The effects of resveratrol supplementation on PPARα, p16, p53, p21 gene expressions, and sCD163/sTWEAK ratio in patients with type 2 diabetes mellitus: A double-blind controlled randomized trial

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The Effects of Resveratrol Supplementation on *PPARα, p16, p53, p21* Gene Expressions, and sCD163/sTWEAK ratio in Patients with Type 2 Diabetes Mellitus: A Double-Blind Controlled Randomized Trial

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ABSTRACT

The present study sought to evaluate the effect of resveratrol supplementation on mRNA expression levels of peroxisome proliferator-activated receptor alpha (*PPARα*), *p53*, *p21*, *p16* and serum levels of cluster of differentiation 163 (CD163) to TNF-like weak inducer of apoptosis (TWEAK) ratio in patients with type 2 diabetes. In this double-blind randomized controlled trial, 71 patients were randomly assigned to receive either 1000 mg trans-resveratrol or placebo (methyl cellulose) for 8 weeks. Expression levels of genes of interest, and serum levels of sCD163 and sTWEAK, were assessed at baseline and end of the study. Resveratrol supplementation significantly increased mRNA expression levels of *p53* and *p21* genes, compared with the placebo group (fold change of *p53*=1.29, P=0.04; fold change of *p21*=1.46, P=0.006). However, no significant effect on expression levels of *PPARα* and *p16* genes were observed after supplementation. In addition, resveratrol significantly reduced serum levels of sCD163/sTWEAK ratio compared with the placebo group (P=0.003). Resveratrol supplementation resulted in significant changes in *p53* and *p21* genes expression, whilst serum levels of sCD163/sTWEAK ratio also improved in the resveratrol group, without any significant change in adjusted sCD163 levels. More research is needed to confirm the beneficial effects of resveratrol for patients with diabetes.

Keywords: *Cardiovascular Diseases; Resveratrol; sCD163; sTWEAK; Type 2 diabetes mellitus*

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major cause of morbidity and mortality, globally (Chen, Magliano, & Zimmet, 2012; Fowler, 2007; Whiting, Guariguata, Weil, & Shaw, 2011), and related hyperglycemia can result in widespread macro and micro-vascular complications, as well as atherosclerosis (Touchian et al., 2018). The incidence of intimal hyperplasia (IH), following atherosclerotic lesions formation, is prevalent in T2DM, and is associated with cardiovascular events; the most common cause of mortality in patients with diabetes (Fowler, 2008; Polak et al., 2011; O. Touchian, G. Sotoudeh, A. Mansoori, E. Nasli-Esfahani, et al., 2016). Recent studies have indicated that the proliferation of vascular smooth muscle cells (VSMCs), along with other underlying factors, are linked to IH (Florence Gizard et al., 2005a; Wright et al., 2011).

There are swathes of evidence suggesting that one of the nuclear receptors superfamily, named peroxisome proliferator-activated receptors (PPARs), can modulate the proliferation of VSMCs (F. Gizard et al., 2005; F. Gizard et al., 2008). PPARα -as a member of this family- has critical roles in fatty acid oxidation, glucose metabolism, vascular function, plaque stability, and cell proliferation (Chinetti-Gbaguidi, Fruchart, & Staels, 2005; Omid Touchian et al., 2016). In previous animal (Florence Gizard et al., 2005b; Florence Gizard et al., 2008) and human studies (Touchian et al., 2018; O. Touchian, G. Sotoudeh, A. Mansoori, M. Djalali, et al., 2016), the role of activated PPARα in the inhibition of VSMCs proliferation has been proposed through the tumor suppressor *p16* gene. P16, or cyclin-dependent kinase inhibitor 2A, can bind and inhibit the action of D-type cyclin-dependent

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85   kinases (CDK4 and CDK6) and arrest the cell cycle in the transition from G_1 to S phase

86   (Sherr, Beach, & Shapiro, 2016).

87   Resveratrol (3,5,4′-trihydroxy-trans-stilbene), a phytoalexin polyphenol found abundantly

88   in red grapes and nuts (Storniolo & Moreno, 2018), has been introduced as a natural

89   exogenous ligand for PPARα, and it appears capable of suppressing cell cycle progression

90   through the p16 pathway (Inoue et al., 2003; Qin, Lu, & Rodrigues, 2014; Takizawa et al.,

91   2015). Moreover, some studies have posited that this bioactive compound has a putative

92   role in phosphorylation and activation of p53 (Dong, 2003; She, Bode, Ma, Chen, & Dong,

93   2001). P53 is a tumor suppressor and anti-cancer agent that upregulates the cyclin-

94   dependent kinase inhibitor 1 (*p21*) gene, which results in the cell-cycle arrest at the S/G_2

95   phase (Cayrol, Knibiehler, & Ducommun, 1998; Coppé et al., 2008). Some studies have

96   addressed the potential role of p53 in the reduction of intimal thickness in animal models

97   (Chang, Barr, Lu, Barton, & Leiden, 1995; Guevara, Kim, Antonova, & Chan, 1999; Rosso

98   et al., 2006; Tanner et al., 1998).

