Resveratrol as Add-on Therapy in Subjects With Well-Controlled Type 2 Diabetes: A Randomized Controlled Trial

DOI: 10.2337/dc16-0499

Silvie Timmers,[1] Marlies de Ligt,[1] Esther Phielix,[1] Tineke van de Weijer,[1] Jan Hansen,[1] Esther Moonen-Kornips,[1] Gert Schaart,[1] Iris Kunz,[2] Matthijs K.C. Hesselink,[1] Vera B. Schrauwen-Hinderling,[1,3] and Patrick Schrauwen[1]

OBJECTIVE

To determine whether resveratrol supplementation can improve insulin sensitivity and promote overall metabolic health on top of standard diabetes care.

RESEARCH DESIGN AND METHODS

Seventeen well-controlled subjects with type 2 diabetes (T2D) were treated with placebo and 150 mg/day resveratrol (resVida) in a randomized double-blind crossover study for 30 days. The main outcome measure was insulin sensitivity by the hyperinsulinemic-euglycemic clamp technique.

RESULTS

Hepatic and peripheral insulin sensitivity were not affected by resveratrol treatment. Intrahepatic lipid content also remained unaffected by resveratrol; however, the change in intrahepatic lipid content correlated negatively with plasma resveratrol levels ($R = -0.68$, $P = 0.03$). Intramyocellular lipid content increased in type 2 muscle fibers ($P = 0.03$), and systolic blood pressure tended to decrease ($P = 0.09$) upon resveratrol treatment. In addition, resveratrol significantly improved ex vivo mitochondrial function (state 3 and state U respiration upon malate with octanoyl-carnitine, $P < 0.005$). Intriguingly, a correlation was found between plasma levels of a metabolite of resveratrol (dihydroresveratrol) and the metformin dose used by the patients ($R = 0.66$, $P = 0.005$), suggesting an interaction between metformin and resveratrol. It could be speculated that the lack of a resveratrol-induced insulin sensitizing effect is caused by this interaction.

CONCLUSIONS

Resveratrol supplementation does not improve hepatic or peripheral insulin sensitivity. Our results question the generalized value of resveratrol as add-on therapy in the treatment of T2D and emphasize the need to perform studies in drug-naive patients with T2D or subjects with prediabetes.

Exercise and calorie restriction (1) are the primary treatment options for type 2 diabetes (T2D). Both target the cellular energy-sensing route, with activation of AMPK and the sirtuin (SIRT) family of transcription factors, resulting in stimulation of mitochondrial biogenesis and function (2). Nutraceutical compounds, such as resveratrol, can also target these pathways (3). Interest in resveratrol peaked when Howitz et al. (4) identified this polyphenolic compound as a potent SIRT1 activator. Since then, resveratrol has been postulated to alleviate metabolic

[1]Department of Human Biology and Human Movement Sciences, NUTRIM School for Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, the Netherlands
[2]DSM Nutritional Products Ltd., Kaiseraugst, Switzerland
[3]Department of Radiology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center, Maastricht, the Netherlands

Corresponding author: Patrick Schrauwen, p.schrauwen@maastrichtuniversity.nl.

Received 7 March 2016 and accepted 18 September 2016.

Clinical trial reg. no. NCT01638780, clinicaltrials.gov.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc16-0499/-/DC1.

S.T. and M.d.L. contributed equally to this work.

consequences of consumption of energy-dense foods and physical inactivity, including T2D (5,6). Indeed, animal studies suggest that resveratrol may blunt metabolic complications induced by a high-fat diet (3,7–9). These encouraging findings have stimulated the application of resveratrol in clinical trials to investigate its potency in humans with chronic metabolic diseases. Some (10,11), but not all, studies (12–14) in obese humans have reported positive effects of resveratrol on measures of insulin sensitivity. In contrast to studies in obese humans, studies in subjects with T2D have been more consistent in reporting a beneficial effect of resveratrol on blood glucose levels (15–17), insulin levels (16,17), markers of insulin resistance, such as the HOMA insulin-resistance index (15,17), and HbA_{1c} (17,18). None of these studies, however, examined the effect of resveratrol on peripheral and hepatic insulin sensitivity by the gold standard hyperinsulinemic-euglycemic clamp technique. Hence, we performed a randomized, double-blinded, placebo-controlled crossover study to examine if 30 days of resveratrol (resVida) supplementation leads to an improvement in peripheral and hepatic insulin sensitivity in subjects with well-controlled T2D.

RESEARCH DESIGN AND METHODS

The study protocol was approved by the Medical Review Ethics Committee of Maastricht University and Medical Centre. All study participants were informed about the nature and risks of the experimental procedures before their written informed consent was obtained.

