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Six months of resveratrol supplementation has no measurable effect in type 2 diabetic patients. A randomized, double blind, placebo-controlled trial

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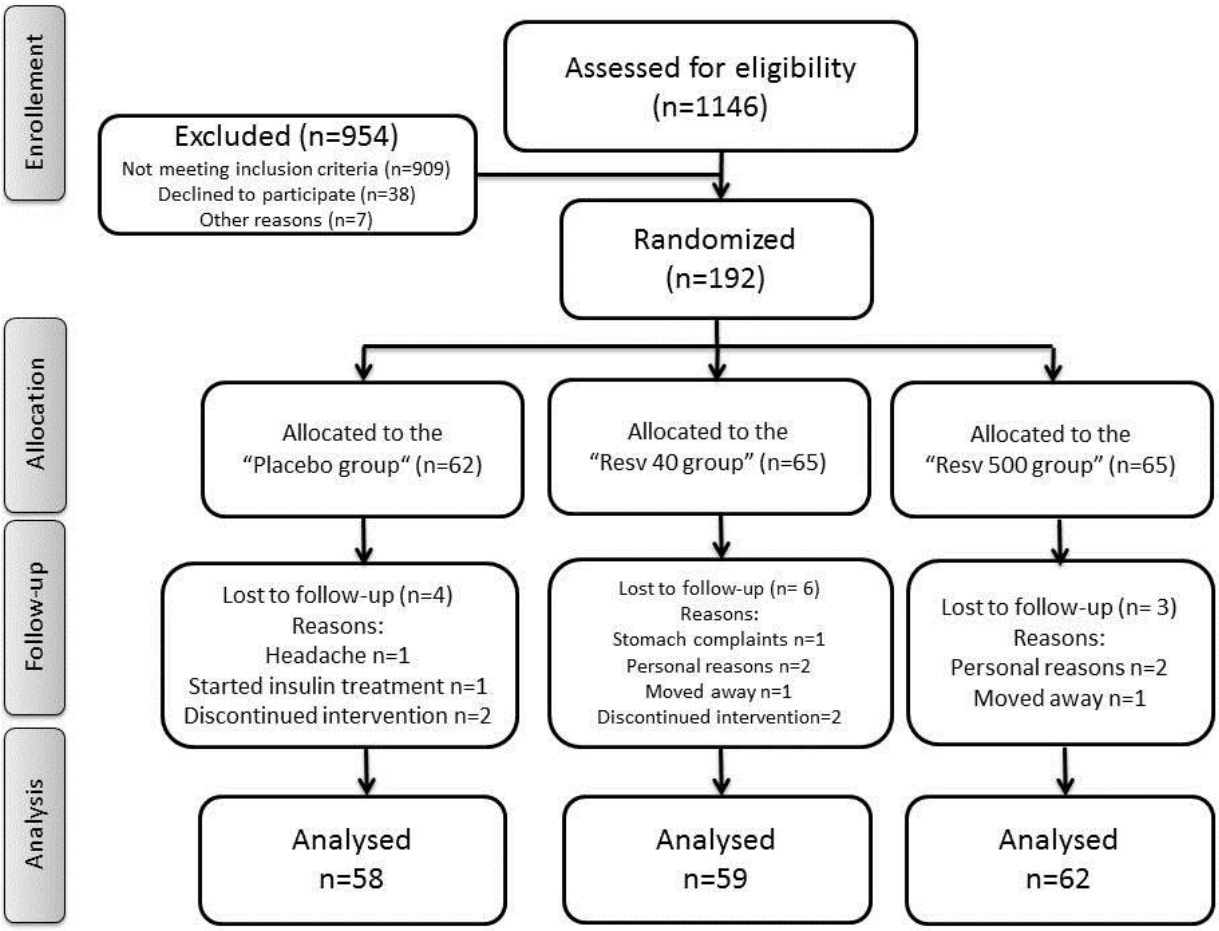
Graphical abstract

Anti-inflammatory and metabolic effects of 6 months of resveratrol *vs* placebo in patients with type 2 diabetes mellitus. A double-blind randomized-controlled trial

Flow diagram of the trial



Abstract

The polyphenol resveratrol is considered to exert many beneficial actions, such as antioxidant, anti-inflammatory, insulin-sensitizer and anticancer effects. Its benefits in patients with type 2 diabetes mellitus (T2DM) are controversial.

Our aims were to determine whether resveratrol supplementation at two different dosages (500 and 40mg/day) for 6 months i) reduced the concentrations of C-reactive-protein (CRP) and ii) ameliorated the metabolic pattern of T2DM patients.

In the present double-blind, randomized, placebo-controlled trial, 192 T2DM patients were randomized to receive resveratrol 500mg/day (Resv500 arm), resveratrol 40mg/day (Resv40 arm) or placebo for 6-months. At baseline and at the trial end, CRP values, anthropometric, metabolic and liver parameters were determined.

No serious adverse event occurred. A dose-dependent, though not significant, CRP decrease of 5.6% (Resv40 arm) and 15.9% (Resv500 arm) was observed *vs* placebo. We failed to detect significant differences in weight, BMI, waist circumference, and values of arterial blood pressure, fasting glucose, glycated hemoglobin, insulin, C-peptide, free fatty acids, liver transaminases, uric acid, adiponectin, interleukin-6, in both the Resv500 and Resv40 arms *vs* placebo. Total cholesterol and triglycerides slightly increased in the Resv500 arm. Subgroup analyses revealed that lower diabetes duration (in both Resv500 and Resv40 arms), and, in the Resv500 arm, younger age, aspirin use and being a smoker were associated with a significantly higher CRP reduction *vs* placebo.

The supplementations with 40 mg/day or 500 mg/day resveratrol did neither reduce CRP concentrations, nor improve the metabolic pattern of T2DM patients.

Abbreviations: alanine amino-transferase (ALT); aspartate amino-transferase (AST); body mass index (BMI); C-reactive proteins (CRP); European Prospective Investigation into Cancer and Nutrition (EPIC); glycated hemoglobin (HbA1c); ɣ-glutamyl-transferase (GGT); Homeostasis Model Assessment-Insulin Resistance (HOMA-IR); interleukin-6 (IL-6); type 2 diabetes mellitus (T2DM).

Keywords: C-reactive protein, resveratrol, type 2 diabetes mellitus

Chemical compound studied in this article: Resveratrol (PubChem CID: 445154)

1. Introduction

A huge number of in vitro and animal studies have provided evidence about the potential benefits of resveratrol (3,5,4'-trihydroxy-*trans*-stilbene), a polyphenolic compound found in several plants, such as root of *Polygonum cuspidatum*, peanuts, berries, and red grapes [1]. The following beneficial properties

have been identified for resveratrol: antioxidant, anti-inflammatory, anti-carcinogenic, anti-platelet aggregation, cardio-protective, neuro-protective, cartilage-protective, insulin sensitizer, anti-aging activities, increasing lifespan, reducing body weight, improving endothelial function and atherosclerosis regression, and mimic calorie restriction [2].

However, human clinical trials have shown conflicting results [3], which may be due to differences in the dosage and the duration of supplementation, the bioavailability of resveratrol, the role of food matrix to improve resveratrol bioactivity, and the characteristics of the patients studied [1,4-5].