99   Chronic inflammation caused by macrophage hyperactivity and overexpression of pro-

100   inflammatory cytokines occurs in diabetes, and especially in obese patients (Kern,

101   Ranganathan, Li, Wood, & Ranganathan, 2001). The cluster of differentiation 163 (CD163)

102   is a macrophage scavenger receptor which is involved in iron recycling and has anti-

103   inflammatory effects (Moestrup & Møller, 2004); indeed, CD163 has been acknowledged

104   as a neutralizing receptor for TNF-like weak inducer of apoptosis (TWEAK), a

105   proinflammatory cytokine which mediates its effects through the Fn14 receptor (Juan A

106   Moreno et al., 2009). Moreover, it has recently been proposed that serum levels of CD163

107   to TWEAK ratio (sCD163/sTWEAK) can be used as an indicator of the vascular disease

108   severity (Llaurado et al., 2012; Moreno et al., 2010; Grazina Urbonaviciene et al., 2011).

109   Despite existing evidence regarding the beneficial effects of resveratrol on cardiovascular

110   diseases, to our knowledge, there is no study that has investigated the effect of this

111   antioxidant on intimal hyperplasia through the proposed mechanisms. Thus, we sought to

112   perform a randomized clinical trial to determine the effect of resveratrol supplementation

113   on the genes expression of *PPARα*, *p16*, *p53*, *p21,* and the serum levels of

114   sCD163/sTWEAK ratio, in patients with T2DM.

115   **MATERIALS AND METHODS**

116   ***Study design and participants***

117   This research was an 8-week, double-blind, randomized controlled trial. This study was

118   conducted in complete agreement with the Helsinki declaration, and the protocol was

119   approved by the Medical Ethics Committee of Yazd University of Medical Sciences and

120   registered in the Iranian Registry of Clinical Trials (www.irct.ir: IRCT20171118037528N1).

121   Male and female volunteers, aged between 30 and 60 years, who had been diagnosed with

122   T2DM, and with body mass index (BMI) between 25 to 30 (kg m^{-2}), were eligible to

123   participate in the study. Patients were excluded if they i) were pregnant or lactating, ii) had a

124   history of any type of cancer, renal or liver failure, gastrointestinal ulcers, Alzheimer's

125   disease and, cardiovascular complications iii) had glycated hemoglobin above 8% or were

126   receiving insulin treatment, iv) consumed any antioxidant supplements (for example vitamin

127   C, E, A or fish oil supplements), anticoagulants, fibrate lipid-lowering agents and anti-

128   inflammatory drugs or drank alcoholic beverages (for at least 6 months before the study).

129 Eligible subjects were invited and, after providing further information, a consent form was

130 signed by willing patients. Stratified block randomization, based on sex and age (30-45 and

131 45-60 years), using computer-generated random numbers, was performed by an independent

132 statistician to allocate participants into intervention and control groups (in a 1:1 allocation

133 ratio).

134 ~~Since, it is~~Given that it has been reported that 1000 mg/day of resveratrol is well tolerated

135 and shows no toxic effects in patients with diabetes (Movahed et al., 2013; Thazhath et al.,

136 2016), participants were instructed to take 1000 mg/day (two 500 mg capsules) micronized

137 trans-resveratrol (Mega-Resveratrol, Danbury, USA) and methylcellulose (Barij essence,

138 Kashan, Iran), in intervention and control groups, respectively, for an 8-week treatment

139 period. Each capsule of resveratrol supplement provided 500 mg of 99.71% micronized

140 trans-resveratrol, with particle sizes lower than 1.9 μm, and free from inactive ingredients,

141 fillers, flavoring agents, and additives. Resveratrol and placebo capsules were completely

142 identical in shape, color, and taste, and packed in the same bottles. Filling and labeling of

143 the containers as A and B was performed by a person who was not involved in the process

144 of the study, and the content of the bottles remained unknown to the participants and

145 researchers. Patients were asked to bring back the containers with remaining capsules at the

146 end of the first month to calculate compliance. More information about the study

147 methodology has been published previously (Abdollahi, Salehi-Abargouei, et al., 2019).

