with higher values of circulating adhesion molecules  
93 (intracellular adhesion molecule-1, selectins), and plasminogen activator inhibitor type I [1-3]. The potential  
94 benefits of dietary phenolics for smokers have been previously demonstrated [4]. A high concentration of  
95 flavonoids and other polyphenols was measured in red wine; furthermore, the reduction in cardiovascular risk by  
96 grapes and grape products is well known (a phenomenon known as the “French Paradox”). Resveratrol is a  
97 polyphenolic compound composed of two phenolic rings connected by a double bond and is found in several  
98 plants, particularly in grapes [5]. It exists in two isoforms, *trans*-resveratrol and *cis*-resveratrol, and the *trans*-  
99 isomer is the more stable form [6]. A growing number of in vitro and animal studies have evaluated the  
100 beneficial properties of resveratrol [7-9]. The following activities have been identified for resveratrol:  
101 antioxidant, anti-inflammatory, anti-carcinogenic, anti-platelet aggregation, cardio-protective, neuro-protective,  
102 cartilage-protective, anti-aging activities. In addition, this compound has been shown to increase lifespan, act as  
103 an insulin sensitizer, reduce body weight, improve endothelial function, and mimic calorie restriction [7-10].  
104 However, the number of published human clinical trials that have evaluated the in vivo effects of resveratrol is  
105 limited [10-11], although several ongoing trials at different stages are available in the clinical trials database  
106 [12]. The anti-inflammatory and antioxidant properties of resveratrol are particularly interesting, because these  
107 effects might account for many of the health benefits reported in laboratory models [10]. Ten individuals  
108 randomized to receive six weeks of an extract containing 40 mg resveratrol exhibited suppressed nuclear factor  
109 kappa B (NFκB) binding, decreased reactive oxygen species (ROS) generation, and reduced concentrations of  
110 tumor necrosis factor alpha, interleukin-6, and CRP with respect to the individuals receiving the placebo [13].  
111 Similarly, a nutritional supplement containing resveratrol was found to have an acute anti-inflammatory and  
112 antioxidant effect after the ingestion of a high-fat, high-carbohydrate meal in 10 healthy females [14].  
113 Resveratrol inhibits both the basal and stimulated release of inflammatory cytokines by alveolar macrophages in  
114 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 ( $\geq 5$  cigarettes/day and a smoking history of  $>20$  packs/year), and mean alcohol consumption  $<30$ g/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<sup>2</sup>, 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  $\gamma$ -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

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

#### 148 *Time schedule*

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

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

#### 162 *Sample size*

163 At least a 30% reduction in CRP values should be detected, with a power of 80% and a two-tailed 0.05  $\alpha$ -value.  
164 Because the distribution of CRP was highly skewed, the log-transformed value of the CRP concentrations was  
165 used to estimate the sample size by the *t*-test for paired-data. Given that a 30%-reduction in the non-transformed  
166 CRP level corresponded to an absolute value reduction of -0.36 for log-CRP and that the standard deviation of  
167 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-  
168 first” group and 25 in the “placebo-first” group) was required to obtain an 80% power and a two-tailed  $\alpha$ -value  
169 of 0.05.

#### 170 *Randomization and allocation concealment*

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

174 *Randomization implementation and blinding*

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

181 *Ethical considerations*

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

185 *Measurements*

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

195 *Statistical analyses*

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

199 The supplementation effect ( $\Delta$ ) on each variable was defined as the within subject difference between the  
200 variable value at the end of resveratrol supplementation and the variable value at the end of placebo  
201 administration. In particular, the difference between variable values at blood collection 2 and at blood collection  
202 4 for resveratrol-first group, and the difference between variable values at blood collection 4 and at blood



203 collection 2 for placebo-first group (Figure 1) were evaluated. The standardized distributions of the  
204 supplementation effects ( $\Delta$ /(standard deviation ( $\Delta$ )), were represented using box-plots.  
205 General linear models (GLM) with patients as random effects were performed to assess possible period and  
206 carry-over effects and to estimate crude and adjusted supplementation effects and 95% confidence intervals (CI).  
207 To facilitate the interpretation of log-transformed variables, only those variables were expressed as the ratio of  
208 the variable value at the end of the resveratrol supplementation period to the variable value at the end of the  
209 placebo treatment period, calculated as exponential of the difference in the logarithmic values.  
210 To assess the baseline imbalance, a sensitivity analysis was performed: the effect of resveratrol supplementation  
211 was estimated by using the covariance analysis, adjusting by the baseline within-subject differences.  
212 Statistical analyses were performed using Stata 11.2 (StataCorp LP, College Station, Texas).