The supposed benefits of resveratrol in type 2 diabetes are particularly attractive, owing to the public health burden of this disease and of its chronic complications [6-7]. Many trials reported improvements in glycemic control, insulin sensitivity and many metabolic parameters after resveratrol supplementation [8-14]. However, more recently, these improvements have not been confirmed [15-18].

Only a few studies that were performed in patients with chronic complications, have evaluated the effects of resveratrol on inflammatory mediators in type 2 diabetes mellitus, a condition characterized by a low-grade chronic inflammatory state [19-20].

The present double-blind randomized placebo-controlled trial tested the hypothesis that resveratrol, when given orally for 6 months, induces a decrease in the circulating concentrations of C-reactive proteins (CRP) and ameliorates the metabolic pattern of patients with type 2 diabetes mellitus when compared to placebo. The efficacy and safety of two different dosages of resveratrol (500 mg/day and 40 mg/day) were evaluated too.

2 Methods

2.1 Recruitment of participants

Participants were recruited from the Diabetic Clinic of the Department of Medical Sciences of the University of Turin during the period October 2013- February 2016.

Inclusion criteria were: type 2 diabetes mellitus, age ≥40 years, body mass index (BMI) <35 kg/m^2, patients on diet and/or hypoglycemic agents other than insulin, willing to give written informed consent and able to understand, participate in and to comply with the study requirements.

Exclusion criteria were: treatment with any antioxidant substance, treatment with insulin, anticoagulants, steroids or anti-inflammatory drugs different from acetyl-salicylic acid, alcohol or substance abuse, uncompensated diabetes, liver or kidney diseases, presence of diabetes-related chronic complications, cardiovascular events or revascularization procedures in the previous four weeks, any severe chronic or life-threatening diseases, pregnancy, allergy to peanuts, grapes, wine, mulberries.

2.2 Study design

Double-blind, randomized, placebo-controlled trial

2.3 Outcomes

The primary outcome was the difference in changes in CRP values from baseline to the end of the trial in patients treated with resveratrol 500 mg/day or resveratrol 40 mg/day *versus* patients treated with placebo.

Secondary outcomes were the differences in changes from baseline to the end of the trial of arterial blood pressure, BMI, waist circumference, body fat percentage, fasting glucose, insulin, C-peptide, interleukin-6 (IL-6), glycated hemoglobin (HbA1c), Homeostasis Model Assessment-Insulin Resistance (HOMA-IR), total and HDL cholesterol, triglycerides, free fatty acids (FFA), adiponectin, uric acid, alanine amino-transferase (ALT), aspartate amino-transferase (AST), ɣ-glutamyl-transferase (GGT) in patients treated with resveratrol 500 mg/day or resveratrol 40 mg/day *versus* patients treated with placebo.

2.4 Intervention

One-hundred and ninety-two patients were randomized respectively to 1 capsule/day of resveratrol 500 mg/day (Resv 500 arm) for 6 months, 1 capsule/day of resveratrol 40 mg/day (Resv 40 arm) for 6 months and 1 capsule/day of placebo (totally inert microcellulose) for 6 months (placebo arm). The capsules were

purchased from Biotivia Bioceuticals (International SrL, Italy). Biotivia prepared all the three types of capsules, which were identical in size, shape, color and taste.

High-pressure liquid chromatography (HPLC) analyses of the 500 mg and 40 mg capsules revealed a 99.7% and a 97.9% purity of trans-resveratrol respectively, while analysis of the placebo capsules showed no resveratrol content.

Data related to health status, the use of drugs or supplements, usual dietary habits and exercise levels, were collected from all subjects.

The patients of the three arms were submitted at baseline and after the 6-months period to the following assessments:

- A validated food-frequency questionnaire
- The Minnesota-Leisure-Time-Physical-Activity questionnaire [21]
- Measurements of body weight, height, waist circumference
- Measurements of fat percentage by dual X-ray densitometry (DXA)
- Measurements of arterial blood pressure
- A fasting blood sample collection to determine the circulating concentrations of CRP, IL-6, glucose, Insulin, C-peptide, HbA1c, total and HDL cholesterol, triglycerides, FFA, adiponectin, uric acid, ALT, AST, GGT.

Every participant was given three identical bottles containing each 60 capsules, and was asked to assume every day one capsule in the morning and to maintain habitual lifestyle and diet for 6 months. All patients were allowed to keep their current hypoglycemic treatment during the trial, but were instructed to abstain from using nutritional supplements or consuming significant amounts of resveratrol-rich foods and beverages.

A food-frequency questionnaire adapted from the EPIC (European Prospective Investigation into Cancer and Nutrition) questionnaire [22], focused on dietary polyphenol intake and previously validated [21], 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). The physical activity level was calculated as the product of the duration and frequency of each activity (in hours/week), weighted by an estimate of the metabolic equivalent (MET) of the activity and summed for the activities performed [21].

Weight, waist circumference, arterial blood pressure were measured by blinded trained researches.

All the laboratory measurements were blindly performed at the Laboratory of Metabolic Diseases of the Department of Medical Sciences, University of Turin.

2.5 Adverse events and compliance

Adverse events and compliance with the study protocol were monitored by monthly phone calls. Participants were instructed to inform the researchers if adverse effects occurred. These cases were then communicated to the statisticians, who monitored the safety of the study.

Participants returned unused capsules at the end of the trial and the pill counting was performed. Furthermore, patients were asked about compliance to prescribed capsules intake during the monthly calls.

2.6 Randomization

A computer generated randomization sequence was centrally developed, using blocks of various length in random sequence, stratified by patients' use of acetyl-salicylic acid and HbA1c levels (cut-point 7%). The procedure was completely concealed to researchers and available on a dedicated web site (www.epiclin.it).

2.7 Blinding

Randomization was performed by a statistician who assigned a number to each patient. Capsules of resveratrol 500 mg, resveratrol 40 mg and placebo were identical. 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 with the number of each patient. She gave the bottles to the researches and did not dispense the capsules to the patients. Patients and researches who dispensed the

capsules and performed the data collection and the measurements were blinded to the content of the bottles. Laboratory determinations were performed blindly.

2.8 Ethics

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. The trial is registered at ClinicalTrials.gov (identifier: NCT02244879).

2.9 Measurements

Body weight was measured to the nearest 0.1 kg, and height was measured to the nearest 0.1 cm with a stadiometer (SECA model 711, Hamburg, Germany), with the participants wearing light clothes and no shoes. Waist circumference was measured at the narrowest level over light clothing by a plastic tape meter to the nearest 0.1 cm. Body composition in terms of lean and fat body mass was determined by DXA (QDR-4500 Hologic Inc., Bedford, MA, USA), using whole-body absorptiometry software. Coefficients of variation (CVs) of the measurements of lean and fat body mass wear near 1.9% and 1.7%, respectively. Arterial blood pressure values were measured from the left arm, in a sitting position, after at least 10 min of rest, with a mercury sphygmomanometer with appropriate cuff sizes (ERKA Perfect-Aneroid, Germany). Two measurements were taken by trained physicians with arm supported at heart level and the values reported were the means of the two.

Blood samples were collected after an overnight fast. All laboratory measurements were centralized. Serum CRP values were determined using a high-sensitivity latex agglutination assay on HITACHI 911 Analyzer (Sentinel Ch., Milan, Italy). The intra-assay and inter-assay CVs were 0.8-1.3% and 1.0-1.5%, respectively. IL-6 circulating concentrations were measured by a quantitative sandwich enzyme immunoassay technique (R&DSystem, Minneapolis, MN, USA) with an intra-assay CV of 6.9% and an inter-assay CV of 7.2 %.