148 Weight and body composition were measured at the beginning and end of the study, in an

149 overnight fasted state, using a body analyzer machine (Tanita BC-418, Tokyo, Japan).

150 Height, waist, and hip circumferences, were measured according to the standard protocol

151 (Kamal, 2006). Body mass index (BMI) was calculated mathematically, as weight (in kg),

152   divided by height in m^2. All participants were asked not to change their dietary habits or

153   physical activity during the study period. However, a physical activity questionnaire (MET)

154   (Aadahl & Jørgensen, 2003) and 3-day food records were obtained at week 0 and week 8 of

155   the study. Data from diet records were analyzed using Nutritionist IV software and

156   converted into macronutrient and micronutrient intakes. Dietary total flavonoid also was

157   calculated using the USDA Database for the Flavonoid Content of Selected Foods

158   (Bhagwat, Haytowitz, & Holden, 2014). Dietary intake, physical activity, as well as

159   anthropometric data have been reported previously (Abdollahi, Salehi-Abargouei, et al.,

160   2019).

161   *Biochemical measures*

162   After overnight fasting, a venous blood sample (10 ml) was collected at the beginning and

163   end of the study for biochemical and gene expression assessments. Aliquots of serum

164   samples were stored in −70°C after centrifugation (3000 × g, 10 min at room temperature;

165   Eppendorf AG, Hamburg). Serum levels of sCD163 and sTWEAK were assessed by

166   enzyme-linked immunoassay (ELISA) method according to the manufacturer protocol

167   (ZellBio, Germany; inter and intra-assay coefficient of variations were <12% and <10%,

168   respectively, for both sCD163 and sTWEAK). For gene expressions assessment, total RNA

169   was extracted directly from whole blood using GeneAll Hybrid-R RNA purification kit

170   (GeneAll Biotechnology Co., Seoul, South Korea). After checking the quality and purity

171   (260/280 nm ratio between 1.8 to 2.2) of the RNA (NanoDrop, Thermo Scientific, USA),

172   total mRNA was reverse-transcribed to the first-strand cDNA by cDNA synthesis kit

173   (GeneAll Biotechnology Co., Seoul, South Korea). The cDNA was amplified by real-time

174 polymerase chain reaction (RT-PCR), and SYBR Green method (Takara Bio Inc., Japan) to

175 determine the gene expression levels of *PPARα*, *p53*, *p21* and *p16* (Applied Biosystems,

176 USA). Glyceraldehyde phosphate dehydrogenase (GAPDH) was considered as a

177 housekeeping gene in all assessments. Three primer designing tools (Primer Blast,

178 Oligocalc, and Gene runner 5.0.99) were applied for sequencing the study primers **(Table**

179 **1)**. PCR efficacy and changes in the expression levels were tested using LinRegPCR

180 software (Robledo et al., 2014) and Pfaffl equation (Pfaffl, 2001), respectively.

181 *Statistical analysis*

182 As, at the time of study design, there were no human studies on the effect of resveratrol on

183 the interested gene expressions, the sample size was calculated based on a previous study

184 investigating the PPARα expression in peripheral blood mononuclear cells (PBMCs)

185 (D'Amore et al., 2013). Considering α=0.05, power of 80%, and a 20% drop-out rate, the

186 final sample size was set to be 36 participants in each group. However, a power analysis was

187 conducted to assess quantity of sample size for outcomes reported in current manuscript. The

188 results of the power analysis showed idealindicated sufficient power for all outcomes (Table

189 3, and Supplementary Table 3). Data entry and statistical analyses were performed using

190 SPSS for Windows (SPSS, Chicago, IL, USA), version 23.0. Data were presented as

191 proportions or mean ± standard deviation for categorical or continuous data, respectively. A

192 one-sample Kolmogorov-Smirnov test was conducted on each variable to test the normality

193 distribution of the data. Independent samples and paired samples t-tests were carried out for

194 comparison of continuous data between and within the study groups, respectively. Analysis

195 of covariance (ANCOVA) was applied to adjust the possible confounders (age, gender, and

196 baseline BMI) when assessing between-group differences. Last observation carried forward

197 imputation was applied to manage missing data (Little et al., 2012). Statistical significance

198 was accepted at P≤0.05, for all comparisons.