213

## 214 **Results**

### 215 *Participant flow*

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

### 219 *Baseline data*

220 The baseline clinical and laboratory characteristics of all the enrolled participants by group are shown in **Table**  
221 **1**. No meaningful difference was evident between the two groups. The habitual nutrient intake patterns,  
222 particularly the estimated resveratrol intakes, were very similar between the two groups.

### 223 *Outcomes and estimation*

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

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

233 *Adverse events*

234 No adverse events were reported in either groups after supplementation.

235

236 **Discussion**

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

242 *Anti-inflammatory effects*

243 Both the acute and chronic anti-inflammatory effects of resveratrol have been demonstrated in 10 healthy  
244 subjects [13-14]. However, a phenolic compound containing resveratrol plus vitamin D3, quercetin, and rice  
245 bran phytate did not significantly affect the levels of inflammatory markers in 34 dysmetabolic patients [20], and  
246 150 mg/day of resveratrol did not reduce the CRP level (although it did reduce tumor-necrosis-factor  $\alpha$ ) in 11  
247 obese men [21]. The results were difficult to compare because different preparations were used with different  
248 resveratrol concentrations: 40mg [13], 100mg [14, 20], and 150mg [21]. Indeed, a mixture containing resveratrol  
249 plus green tea extract, polyunsaturated fatty acids, vitamins, and tomato extract [22], a polyphenol-rich grape  
250 preparation [23], and a grape extract [24] have been shown to exert significant anti-inflammatory effects in  
251 overweight or high-risk patients. These compounds contained a low resveratrol concentration (<10mg), but also  
252 other bioactive substances.

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

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

263 inflammatory cytokines by bronchoalveolar lavage fluid macrophages isolated from smokers and patients with  
264 chronic obstructive pulmonary disease was significantly inhibited by resveratrol, thus potentially leading to the  
265 inhibition of neutrophilia and reduced inflammatory cytokine levels in the airways of these patients [15].  
266 Intriguingly, resveratrol proved more effective than corticosteroids under the same experimental conditions [15].  
267 The cellular effects of resveratrol are quite complex [28]. It interacts with multiple receptors and enzymes, and in  
268 particular, it stimulates the activities of sirtuin 1 (SIRT1) and adenosine monophosphate-activated protein kinase,  
269 both of which regulate metabolism in many tissues. Resveratrol also inhibits cyclooxygenases, adhesion  
270 molecules, inducible NO synthase, and activated immune cells, as extensively reviewed elsewhere [10,29-31].  
271 The mechanisms by which resveratrol exerts its anti-inflammatory effects in humans may include the following:  
272 the increased expression of SIRT1, with a subsequent reduction in the expression of phosphotyrosine  
273 phosphatase-1B, which is induced by inflammation [13]; the suppression of the intranuclear binding of NFκB,  
274 the major pro-inflammatory transcription factor [13, 22] or the activator protein-1; the suppression of the  
275 expression of two major pro-inflammatory kinases (jun-N-terminal kinase-1 and inhibitor of κB-kinase) [13]; the  
276 suppression of cytokine signaling 3 [13-14], and of pro-inflammatory cytokines by mononuclear cells [13-14];  
277 the increase in the level of anti-inflammatory eicosanoid production [22]; the up-regulation of anti-inflammatory  
278 genes; and the decreased expression of pro-inflammatory genes [21,23]. Therefore, the activity of resveratrol  
279 cannot be ascribed to a single mechanism of action.

#### 280 *Anti-oxidant effects*

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

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

#### 294 *Change in the triglyceride concentrations*

295 Resveratrol supplementation significantly reduced the triglyceride levels in our study population. Significant  
296 changes in the concentrations of medium and long chain triglycerides, decreased apolipoprotein C-III (apo CIII)  
297 and hepatic acyl-CoA cholesterol acyl-transferase activity, and the up-regulation of genes involved in lipid  
298 metabolism, resulting in a reduction in plasma triglycerides, have been observed after resveratrol  
299 supplementation [22-23]. Timmers has hypothesized that fat is liberated from peripheral depots to be  
300 metabolized by the muscle after resveratrol supplementation, as suggested by the increased intramyocellular  
301 lipid levels, improved muscle fat oxidative capacity, and reduced intrahepatic lipid content and plasma  
302 triglyceride concentrations [21]. Thus, resveratrol has been suggested to mimic the effects of endurance training  
303 [21].