Serum glucose was measured by the glucose oxidase method (Sentinel Ch., Milan) with an intra-assay CV of 1.1 % and an inter-assay CV of 2.3%. Triglycerides and cholesterol were assayed by enzymatic colorimetric assays (Sentinel Ch., Milan) with an intra-assay CV of 3.0 % and an inter-assay CV of 3.5% for triglycerides and with an intra-assay CV of 2.2 % and an inter-assay CV of 3.4 % for cholesterol. HDL-cholesterol was determined by enzymatic colorimetric assay after precipitation of LDL and VLDL fractions using heparin-MnCl2 solution and centrifugation at 4°C and it had an intra-assay variation CV of 2.5 % and an inter-assay CV of 4.1%. FFA values were assayed by an enzymatic colorimetric method (RANDOX, UK).

AST, ALT, GGT were measured by a kinetic determination (Sentinel Ch., Milan) according to the IFCC recommendations. Insulin was measured by a biotin labelled antibody based sandwich enzyme immunoassay (LDN, Germany). The kit had a sensitivity of less than 1.8 µU/ml and a range of 0 to 100 µU/ml. The intra-assay and inter-assay CVs were respectively 1.8–2.6% and 2.9-6.0%. Hba1c was determined with a latex-based method (Sentinel Ch., Milan). The intra-assay and inter-assay CVs were respectively 1.1–1.5% and 1.1– 1.6%. Adiponectin was measured by sandwich enzyme-linked immunosorbent assays (BioVendor, Brno, Czech Republic). The kit has a sensitivity of 470 ng/ml and a range of 5000 to 150000 ng/ml. The intra- and inter-assay CVs were 4.1% and 6.9%, respectively. Uric acid was assayed by uricase-based enzymatic colorimetric assays (Sentinel Ch., Milan) with an intra-assay CV of 2.1 % and an inter-assay CV of 1.7%.

The HOMA-IR was calculated according to the published algorithm [23].

2.10 Sample size calculation and statistical analyses

Based on our previous results [24], we expected that the administration of resveratrol could induce a reduction of CRP values (mg/l) corresponding to an effect size of at least 0.50. To detect this effect, a total sample size of 192 patients (about 64 per arm) was calculated to reach a statistical power of 80% considering an overall type 1 error of 5%.

According to protocol, to account for differences between arms of the analyzed variables at enrollment, comparisons of change from baseline of the primary and secondary endpoints between the resveratrol and

placebo arms were performed by ANCOVA, to adjust for baseline imbalances of the analyzed endpoints and to account for the stratification variables used in the randomization (use of acetyl-salicylic acid and HbA1c levels).

To preserve the overall type 1 error of 5%, a gatekeeping strategy was adopted accounting for the hierarchical structure of multiple comparisons in the first step. The Resv 500 arm was first compared with placebo and only if this test was statistically significant at $p<0.05$, a comparison between Resv 40 arm and placebo with a test $p<0.05$ was considered as significant. The same hierarchical approach was used to analyze the secondary endpoints.

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

3. Results

3.1 Tolerability and compliance

Of the 192 participants, respectively 4, 6, and 3 from the placebo, Resv 40 and Resv 500 arms dropped out. All doses of resveratrol were well tolerated and there were no serious adverse effects or alarming changes in laboratory parameters during the trial. Most patients discontinued supplementation because they no longer wanted to continue, or moved away. Data from 179 participants were thus analyzed. The flow diagram of the trial is presented in **Figure 1**.

A compliance audit of returned capsules indicated more than 95% compliance in all arms (data not shown).

3.2 Baseline data

Table 1 outlines the baseline characteristics of the participants. There was by chance, a higher proportion of females in the Resv 40 group, since no stratification by gender was planned. As a consequence, baseline CRP values were higher in this arm, owing to the demonstrated positive association between female gender and CRP values [25]. Overall, there was homogeneity among placebo and the resveratrol arms for the other variables, in particular for dietary habits and use of medications.

3.3 Outcomes

Table 2 shows the impact of resveratrol 40mg/day and of resveratrol 500mg/day *versus* placebo at the end of the trial with respect to the corresponding baseline values. A dose-dependent decrease of 5.6% (Resv 40 arm) and 15.9% (Resv 500 arm) was observed for mean CRP concentrations. Even though CRP decreased in both resveratrol arms, no significant differences with respect to the Placebo arm were found.

We failed to detect significant differences in weight, BMI, waist circumference, and values of arterial blood pressure, IL-6, fasting glucose, HbA1c, insulin, HOMA-IR, C-peptide, AST, ALT, GGT, uric acid, adiponectin, FFA, in both the Resv 500 and Resv 40 arms *vs* the Placebo arm.

Resveratrol did not induce improvements in lipid variables, but rather slightly increased the circulating concentrations of total cholesterol and triglycerides, at the dosage of 500mg.

No significant change was observed in total daily energy, nutrient intakes, estimated resveratrol intake and exercise levels within each arm at the end of the trial (data not shown).

3.4 Subgroup analyses

Exploratory analyses were performed to investigate possible modifying factors on the results (**Figures 2-3**). There was no heterogeneity of effects for gender, alcohol intake, statin use, and Hba1c values in the adjusted mean difference on changes from baseline of CRP values in both Resv 500 and Resv 40 arms *vs* the Placebo arm. However, lower diabetes duration, younger age, aspirin use and being an actual smoker were associated with a significantly higher CRP reduction in the Resv 500 arm with respect to the Placebo arm (Figure 2). A statistically significant interaction (p=0.02) was noted according to diabetes duration: CRP values decreased in the Resv 40 arm *vs* placebo in patients with a lower diabetes duration and increased in those with a longer diabetes duration.

In **Supplementary Tables S1-S2**, the stratified comparisons by disease duration of change from baseline of all the endpoints are reported. Hba1c values in both Resv 500 and Resv 40 arms *vs* Placebo arm were decreased in patients with lower diabetes duration.

Finally, we repeated the analyses by taking into account the treatment with statins and with the different hypoglycemic drugs, and the estimates of the differences between resveratrol and placebo arms did not change (data not shown).

4. Discussion

The results of the present trial did not support the hypothesis that resveratrol reduces the concentrations of CRP in patients with type 2 diabetes. Furthermore, we failed to detect any beneficial metabolic effect for this substance, but rather a slight increase in total cholesterol and triglyceride values. Even if subgroup analyses suggest the possibility that patients with lower diabetes duration may benefit from the use of resveratrol, the present data provided evidence against the supplementation with this polyphenol.

4.1 Effects of resveratrol on CRP concentrations

In healthy individuals, resveratrol exerts an anti-inflammatory effect [24,26-28]. The mechanisms proposed for its anti-inflammatory effects in humans were multiple and complex, such as the increased expression of sirtuin (SIRT)-1, the inhibition of NFκB, the major pro-inflammatory transcription factor, and of NFκB-related inflammatory and autoimmune markers, the suppression of the expression of pro-inflammatory kinases or other pro-inflammatory enzymes, cytokines and endothelial growth factors, the attenuation of monocyte adhesion to the endothelium, the diminished activity of T- and B-cells and macrophages, the increase in the level of anti-inflammatory eicosanoid production, the up-regulation of anti-inflammatory genes and the decreased expression of pro-inflammatory genes, the antioxidant and scavenger capacity [1,8,24,26-27].