199 **RESULTS**

200 A total of 76 patients were enrolled in this 8-week intervention study. Five of the

201 participants (three patients in resveratrol group and two patients in the placebo group)

202 dropped out because of disinclination to continue participating in the study (n=3),

203 pregnancy (n=1), and traveling (n=1). Finally, 71 participants (35 patients in the resveratrol

204 group and 36 patients in the placebo group) completed the study (**Figure 1**). No significant

205 study-related adverse event was observed; although, two patients reported tolerable

206 gastrointestinal distress following resveratrol supplementation. The compliance rate of

207 participants was calculated 93.1% in the resveratrol group and 92.6% in the placebo group,

208 based on the residual capsules.

209 Baseline characteristics of the participants are summarized in **Table 2**. Lifestyle factors,

210 including dietary intake, total flavonoid, and physical activity remained unchanged during

211 the study (**data not shown**).

212 The effect of the intervention on relative changes in mRNA expression levels of *p16, p21,*

213 *p53,* and *PPARα* is detailed in **Figure 2**. The analyses revealed that there was a 1.29-fold

214 increase in *p53* gene expression levels (P= 0.04), and 1.46-fold increase in gene expression

215 levels of *p21* (P= 0.006), in the resveratrol group compared with the placebo group. However,

216 there were no significant differences between the two groups in the expression of *p16* and

217 *PPARα* genes.

218 Furthermore, in comparison with placebo, resveratrol supplementation yielded a significant

219 reduction in sCD163 levels in the unadjusted model (-176.85 ± 310.7 ng/ml, P= 0.04).

220 However, no significant change was observed after adjusting for age, gender, and baseline

221 BMI. We also found a significant increment in sTWEAK levels, in both unadjusted and

222 adjusted models, compared with the control group (394.2 ± 903.9 pg/ml, P<0.05). Moreover,

223 comparing the mean changes in sCD163/sTWEAK ratio highlighted significant differences

224 between two groups (-0.09 ± 0.13, P= 0.001); which remained unchanged after controlling

225 for possible confounders (P= 0.003) **(Table 3)**. The results remained stable when intention-

226 to-treat analysis was applied (**Supplementary Tables 2 and 3**)

227 **DISCUSSION**

228 The results of this study showed that 8-weeks resveratrol administration, in patients with

229 T2DM, upregulated mRNA expression of *p53* and *p21* genes, without any significant effect

230 on the expression of *PPARα* and *p16* genes. In addition, serum levels of sCD163 to

231 sTWEAK ratio significantly decreased as a result of resveratrol supplementation.

232 To our knowledge, no study has investigated the effect of resveratrol on genes expression of

233 *PPARα*, *p16*, *p53*, *p21,* and the serum levels of sCD163/sTWEAK ratio, in patients with

234 T2DM. However, there are some cell line studies in this regard; for instance, Zakar et al., in

235 a study on VSMCs, showed that resveratrol can stimulate the cellular signaling of p53 in both

236 the nucleus and cytoplasm (Z. H. Mnjoyan & K. Fujise, 2003). Another study suggested that

237 resveratrol activates p53 in a dose-dependent manner (Howitz et al., 2003), and it has been

238 proposed that acetylation and phosphorylation at the serine-15 residue of p53 by resveratrol

239 can increase the activity and stability of this tumor suppressor (She et al., 2001). Resveratrol

240 has also been shown to inhibit p53 deacetylation, which is mediated by NAD-dependent

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241 deacetylase sirtuin-1 (SIRT1) (Howitz et al., 2003). Similarly, this phenol can increase p21

242 levels in a dose-dependent manner, and seems to be related to elevated p53 and its signaling

243 pathways (Zakar H Mnjoyan & Ken Fujise, 2003). There is some evidence supporting the

244 effect of resveratrol in p53 activation and p21 -as its target gene- in cancerous cells (Lu, Ho,

245 Ghai, & Chen, 2001; Shih, Davis, Lin, & Davis, 2002), but the present study is the first to

246 propose the same changes in PBMC of patients with diabetes.

247 In our study, mRNA genes expression level of *PPARα* or *p16* did not change following

248 resveratrol supplementation. It has been reported that resveratrol can bind to the PPARs and

249 stimulate their transcription activities (Inoue et al., 2003), although the activation of PPARs

250 by resveratrol has been observed in in-vitro studies, results are inconsistent across different

251 cell lines, based on the presence or absence of coactivators or corepressors (Calleri et al.,

252 2014). It seems that tissue distribution of PPARα is an important factor that should be

253 considered, whilst it should also be acknowledged that the *PPARα* gene is expressed at a low

254 level in PBMC, and this can explain our findings. Consequently, the theory of upregulation

255 of p16 by PPARα activation, through binding to the PPAR response element in p16 promoter

256 (Florence Gizard et al., 2005a), was not approved by our results.