Participants

Seventeen men (age 40–70 years, BMI 27–35 kg/m^2, body fat percentage >25%) with well-controlled T2D (HbA_{1c} < 8.0% [<64 mmol/mL]) participated in the study. Sixteen participants were treated with the oral glucose-lowering medication metformin, six of whom were treated in combination with sulfonylurea derivatives (SUDs) (Supplementary Table 1). Most of the participants received additional medications to lower cholesterol (n = 11) and/or blood pressure (n = 12). Exclusion criteria were unstable body weight (weight gain or loss >3 kg in the previous 3 months), engagement in programmed exercise >2 h per week, impaired renal and/or kidney function, intake of dietary supplements (except vitamins and minerals), alcohol consumption >20 g/day, diabetes comorbidities, and insulin treatment.

Outcomes

The primary outcome measure was the effect of resveratrol treatment on insulin sensitivity compared with placebo. Secondary outcome measures were intrahepatic lipid content (IHL), intramyocellular lipids (IMCL), mitochondrial function (in vivo and ex vivo), blood pressure, and cardiac function.

Clinical Study Design

The study was conducted at Maastricht University Medical Center, the Netherlands, between June 2012 and June 2014. In randomized order, participants underwent two experimental trials: a placebo and a resVida (150 mg/day *trans*-resveratrol [99.9%]; provided by DSM Nutritional Products Ltd.) condition, with a washout period of at least 30 days. The resveratrol dosage was based on our previous study in healthy obese subjects in which we found activation of the energy-sensing pathway AMPK-SIRT1-peroxisome proliferator-activated receptor γ coactivator 1-α (PGC1-α) in combination with metabolic improvements (10). Randomization was performed according to standard procedures as described in *Statistical Methods* by Snedecor and Cochran (19).

Participants were instructed to take the first supplement on the day of the baseline measurements (day 0) the last supplement in the evening of day 29, and to abstain from food and beverages containing substantial amounts of resveratrol (e.g., wine, grapes, peanuts, and berries) and from food supplements. In addition, instructions to maintain their normal living, activity and sleeping patterns were given. At the start (day 0) and end (day 30) of both intervention periods, blood samples were analyzed for general safety parameters (creatinine, aspartate aminotransferase, alanine aminotransferase, γ- glutamyl transferase , bilirubin), blood pressure was measured after an overnight fast, as previously described (10), and a 12-lead electrocardiogram was performed. Body fat percentage was determined by DXA on day 0 of the first intervention period for patient characterization.

Each experimental trial lasted 30 days, and the participants came to the university weekly (days 0, 7, 14, 21, and 29). The weekly checkup took place in the morning in overnight fasted state and included body weight measurement and drawing of a blood sample for analysis of resveratrol (parental and metabolites) to confirm compliance to the protocol. On day 29 at 4 P.M., subjects came to the university for cardiac function measurements by M-mode, two-dimensional and Doppler echocardiography, using a Vivid 7 ultrasound system (GE Healthcare, Milwaukee, WI) with 3.5-MHz cardiac transducer. Results were interpreted according to the criteria of the American Societies of Echocardiography. Subsequently, by proton magnetic spectroscopy (^{1}H-MRS) IHL content was quantified, as described earlier (20), on a 3T whole-body scanner (Achieva Tx; Philips Healthcare, Best, the Netherlands) using an echo time of 32.5 ms. Spectra were fitted with a home-written script (21) in MATLAB R2014b (MathWorks, Natick, MA). Values are given as T2-corrected ratios (22) of the CH_2 peak, relative to the unsuppressed water resonance (as percentage). Postexercise phosphocreatine (PCr) recovery was assessed by ^{31}P-MRS to estimate in vivo mitochondrial function in vastus lateralis muscle on a 3T whole-body scanner, described elsewhere (21). To standardize food intake before these measurements, subjects consumed a standardized lunch at a fixed time and afterward stayed fasted until the measurements were completed.