Only a few trials confirmed the anti-inflammatory properties of resveratrol in patients with pathological conditions [14,29-31]. Indeed, in patients with the metabolic syndrome [32], slightly overweight [33], obese [15,17], with non-alcoholic fatty liver disease [16], impaired glucose tolerance [9], stable coronary artery disease [34], and in the elderly [35], resveratrol failed to reduce the concentrations of inflammatory markers. In patients with diabetes, resveratrol did not decrease CRP concentrations, in line with our results,

but reduced the concentrations of fibrinogen [20], interleukin-6 and down-regulated the transcript levels of key pro-inflammatory mediators such as the chemokine (C-C motif) ligand 3, tumor necrosis α, interleukin 1-ß, with the involvement of inflammatory-related microRNA in peripheral blood mononuclear cells [18].

To explain these divergent results, the very low bioavailability of resveratrol has been called into question (plasmatic concentrations are almost undetectable because of the rapid first-pass metabolism of absorbed resveratrol) [5], together with the effect of different food matrices on resveratrol bioactivity, the high number of resveratrol metabolites with a possible different significance, and the pronounced intra-individual differences in resveratrol metabolism by human gut microbiota [1,36]. Differences in the dosage and duration of supplementation and in the characteristics of the patients studied might also contribute to the discrepancies among studies.

We found a decrement in CRP concentrations, after resveratrol supplementation in patients with a lower diabetes duration. This is likely, because subclinical chronic inflammation is a common feature in the natural course of diabetes, and correlate with its chronic complications [37]; realistically, long-lasting diabetes with its inveterate chronic low-grade pro-inflammatory and pro-oxidant status does not seem sensitive to this supplementation. As expected, younger patients, those on aspirin, and, intriguingly, smokers showed a higher CRP reduction after the consumption of resveratrol 500mg. Accordingly, we have found that in healthy smokers, a short period of supplementation with resveratrol exerted anti-oxidant effects and induced a significant reduction in CRP blood concentrations [24]. Smokers are characterized by an oxidant-antioxidant imbalance, a low-grade systemic inflammatory condition with elevated concentrations of inflammatory mediators, and endothelial dysfunction [38-39].

It could be hypothesized that the favorable effects of resveratrol might be observed in the presence of a chronic pro-inflammatory state, but not in advanced and inveterate conditions, as those present in patients with a long history of type 2 diabetes. Indeed, subgroup analyses are based on a very limited number of patients and their clinical relevance is highly questionable. Furthermore, no significant effect on IL-6 concentrations were found, further questioning the anti-inflammatory effect of resveratrol.

4.2 Effects of resveratrol on metabolic variables

The metabolic benefits of resveratrol is another highly controversial topic. Many trials reported a beneficial role for resveratrol on different metabolic variables [8-14,40]. Most of them were criticized because of concerns due to small sample size, short follow-up, lack of adjustments for appropriate covariates in the statistical analyses [1].

Indeed, in overweight/obese or dysmetabolic patients, resveratrol supplementation determined a reduced resting metabolic rate and lower fat oxidation [8], did not affect glucose metabolism or insulin sensitivity [15-17, 32-33, 41-42], lipid profile [9,15-17,19,32-33,41], blood pressure [15,17,19,32], and adiponectin values [9,15-16,32]. Furthermore, when insulin action was more carefully studies, by using the gold-standard assessment method, i.e. the hyperinsulinemic-euglycemic clamp procedure, no effect of resveratrol supplementation was found both in insulin sensitivity (at liver, adipose tissue, skeletal muscle levels) and in glucose and fatty acid kinetics [15-16,33].

Trials on diabetic patients which found a beneficial effect of resveratrol were performed in younger patients [10-12,39], while older patients showed no benefits from resveratrol in terms of blood glucose, HbA1c values, body weight and glucagon-like peptide 1 secretion [18]. In diabetic patients with foot ulcers, changes in metabolic variables were similar between the placebo and resveratrol groups [20], and in diabetic patients with stable coronary artery disease, a significant decrease of adiponectin levels and increment in HbA1c values were reported [30]. Accordingly, we found a significant difference by disease duration in HbA1c changes, with a trend toward a HbA1c increment in those with ≥8-y diabetes and a trend toward a reduction in patients with <8y diabetes.

Furthermore, we found an adjusted mean increment from baseline in total cholesterol and triglycerides mean values in the Resv 500 *vs* placebo, which was statistically significant, but not clinically significant. We cannot exclude that statin treatment may be the responsible of the effects on lipid concentrations, because of the high proportion of our patients on statins. Indeed, a synergistic effect between statins and resveratrol supplements has been demonstrated [11-12]. However, when we analyzed adjusted mean difference on change of total cholesterol and triglycerides from baseline by statin use, the results did not

change (data not shown). Furthermore, other trials found a slight increment in lipid variables, after resveratrol supplementation [16] and a recent meta-analysis of randomized controlled trial failed to find any benefits on plasma lipid parameters by resveratrol [43].

We did not find any relevant change in anthropometric variables in our patients, in line with other authors, who reported no differences in both total body mass, lean body mass, total fat body mass or visceral and abdominal subcutaneous fat volumes, and liver and skeletal muscle lipid content [10-11,15-16,18-19,44]. Finally, only a few trials have evaluated liver function tests after resveratrol supplementation, reporting no significant variations [15,24,33], in line with our results. However, in non-alcoholic fatty liver disease, both a significant increase in AST and ALT concentrations after supplementation with high doses of resveratrol [16] and a significant decrease in AST and ALT concentrations with moderate doses of resveratrol [31,45] were reported, highlighting once again the highly controversial results available about this supplement.

It could be hypothesized that different dosage or duration of resveratrol supplementation might justify the discrepancy among studies. However, conclusions from previous studies were consistent neither with a proportional dose-effect of resveratrol, since very low dose (8-10 mg/day) seemed effective [12,14,30], but not higher dosages (≥1g/day) [15-16,32], nor with a time-dependent effect, because short-follow-up trials [8,10-12,31,41], but not 1-y supplementation [19], ameliorated the metabolic pattern of participants.

4.3 Safety

Both 40 mg and 500 mg/day resveratrol were well tolerated and no major adverse effects were found. Indeed, previous trials with longer periods of supplementation [14,19,30] or employing higher dosages (up to 3g/day) [9,11,15-16], did not report major adverse effects.

4.4 Limitations

The study was powered to detect an effect size of 0.5 on CRP values; the standard deviation of CRP values in this study was close to 8 mg/l, whereas our trial was powered to detect mean differences of 4 mg/l between arms. The benefit observed in the Resv 500 arm corresponded to a lower effect size than expected of approximately 0.3. Therefore, the sample size may have been too small to unmask a smaller, but still meaningful, anti-inflammatory effect. Indeed, considering also the exploratory analyses, our results were plausible and in line with the evidences available in literature.

Because no stratification by gender was planned, by chance, a higher proportion of females resulted in the Resv 40 group. As a consequence of the differences by gender in CRP values, at baseline those individuals showed increased CRP concentrations. Indeed, baseline imbalances should not bias the results, because we evaluated intra-individual before-after changes, and these changes were compared among arms. In addition, the comparisons were also adjusted for the baseline CRP values by using ANCOVA.