#### 304 *Other variables*

305 We did not find any effects of resveratrol on other metabolism-related variables. Increased HDL-cholesterol and  
306 apolipoprotein A1 (apo A-1) values [22,24], reduced LDL-cholesterol levels [23-24], decreased oxidized-LDL  
307 [24] and glucose concentrations [23], improved insulin sensitivity [38], reduced arterial blood pressure and  
308 reduced hepatic liver content [21] have reported. However, other authors did not find any effects on body weight  
309 [20-21], blood pressure, insulin resistance, the lipid profile [20], or the glucose [24], and insulin values [38].  
310 These differences might be due to the preparations used, with contained different concentrations of resveratrol or  
311 other substances (e.g. fish oil, green tea, antioxidant vitamins), the different durations of the follow-up, and,  
312 above all, the different populations studied. Other cohorts included overweight [21-24], hypertensive [24], or  
313 diabetic [24,37] individuals. Therefore, it could be more difficult to improve values that are already within the  
314 reference range at baseline, as in the case of our patients. We observed minor variations in the liver enzyme  
315 values, in line with the results of another study [24], suggesting that resveratrol does not harm the liver.

#### 316 *Limitations*

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

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

### 326 *Conclusions*

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

330

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

336

337

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341 analysis, or the manuscript preparation.

342

### 343 **Conflict of interest.**

344 NONE

## References

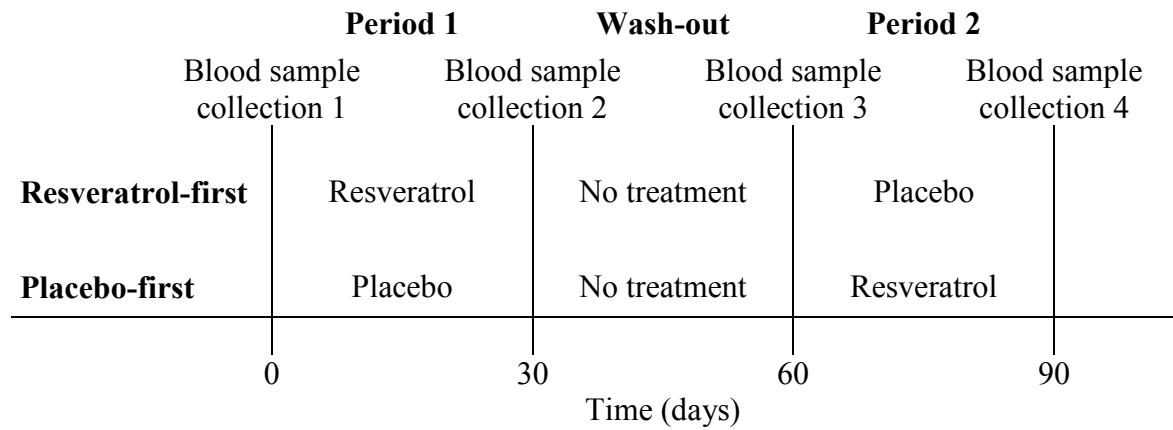
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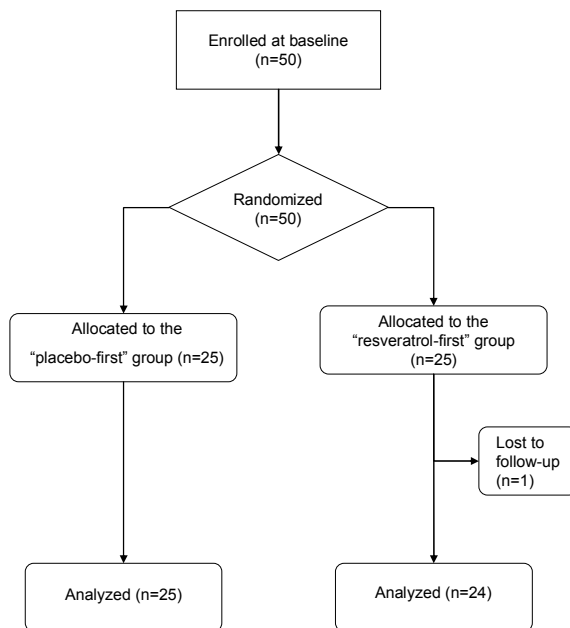
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**Figure.1 Time schedule of the study**