After the MRS measurements, participants entered the respiration chamber for 12 h starting at 7 P.M. to allow measurement of sleeping metabolic rate (23). Before the respiration chamber measurement started, a standardized evening meal (pasta Bolognese and fruit yogurt) was provided. The energy provided was based on individual daily energy requirements, calculated with the Harris and Benedict equation. In the morning of day 30, participants left the respiration chamber at 7 A.M. in the overnight fasted state and a biopsy specimen was taken from the vastus lateralis muscle under local anesthesia (2% lidocaine), as previously described (24). A portion of the muscle tissue was directly frozen in melting isopentane for immunohistochemistry and stored at −80°C until assayed. Another portion (~30 mg) was immediately placed in ice-cold preservation medium for determination of ex vivo mitochondrial respiration. From the

muscle tissue in the preservation medium, permeabilized muscle fibers were immediately prepared (24). The permeabilized muscle fibers (~2.5 g wet weight) were analyzed for mitochondrial function using an oxygraph (OROBOROS Instruments, Innsbruck, Austria) (10). Fresh cryosections (5 μm) were stained for IMCL by Oil Red O staining combined with fiber typing and immunolabeling of the basal membrane marker laminin to allow quantification of IMCL (25). Mitochondrial DNA copy number and protein expression of oxidative phosphorylation (OXPHOS) and PGC1-α by Western blot were performed according to standard procedures as described previously (10).

After the muscle biopsy was taken, a two-step hyperinsulinemic-euglycemic clamp was performed to assess peripheral and hepatic insulin sensitivity. Three days before the clamp, subjects were instructed to refrain from strenuous activities and to continue their antidiabetic medication, with the last dose on the morning of the test. The clamp started by giving the subjects a primed continuous infusion of D-[6,6-$^2H_2$]glucose (0.04 mg/kg/min) to determine rates of endogenous glucose production (EGP), glucose appearance (R_a), and glucose disposal (R_d) (26). After 120 min, a low insulin infusion was started (10 mU/m^2/min) for 3 h, after which a high insulin infusion was started (40 mU/m^2/min) for 2 h. During the last 30 min of each insulin infusion step (0, 10, and 40 mU/m^2/min), blood samples were collected, and substrate utilization was measured by indirect calorimetry. Steele's single pool nonsteady-state equations were used to calculate glucose R_a and R_d (27). Volume of distribution was assumed to be 0.160 L/kg for glucose.

Compliance

To check compliance, resveratrol metabolites were measured by mass spectroscopy in plasma on days 0, 7, 14, 21, and 30 (10). In addition, unused capsules were counted.

Sample Size

The sample size was determined based on demonstrating the statistical superiority of resveratrol on insulin-stimulated glucose disposal in muscle compared with placebo treatment. We estimated 14 subjects were required to achieve 80% power, with an assumed treatment difference of 1.4 mg/kg fat-free mass/min after 30 days and an assumed SD of 1.7 mg/kg fat-free mass/min for a hyperinsulinemic clamp. A dropout of 20% was taken into account, so 17 subjects were recruited. The expected effect size and SD of a hyperinsulinemic-euglycemic clamp was based on a previous study in our research group with a pre- and postintervention clamp in subjects with T2D (28).

Statistical Analysis

Results are presented as means ± SEM when normally distributed and as median (95% CI) when this was not the case (using Shapiro-Wilk normality test). The Student paired *t* test was used to compare placebo and resveratrol supplementation in normally distributed data; otherwise, the Wilcoxon signed-rank test was used. Treatment comparisons of plasma parameters measured at the beginning and end intervention were assessed by two-way repeated-measures ANOVA. Linear regression analyses were conducted to identify correlations between variables. On normally distributed data Pearson correlation was used, otherwise Spearman correlation was used. Potential carryover effect between treatment and period was examined by unpaired *t* test analyses according to Pocock et al. (29). A *P* value of <0.05 was considered statistically significant. Analyses were performed using SPSS 22.0 software.

RESULTS

Study Design and Plasma Biochemistry

The study participants were 17 well-controlled male subjects with T2D (baseline characteristics, Supplementary Table 1). To check compliance with the study protocol and to confirm systemic conversion of resveratrol to dihydroresveratrol (DHR), parental resveratrol and DHR (aglycone + glucuronide conjugates) levels were determined weekly in the plasma. Although no resveratrol or DHR could be detected during the placebo treatment, both compounds were present in the plasma of all resveratrol-supplemented subjects. Plasma levels were 378.59 ± 40.65 ng/mL for parental resveratrol and 444.69 ± 101.81 ng/mL for DHR at day 30 of the resveratrol treatment. These levels are representative for the entire study period. In addition, no carryover effect was found between treatment and period for the primary study outcome, confirming the washout period was sufficient.