257 TWEAK belongs to the TNF superfamily, is generated mainly from macrophages, and is

258 released into the bloodstream in its functional form (sTWEAK) (Chicheportiche et al., 1997).

259 Studies have reported unexpected reductions in sTWEAK in inflammatory diseases, such as

260 T2DM (Jelic-Ivanovic et al., 2009; Kralisch et al., 2008), and although the exact cause of the

261 reduction is not clear, some mechanisms related to the CD163 and Fn14 receptor have been

262 suggested (J. A. Moreno et al., 2009; G. Urbonaviciene et al., 2011).

263 Indeed, studies have shown that both serum and mRNA expression levels of CD163 are

264 higher in inflammatory conditions, such as T2DM, obesity, or atherosclerosis

265 (Kawarabayashi et al., 2017). CD163 is a scavenger receptor located on macrophages and

266 involved in the hemoglobin-haptoglobin complexes removal, and is also known as a

267 scavenger of sTWEAK (Bover et al., 2007; Fabriek, Dijkstra, & van den Berg, 2005).

268 Resveratrol is an antioxidant and can activate a variety of antioxidant enzymes, such as

269 catalase, glutathione peroxidase, glutathione transferase, and superoxide dismutase, which

270 can elicit a consequential reduction of monocyte/macrophage activity and CD163 (Ingl et al.,

271 2014; O. Toupchian, G. Sotoudeh, A. Mansoori, E. Nasli-Esfahani, et al., 2016). Therefore,

272 sTWEAK levels are increased following CD163 reduction. Resveratrol also upregulates

273 expression levels of SIRT1 and 5' AMP-activated protein kinase (AMPK), which have

274 inhibitory interactions with NF-κB (Xu, Botchway, Zhang, Zhou, & Liu, 2018), and, as a

275 result, the TWEAK-Fn14 pathway is suppressed, which can lead to increases in sTWEAK

276 levels. However, significant improvement in sCD163 disappeared after adjustment for age,

277 gender, and baseline BMI, in our results. It has been reported that higher white adipose tissue

278 is associated with higher sCD163 concentration, representing obesity-induced inflammation,

279 and macrophage activity (Kračmerová et al., 2014). In accordance with previous study

280 visceral adipose tissue is an important predictor of sCD163 concentration in patients with

281 type 2 diabetes (Sørensen et al., 2015). As, visceral adipose tissue is greater in female than

282 male, sex differences also may confound results (Karastergiou, Smith, Greenberg, & Fried,

283 2012).

284 The present study is the first human trial to investigate the effects of resveratrol

285 supplementation on the cellular factors associated with intimal hyperplasia and the first

286   randomized controlled trial that used micronized resveratrol supplement as a natural ligand

287   for PPARα. However, a few limitations need to be considered. One of the limitations of our

288   study was the relatively short-term intervention, which did not allow investigators to

289   discern the long term effects of resveratrol on the studied variables. Also, in this study, we

290   assessed the mRNA expression levels of PPARα, however, it seems PPARα activity may

291   be more important for interpreting results. Another limitation in this study was the surrogate

292   markers that were used for endothelial function assessment, instead of gold-standard

293   methods, such as flow-mediated dilation (FMD) or peripheral arterial tonometry (PAT).

294   **CONCLUSIONS**

295   We found that 8 weeks supplementation with micronized resveratrol, in patients with

296   T2DM, improved sCD163/sTWEAK ratio, despite no significant change in adjusted

297   sCD163 levels, *p53* and *p21* gene expressions were upregulated. However, there is a need

298   for further long-term trials to confirm the veracity of these results.

305   **CONFLICT OF INTEREST**

306   The funding bodies had no role in the design of the study, writing, or decision to submit the

307   manuscript for publication. They also had no role in any aspect of the described data

308 management, analysis, or the reporting of results. The authors declare that they have no

309 conflict of interest.

310 **AUTHOR CONTRIBUTION**

311 SA, AS-A, OT, JH and HM-K were involved in initial idea of this study and designing the

312 trial. OT, SA, AS-A, CC and HM-K ~~were~~ contributed ~~in~~ to writing the manuscript and ~~getting~~

313 securing the grant. OT and JH were co-investigators and involved in collecting data,

314 concealment procedure and counselling patients. HF provided statistical expertise in clinical

315 trial design, sample size calculation and blinding. MHS ~~was~~ contributed to the design of

316 biochemical procedures. All authors read and approved the final manuscript.