Most of our patients were treated with statins or metformin, whose effects could interact with those of resveratrol and may have affected the results. However, after controlling for the use of these drugs, the results did not change.

Since plasmatic concentrations of resveratrol or its metabolites were not measured, the actual exposure to the substance cannot be determined. However, on the basis of phone calls and capsule counts we consider that compliance to the study protocol was adequate.

5. Conclusion

The present study demonstrates that 6-months supplementations with 40 mg/day or 500 mg/day resveratrol did neither reduce CRP circulating concentrations, nor improve the metabolic pattern of type 2 diabetic patients. Even if we cannot exclude the possibility that specific subgroups of patients, such as those with lower diabetes duration, may benefit from the use of resveratrol, our results did not provide evidence in favor of this supplementation. Furthermore, despite the remarkable accumulation of evidence on biochemical results and other surrogates, the clinical implication of any of these potential effects in diabetic patients remains unclear.

Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

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Figure 1. Flow diagram of the trial

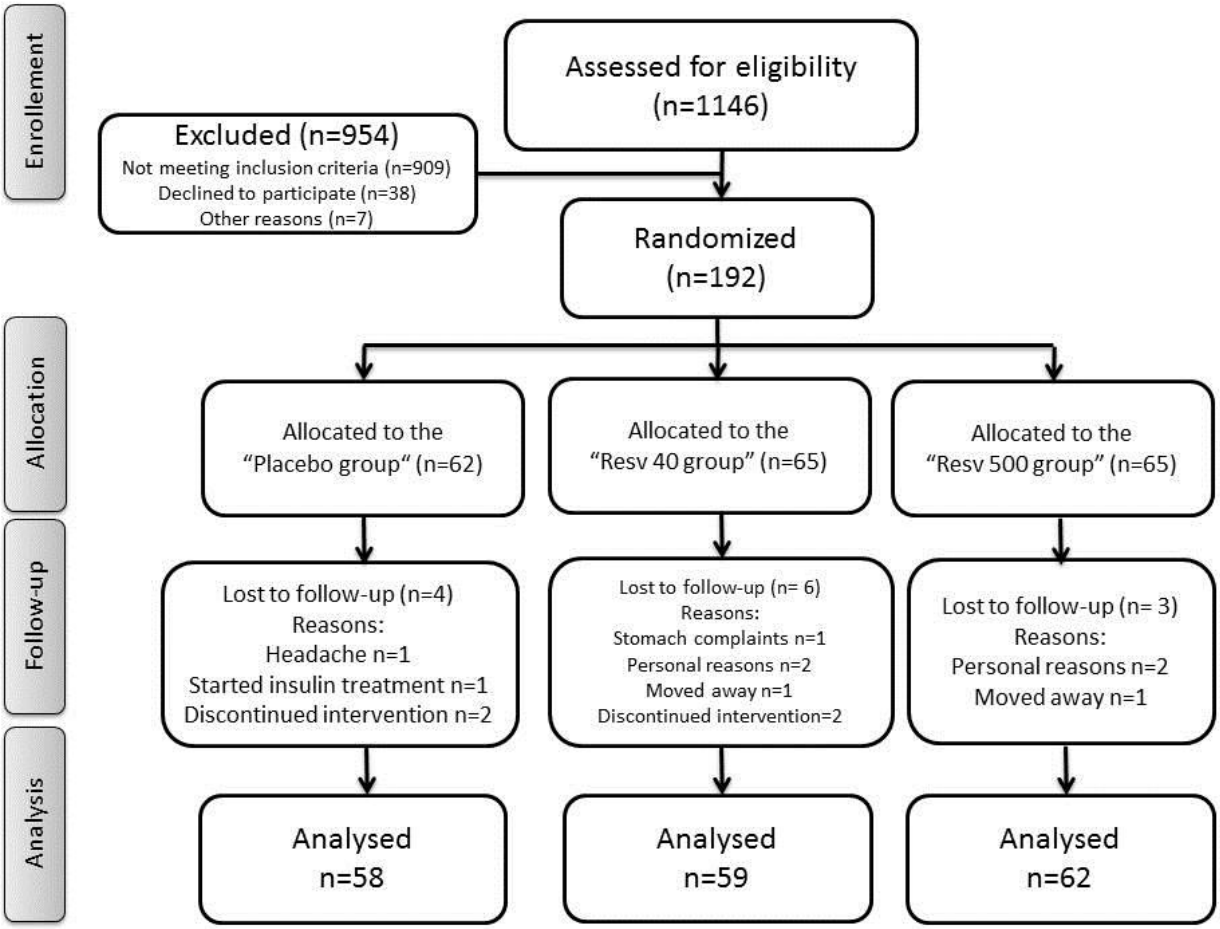

Figure 2. Adjusted mean difference on change from baseline (95%CI) of CRP values (Resv 500 arm *vs* Placebo arm)

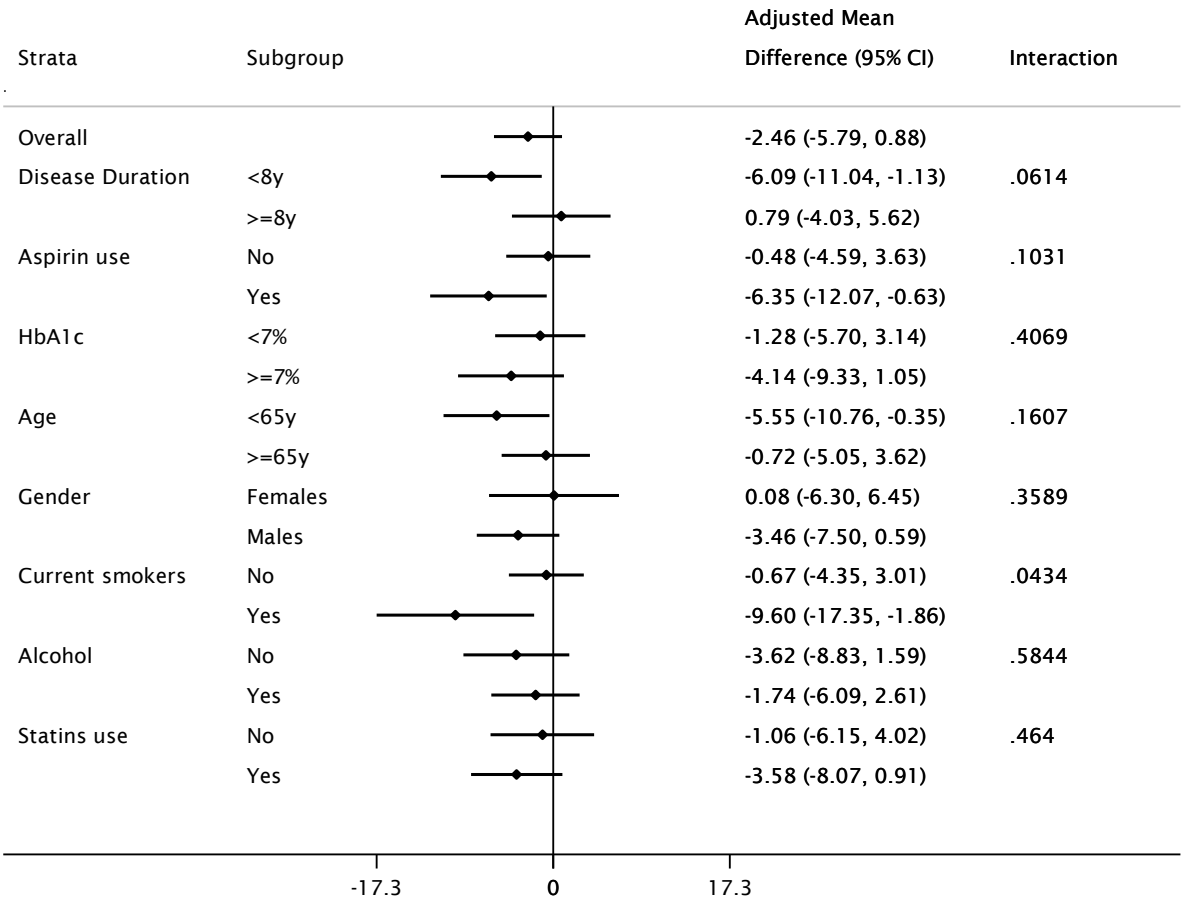

Figure 3. Adjusted mean difference on change from baseline (95%CI) of CRP value (Resv 40 arm *vs* Placebo arm)