**Figure.2 Flow of the participants.**

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

	<b>Total</b>	<b>Placebo-first</b>	<b>Resveratrol-first</b>
Age (years)	35.0 [9.8] <sup>2</sup>	35.4 [10.4]	34.7 [9.4]
Males (%)	15 [34.0]	8 [36.0]	7 [32.0]
Dietary variables <sup>1</sup> :			
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) <sup>3</sup>	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) <sup>3</sup>	7.4 [3.1]	7.8 [4.2]	7.4 [2.4]
HOMA-IR (mmol/L x μU/mL) <sup>3</sup>	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) <sup>3</sup>	78.0 [46.0]	77.0 [28.0]	90.0 [56.0]
AST (U/L) <sup>3</sup>	28.0 [13.0]	27.0 [14.0]	29.0 [9.0]
ALT (U/L) <sup>3</sup>	18.0 [10.0]	18.0 [10.0]	19.0 [10.0]
GGT (U/L) <sup>3</sup>	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 <sup>2</sup> )	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]

<sup>1</sup>Nutrient dietary intake was energy-adjusted <sup>2</sup> Mean [SD] (all such values, with the exception of variables marked with <sup>3</sup>) <sup>3</sup> 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<sup>1</sup> (left) and ratio (right) from effects of placebo administration.**

	<b>Effects (difference)</b>	<b>95% CI</b>	<b>p-value</b>	<b>Effects (ratio)</b>	<b>95% CI</b>
CRP <sup>2</sup>	-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 <sup>1</sup>	-0.06	[-0.14,0.02]	0.14	0.94	[0.87,1.0]
HOMA-IR <sup>2</sup>	-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 <sup>2</sup>	-0.35	[-0.44,-0.25]	<0.001	0.71	[0.65,0.78]
AST <sup>2</sup>	-0.06	[-0.14,0.02]	0.15	0.94	[0.87,1.0]
ALT <sup>2</sup>	-0.06	[-0.18,0.06]	0.31	0.94	[0.83,1.1]
GGT <sup>2</sup>	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		