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608   **Table 1** Real-time PCR primer sequences

	Forward	Reverse
p53	5'GAGCTGAATGAGGCCTTGGA3'	5'CTGAGTCAGGCCCTTCTGTCTT3'
p21	5'TGGAGACTCTCAGGGTCGAAA3'	5'GGCGTTTGGAGTGGTAGAAATC3'
p16	5'CTTCCTGGACACGCTGGTG3'	5'GCATGGTTACTGCCTCTGGTG3'
PPARα	5'CTATCATTTGCTGTGGAGATCG3'	5'AAGATATCGTCCGGGTGGTT3'
GAPDH[*]	5'TGGTATCGTGGAAGGACTCATG3'	5'GCTTCACCACCTTCTTGATGTC3'

609   GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase; PCR, Polymerase chain reaction; PPARα,

610   Peroxisome proliferator activated receptor alpha.

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625 **Table 2** Baseline characteristics of the study participants

Variables[1]	Resveratrol (n=35)	Placebo (n= 36)
Age (years)	50.14 ± 7.38	50.06± 7.69
Diabetes duration (years)	9.40 ± 7.07	8.11 ± 6.90
Gender (female), n (%)	15 (42.9)	16 (44.4)
Height (cm)	164.94 ± 7.22	162.08 ± 11.29
Weight (kg)	73.69 ± 8.24	72.71 ± 10.52
BMI (kg m^{-2})	27.10 ± 2.69	27.66 ± 2.71
Smoker, n(%)	5 (14.3)	2 (5.6)

Complications

Hypertension, n (%)	11 (31.4)	7 (19.4)
Kidney disorders, n (%)	2 (5.7)	3 (8.3)
Hepatic disorders, n (%)	3 (8.6)	2 (5.6)
Neuropathy, n (%)	2 (5.7)	2 (5.6)
Retinopathy, n (%)	5 (14.3)	5 (13.9)
Family T2DM History, n (%)	25 (71.4)	30 (83.3)
Medications		
Metformin, n (%)	30 (85.7)	31 (86.1)
Glibenclamide, n (%)	11 (31.4)	16 (44.4)
Statins, n (%)	3 (8.6)	4 (11.1)
Blood pressure lowering drugs, n (%)	6 (17.1)	5 (13.9)

626 [1]Data are expressed as mean± SD for continuous variables or as frequency (percentage) for categorical

627 variables.

628 BMI, Body mass index; CVD, Cardiovascular disease

Table 3 Serum levels of CD163 and TWEAK values during study in resveratrol and placebo groups

	Resveratrol (n=35)			P-value[2]	Placebo (n= 36)			P-value[2]	P-value[3]	P-value[4]	Power
	Before	After	Change		Before	After	Change				
sCD163 (ng/mL)	1353.43 ± 339.2	1176.57 ± 282.4	-176.85 ± 310.7	0.002	1100 ± 383.14	1086 ± 289	-18.8 ± 343.3	0.74	0.04	0.8	0.84
sTWEAK (pg/mL)	3343.14 ± 918.3	3737.4 ± 1193.4	394.2 ± 903.9	0.01	3648 ± 1017.5	3308.5 ± 1173.6	-339.4 ± 1123	0.08	0.004	0.001	0.8
sCD163/sTWEAK	0.43 ± 0.15	0.34 ± 0.11	-0.09 ± 0.13	<0.001	0.33 ± 0.19	0.36 ± 0.15	0.25 ± 0.14	0.29	0.001	0.003	0.81

[1]All variables are expressed as mean ± SD. [2]The presented P-values are associated with before and after intervention comparisons obtained from paired t test. [3]The presented P-values are associated with mean changes comparisons obtained from independent-samples t test. [4]The presented P-values are associated with mean changes comparisons adjusted for age, gender, and baseline BMI obtained from analysis of covariance (ANCOVA).

sCD163, Serum level of cluster of differentiation 163; sTWEAK, Serum level of TNF-like weak inducer of apoptosis

Figure legends

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

Figure 2 Fold changes (means ± SDs) in gene expression levels of *PPARα, p16, p53* and *p21* in patients with T2DM receiving resveratrol (n=35) or placebo (n=36). The presented P-values are associated with fold changes comparisons adjusted for age, gender, and baseline BMI obtained from analysis of covariance (ANCOVA).

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