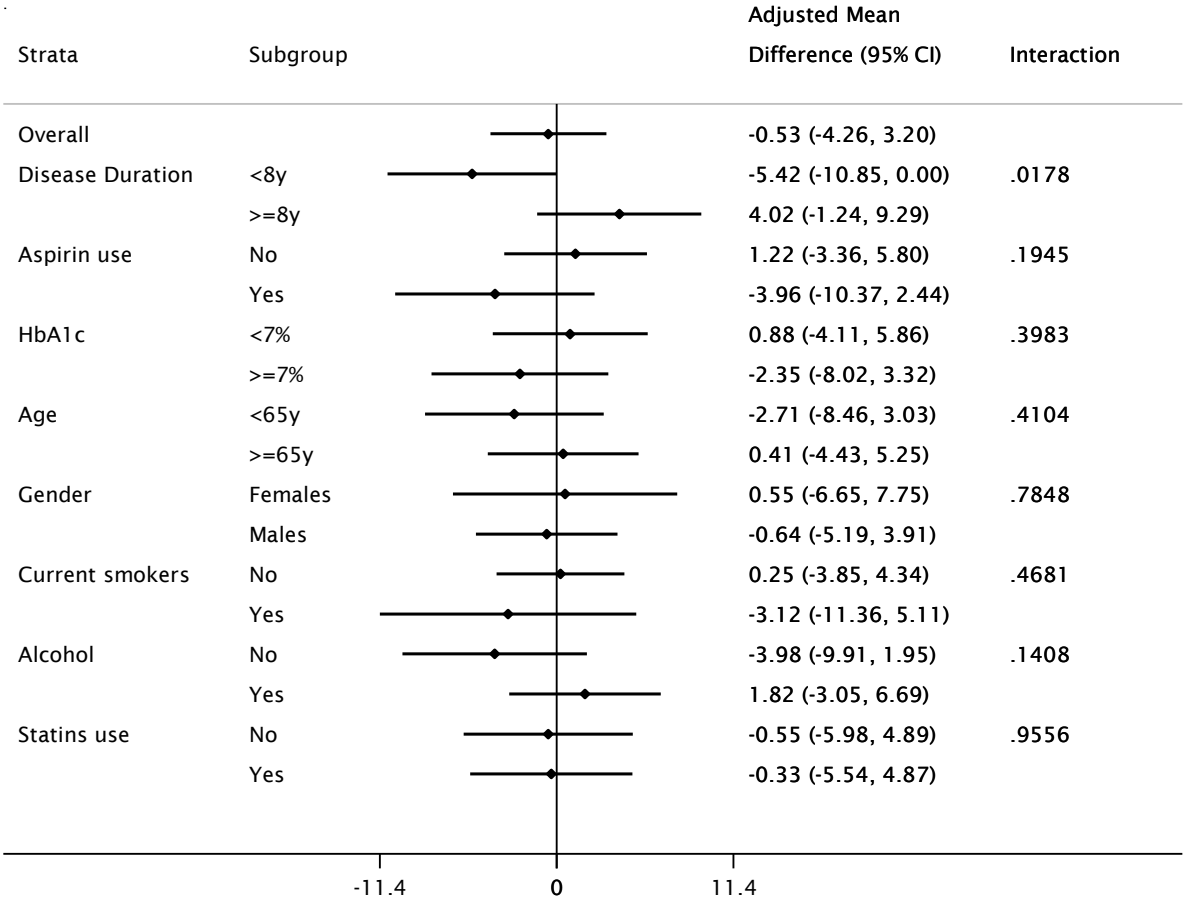

Table 1. Baseline characteristics by arm of the trial

	Placebo	Resv 40	Resv 500
Number	62	65	65
Age (years)	65.4±8.8	64.9±8.6	65.0±7.6
Males (%)	75.8	58.5	63.1
Actual smokers (%)	22.6	18.5	18.5
Diabetes duration (years)	8.0 (10.0)	7.0 (13.0)	7.0 (10.0)
Aspirin use (%)	35.5	35.4	35.4
Drop-outs (%)	6.5	9.2	4.6
METS (h/week)	29.1 (30.7)	30.3 (37.7)	30.5 (35.7)
Kcal/day	1815.6±354.2	1753.1±302.0	1816.1±391.8
Carbohydrate (% kcal)	50.5±4.4	50.3±6.7	50.7±6.0
Protein (% kcal)	15.1±2.4	14.9±2.4	15.2±2.7
Total fat (% kcal)	34.4±4.4	34.8±5.7	34.1±5.0
Saturated fatty acids (% kcal)	6.7±1.5	6.6±1.5	7.0±1.8
Monounsaturated fatty acids (% kcal)	13.2±2.8	13.7±3.2	13.7±3.3
Fiber (g/day)	20.0±7.8	20.4±7.7	20.9±7.6

Resveratrol (mg/day)	0.61 (1.68)	0.61 (1.78)	0.65 (1.79)
Alcohol drinking (%)	58.1	56.9	58.5
Weight (kg)	80.5±12.7	80.3±14.8	78.8±14.7
BMI (kg/m^2)	28.2±3.9	29.5±3.8	28.8±3.9
Waist circumference (cm)	101.7±11.2	103.3±10.7	101.2±9.7
Fat mass (%)	31.7±8.5	33.4±7.0	32.0±7.2
Systolic blood pressure (mmHg)	134.1±8.8	133.1±10.1	131.8±10.0
Diastolic blood pressure (mmHg)	81.3±7.5	80.9±7.5	81.4±8.6
Fasting glucose (mg/dl)	143.3±40.2	146.4±48.5	138.2±29.5
HbA1c (%)	6.9±1.0	7.2±1.3	6.9±1.2
Insulin (µU/ml)	13.6 (9.63)	17.3 (12.1)	15.3 (9.69)
HOMA-IR (mmol/L x µU/ml)	4.49 (3.42)	5.29 (5.31)	5.25 (3.45)
C-peptide (nmol/l)	0.84±0.4	0.97±0.5	0.84±0.5
Total cholesterol (mg/dl)	147.5±35.1	173.3±41.8	185.0±36.8
HDL cholesterol (mg/dl)	45.9±14.6	44.5±14.0	47.8±14.0
LDL cholesterol (mg/dl)	103.7±32.0	103.0±33.9	109.2±34.9
Triglycerides (mg/dl)	105.0 (72.0)	109.0 (87.0)	122.0 (74.0)
Free fatty acids (mmol/l)	0.66±0.21	0.66±0.19	0.66±0.20
Uric acid (mg/dl)	5.6±1.3	5.2±1.4	5.4±1.4
AST (U/l)	20.5±5.4	22.8±7.5	21.4±5.6