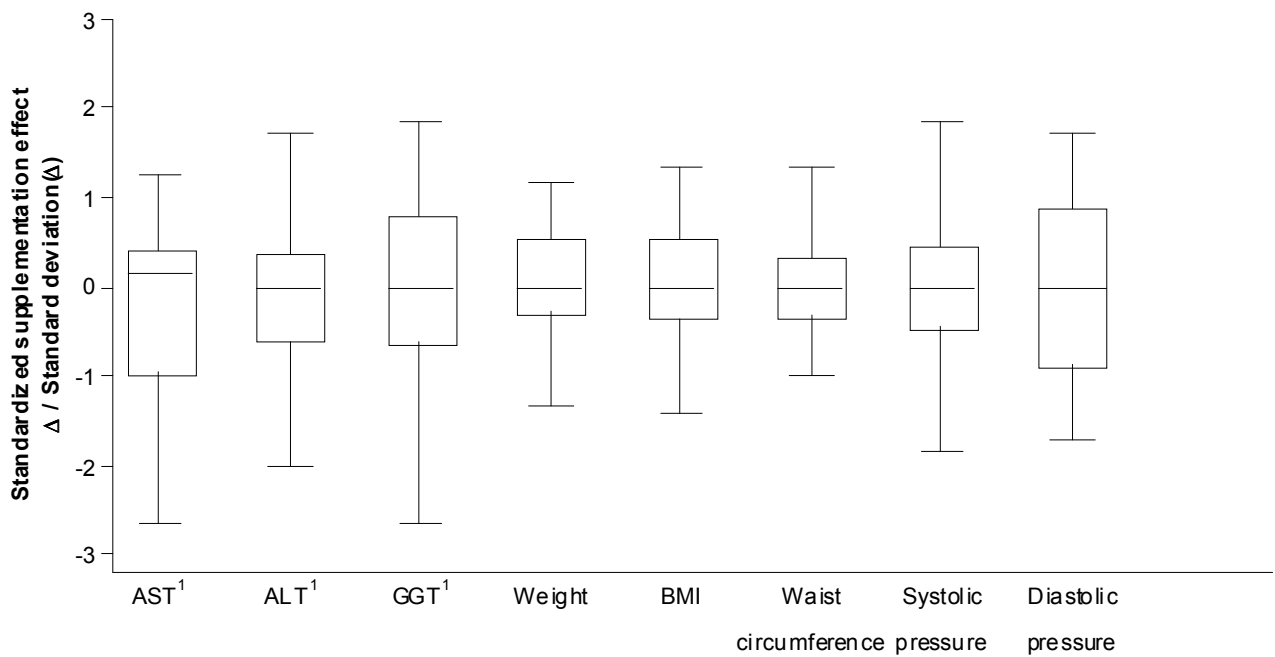
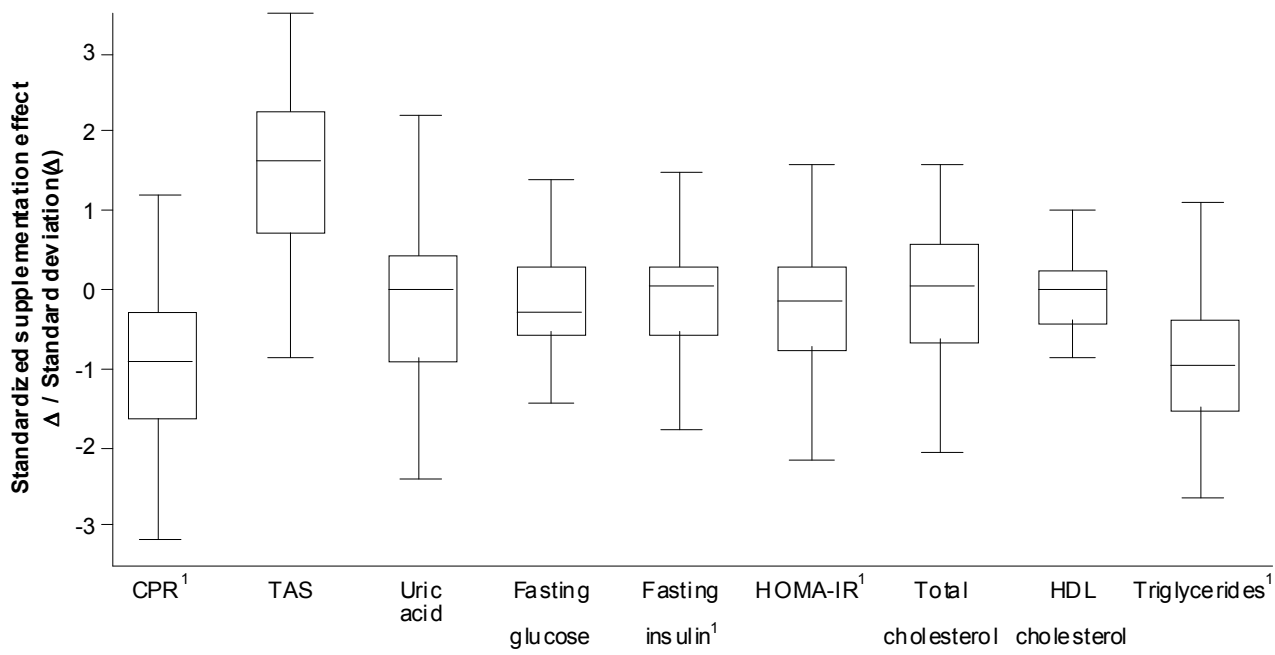
<sup>1</sup> Adjusted estimated effects of resveratrol supplementation as difference (left) and ratio (right) from effects of placebo administration; 95% CI and p-values were estimated by general linear models with patients as random effects, adjusted for period and carry-over effects.

<sup>2</sup> log-transformed variable

Alanine aminotransferase (ALT); aspartate aminotransferase (AST); C-reactive protein (CRP);  $\gamma$ -glutamyl transferase (GGT).

**Legend to Figure 3.**

Box-plots of standardized supplementation effects ( $\Delta$ /(standard deviation ( $\Delta$ ))); 5th and 95th percentile ( $\perp$ ), upper and lower quartile ( $\square$ ); median ( $—$ ); <sup>1</sup>log-transformed values.



<sup>1</sup> Log-transformed