ALT (U/l)	16.4±6.3	19.5±10.8	17.8±7.6
GGT (U/l)	23.0 (18.0)	22.0 (14.0)	25.0 (17.0)
Adiponectin (ng/ml)	7901.1 (5640.0)	6871.2 (6175.8)	8106.4 (4699.0)
CRP (mg/l)	1.39 (2.63)	2.78 (2.91)	1.27 (2.10)
IL-6 (pg/ml)	2.83 (1.91)	2.71 (2.37)	2.55 (2.15)
Treatment with statins (%)	58.1	55.4	55.4
Anti-hypertensive drugs (%)	71.0	70.8	69.2
Metformin use (%)	66.1	67.7	67.7
Sulphonylureas use (%)	37.1	32.3	35.4
Incretin use (%)	24.2	29.2	26.2

Alanine aminotranferase (ALT); aspartate aminotransferase (AST); C-reactive protein (CRP); γ-glutamyl transferase (GGT); metabolic equivalent of activity (MET); Interleukin-6 (IL-6).

Mean ± SD (all such values); median (inter-quartile range) (all such variables)

Table 2. Comparisons on change from baseline for study endpoints. Analyses performed by using ANCOVA (adjusted for baseline level and stratification variable)

	Placebo	Resv 40 *vs* Placebo			Resv 500 *vs* Placebo		
	Mean change from baseline	Mean change from baseline	Adjusted mean difference on change from baseline (95%CI)	P	Mean change from baseline	Adjusted mean difference on change from baseline (95%CI)	P
Weight (kg)	-0.25	-0.48	-0.03 (-1.33 1.28)	0.97	-0.36	0.10 (-0.97 1.17)	0.85
BMI (kg/m^2)	-0.08	-0.19	-0.06 (-0.53 0.41)	0.80	-0.15	0.00 (-0.38 0.38)	0.99
Waist circumference (cm)	0.27	0.05	0.03 (-1.91 1.98)	0.97	0.12	-0.32 (-1.89 1.26)	0.69
Fat mass (%)	0.72	0.80	0.11 (-0.75 0.97)	0.80	0.96	0.3 (-0.42 1.01)	0.42
Systolic BP (mmHg)	-0.04	-3.27	-3.45 (-7.41 0.51)	0.09	-2.67	-3.29 (-7.23 0.66)	0.10
Diastolic BP (mmHg)	-1.46	-1.42	-0.55 (-3.52 2.43)	0.72	-1.45	0.04 (-2.74 2.82)	0.98
Fasting glucose (mg/dl)	2.91	-5.09	-6.59 (-18.14 4.96)	0.26	4.38	-0.35 (-10.44 9.74)	0.95
HbA1c (%)	0.18	0.07	0.04 (-0.31 0.39)	0.82	0.31	0.10 (-0.20 0.40)	0.51
Insulin (μU/ml)	1.24	1.05	2.02 (-0.76 4.81)	0.15	2.05	0.97 (-1.83 3.78)	0.49
HOMA-IR (mmol/l xμU/ml)	-4.06	-6.15	0.11 (-0.03 0.26)	0.13	-4.33	0.06 (-0.07 0.20)	0.36
C-peptide (nmol/l)	0.04	-0.02	0.01 (-0.13 0.15)	0.86	0.04	0.04 (-0.10 0.17)	0.59
Total CT (mg/dl)	-6.95	1.14	8.19 (-2.36 18.74)	0.13	1.57	11.94 (2.55 21.33)	0.01

HDL CT (mg/dl)	0.36	1.48	0.35 (-2.77 3.47)	0.83	0.23	-0.13 (-3.38 3.11)	0.94
LDL CT (mg/dl)	-6.34	-0.39	6.71 (-2.42 15.84)	0.15	-1.98	6.37 (-2.83 15.58)	0.17
Triglycerides (mg/dl)	3.68	-8.76	1.58 (-14.61 17.77)	0.85	23.78	25.7 (-0.12 51.48)	0.05
Free fatty acids (mmol/l)	0.006	-0.005	-0.01 (-0.02 0.01)	0.25	-0.017	-0.02 (-0.07 0.03)	0.51
Uric acid (mg/dl)	0.29	0.29	-0.05 (-0.45 0.35)	0.80	0.26	-0.04 (-0.43 0.36)	0.86
AST (U/l)	2.14	2.03	-0.17 (-3.18 2.83)	0.91	4.53	2.57 (-1.25 6.40)	0.19
ALT (U/l)	3.57	1.49	-0.23 (-4.65 4.18)	0.92	4.30	1.62 (-4.18 7.42)	0.59
GGT (U/l)	10.04	4.37	-5.25 (-23.83 13.34)	0.58	1.63	-7.50 (-24.26 9.27)	0.38
Adiponectin (ng/ml)	-786.78	735.59	1497.18 (-277.9 3272.2)	0.10	1726.30	1498.05 (-431.7 3427.8)	0.13
IL-6 (pg/ml)	0.22	0.04	-0.18 (-1.30 0.94)	0.75	-0.09	-0.64 (-1.71 0.44)	0.24
CRP (mg/l)	1.91	-0.25	-0.53 (-4.26 3.20)	0.78	-0.53	-2.46 (-5.79 0.88)	0.15

Alanine aminotranferase (ALT); aspartate aminotransferase (AST); blood pressure (BP); cholesterol (CT); C-reactive protein (CRP); γ-glutamyl transferase (GGT); Interleukin-6 (IL-6).

Table S1: Stratified comparisons by disease duration on change from baseline for the study endpoints: Resv 500 *vs* Placebo

	Diabetes Duration <8 years			Diabetes Duration>=8 Years			
	Mean Change from baseline Placebo	**Mean Change from baseline Resv 500**	**Adjusted mean difference on change from baseline (95%CI)**	**Mean Change from baseline Placebo**	**Mean Change from baseline Resv 500**	**Adjusted mean difference on change from baseline (95%CI)**	**Interaction-p**
Weight (kg)	0.04	-0.72	-0.81 (-2.44 0.81)	-0.52	0.03	0.95 (-0.61 2.51)	0.14
BMI (kg/m^2)	0.03	-0.31	-0.37 (-0.94 0.19)	-0.18	0.01	0.35 (-0.2 0.9)	0.08
Waist circumference (cm)	0.47	-0.32	-1.19 (-3.58 1.21)	0.09	0.59	0.46 (-1.84 2.77)	0.35
Fat Mass (%)	0.61	0.86	0.5 (-0.6 1.59)	0.83	1.06	0.11 (-0.94 1.17)	0.63
Systolic BP (mmHg)	-0.74	-3.71	-3.74 (-9.73 2.25)	0.62	-1.55	-2.75 (-8.51 3.01)	0.82
Diastolic BP (mmHg)	-1.30	-0.81	-0.14 (-4.37 4.1)	-1.62	-2.14	0.11 (-3.96 4.17)	0.94
Fasting glucose (mg/dl)	3.37	3.32	-2.94 (-18.12 12.23)	2.48	5.51	2.4 (-12.6 17.4)	0.64
HbA1c (%)	0.38	-0.09	-0.24 (-0.68 0.21)	-0.01	0.74	0.44 (0.02 0.87)	0.04
HOMA-IR (mmol/l xμU/ml)	-3.78	-4.71	0.01 (-0.19 0.22)	-4.32	-3.93	0.12 (-0.08 0.32)	0.48
Insulin (μU/ml)	0.45	0.98	-0.04 (-4.25 4.16)	1.98	3.19	2.05 (-2.06 6.17)	0.50
C-peptide (nmol/l)	-0.06	-0.06	-0.04 (-0.24 0.16)	0.12	0.14	0.12 (-0.08 0.31)	0.29
Total CT (mg/dl)	-10.63	-2.06	16.64 (2.37 30.91)	-3.52	5.45	7.97 (-5.68 21.63)	0.41
HDL CT(mg/dl)	1.93	-1.64	-1.74 (-6.63 3.15)	-1.10	2.24	1.63 (-3.12 6.37)	0.35
LDL CT (mg/dl)	-11.96	-8.90	8.15 (-5.72 22.03)	-1.10	5.41	5.07 (-8.36 18.51)	0.76
Triglycerides (mg/dl)	7.18	46.71	38.26 (0.1 76.41)	0.41	-0.72	10.79 (-25.74 47.33)	0.32
Free fatty acids (mmol/l)	0.007	0.01	0.001 (-0.08 0.08)	0.004	-0.05	-0.04 (-0.11 0.04)	0.49
Uric acid (mg/dl)	0.22	0.52	0.27 (-0.33 0.86)	0.35	-0.01	-0.34 (-0.91 0.23)	0.16
AST (U/l)	3.00	5.13	1.02 (-4.8 6.85)	1.34	3.90	3.81 (-1.78 9.39)	0.51
ALT (U/l)	4.70	0.39	-4.00 (-12.77 4.77)	2.52	8.48	7.11 (-1.25 15.47)	0.08
GGT (U/l)	17.81	2.58	-14.98 (-40.35 10.39)	2.79	0.62	-1.23 (-25.77 23.3)	0.46
Adiponectin (ng/ml)	-719.27	2622.30	2853.93 (-50.6 5758.4)	-849.63	768.50	220.92 (-2567.2 3009)	0.21
IL-6 (pg/ml)	-0.20	-0.43	-1.31 (-2.95 0.33)	0.59	0.28	-0.04 (-1.61 1.54)	0.29
CRP (mg/l)	3.29	-1.91	-6.09 (-11.04 -1.13)	0.62	0.94	0.79 (-4.03 5.62)	0.06

Alanine aminotranferase (ALT); aspartate aminotransferase (AST); blood pressure (BP); cholesterol (CT); C-reactive protein (CRP); γ-glutamyl transferase (GGT); interleukin-6 (IL-6)

Table 2S. Stratified comparisons by disease duration on change from baseline for study endpoints: Resv 40 *vs* Placebo.

	Diabetes Duration <8 years			**Diabetes Duration>=8 Years**			
	Mean Change from baseline Placebo	**Mean Change from baseline Resv 40**	**Adjusted mean difference on change from baseline (95%CI)**	**Mean Change from baseline Placebo**	**Mean Change from baseline Resv 40**	**Adjusted mean difference on change from baseline (95%CI)**	**Interaction-p**
Weight (kg)	0.04	-0.79	-0.93 (-2.88 1.02)	-0.52	-0.14	0.82 (-1.09 2.73)	0.23
BMI (kg/m^2)	0.03	-0.29	-0.37 (-1.06 0.32)	-0.18	-0.09	0.24 (-0.45 0.92)	0.23
Waist circumference (cm)	0.47	-0.63	-1.31 (-4.18 1.56)	0.09	0.80	1.38 (-1.44 4.21)	0.20
Fat Mass (%)	0.61	0.92	0.14 (-1.13 1.41)	0.83	0.65	0.07 (-1.21 1.34)	0.94
Systolic BP (mmHg)	-0.74	-2.26	-2.46 (-8.35 3.44)	0.62	-4.39	-4.45 (-10.23 1.32)	0.64
Diastolic BP (mmHg)	-1.23	-1.23	-1.62 (-6.04 2.81)	-1.62	-1.64	0.37 (-3.96 4.69)	0.54
Fasting glucose (mg/dl)	3.37	-6.97	-11.55 (-28.59 5.48)	2.48	-3.00	-1.03 (-17.77 15.71)	0.40
HbA1c (%)	0.38	-0.25	-0.370 (-0.87 0.13)	-0.01	0.43	0.48 (-0.01 0.97)	0.02
HOMA-IR (mmol/l xμU/ml)	-3.78	-4.60	0.1 (-0.11 0.3)	-4.32	-7.87	0.15 (-0.06 0.36)	0.71
Insulin (μIU/ml)	0.45	1.49	1.77 (-2.21 5.74)	1.98	0.55	2.66 (-1.4 6.72)	0.76
C-peptide (nmol/l)	-0.06	-0.02	0.01 (-0.19 0.22)	0.12	-0.01	0.02 (-0.18 0.23)	0.94
Total CT (mg/dl)	-10.63	1.19	14.45 (-1.13 30.03)	-3.52	1.07	2.62 (-12.69 17.93)	0.30
HDL CT (mg/dl)	1.93	2.87	1.88 (-2.69 6.44)	-1.10	-0.07	-1.24 (-5.81 3.32)	0.35
LDL CT (mg/dl)	-11.96	-3.06	9.65 (-3.82 23.13)	-1.10	2.57	4.49 (-8.75 17.73)	0.60
Triglycerides (mg/dl)	7.18	6.90	6.46 (-17.38 30.3)	0.41	-26.11	-4.25 (-27.74 19.23)	0.54
Free fatty acids (mmol/l)	0.007	-0.009	-0.016 (-0.04 0.007)	0.004	0.00	0 (-0.02 0.02)	0.40
Uric acid (mg/dl)	0.22	0.43	0.19 (-0.42 0.79)	0.35	0.12	-0.27 (-0.85 0.31)	0.30
ALP (U/l)	14.48	4.97	-12.17 (-30.62 6.28)	10.34	15.50	9.11 (-8.73 26.95)	0.11
AST (U/l)	3.00	0.42	-2.7 (-7.05 1.65)	1.34	3.82	2.66 (-1.66 6.99)	0.09
ALT (U/l)	4.70	-0.52	-3.45 (-9.87 2.97)	2.52	3.71	3.41 (-2.85 9.68)	0.14
GGT (U/l)	17.81	-0.74	-18.82 (-46.32 8.68)	2.80	10.04	6.79 (-20.11 33.69)	0.20
Adiponectin (ng/ml)	-719.27	1688.14	2552.31 (-71.24 5175.87)	-849.63	-369.36	350.34 (-2212.79 2913.46)	0.25
IL-6 (pg/ml)	-0.20	-0.51	-0.56 (-2.23 1.10)	0.59	0.66	0.16 (-1.47 1.78)	0.55
CRP (mg/l)	3.29	-4.19	-5.42 (-10.85 0)	0.62	4.10	4.02 (-1.24 9.29)	0.02

Alanine aminotranferase (ALT); aspartate aminotransferase (AST); blood pressure (BP); cholesterol (CT); C-reactive protein (CRP); γ-glutamyl transferase (GGT); interleukin-6 (IL-6).