Insulin Sensitivity and Substrate Kinetics Assessed by Hyperinsulinemic-Euglycemic Clamp

Plasma glucose profiles during the hyperinsulinemic-euglycemic clamp procedure were similar for the placebo and the resveratrol conditions (Fig. 1*A*). Both during the low- and high-insulin state, glucose levels were clamped ~5 mmol/L (Fig. 1*A*). Insulin-stimulated glucose disposal, expressed as the change in R_d from basal to the low- or high-insulin state, was not significantly changed by resveratrol supplementation (P_{basal} = 0.65, $P_{low\ insulin}$ = 0.89, and $P_{high\ insulin}$ = 0.66) (Fig. 1*B*). Basal EGP, reflecting hepatic glucose output, and EGP under low insulin–stimulated conditions were also similar between both conditions, suggesting that hepatic insulin sensitivity was unchanged (P_{basal} = 0.22) (Fig. 1*C*). In fact, EGP was similarly suppressed during the low-insulin state of the hyperinsulinemic-euglycemic clamp ($P_{low\ insulin}$ = 0.84, $P_{high\ insulin}$ = 0.74) (Fig. 1*C*), and EGP suppression was nearly complete upon high-insulin infusion in both conditions ($P_{low\ insulin}$ = 0.64, $P_{high\ insulin}$ = 0.73) (Fig. 1*D*). In line with the absence of resveratrol-induced changes in R_d and EGP, nonoxidative glucose disposal also remained unaffected by 30 days of resveratrol supplementation (Supplementary Table 2). In addition, resveratrol did not exert an effect on circulating insulin levels (Supplementary Table 2), thereby excluding an effect of resveratrol on insulin clearance.

As expected, carbohydrate oxidation increased during the clamp at the expense of free fatty acid (FFA) oxidation (Supplementary Table 2). No statistically significant changes in carbohydrate or FFA oxidation rates were observed when basal and insulin-stimulated oxidation rates were compared between placebo and resveratrol (Supplementary Table 2). At the end of the hyperinsulinemic-euglycemic clamp, suppression of FFA was similar between both conditions (*P* = 0.36).

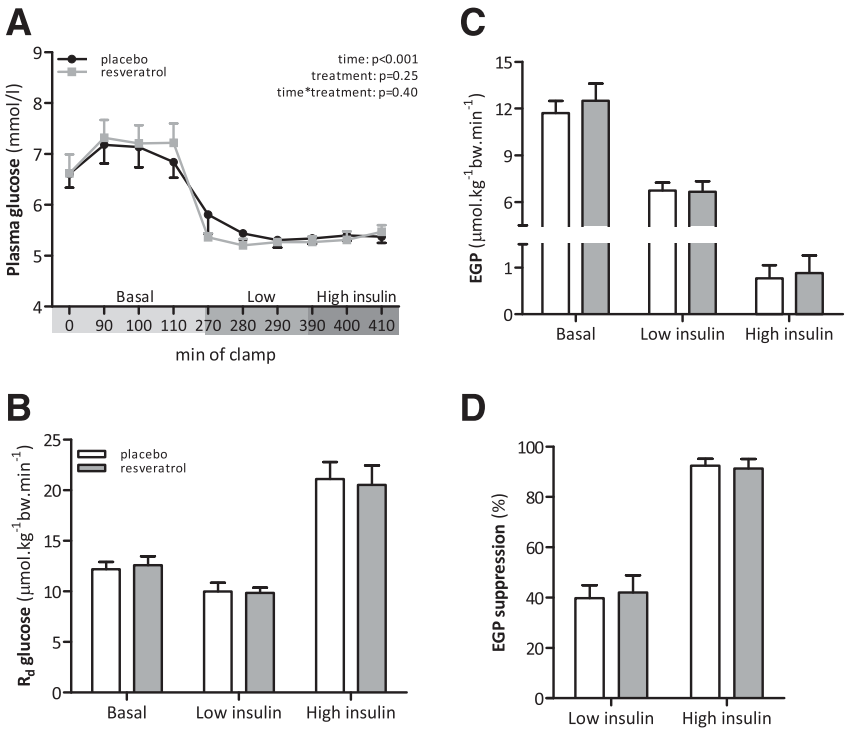

Figure 1—Effect of resveratrol on peripheral and hepatic insulin sensitivity. After 30 days of resveratrol and placebo, peripheral and hepatic insulin sensitivity was assessed with a two-step hyperinsulinemic-euglycemic clamp (t = 0–120 min: D-[6,6-$^2H_2$]glucose tracer infusion; t = 120–300 min: low-insulin infusion; t = 300–420 min: high-insulin infusion). As a result of technical problems, data are only available for 14 subjects (in 1 subject the equipment malfunctioned, in another subject the catheter with the D-[6,6-$^2H_2$]glucose tracer leaked, and in the last subject aspiration of the venous catheter was no longer possible in the late phase of the clamp, which is necessary for regular blood sampling). *A*: Plasma glucose levels during the last 30 min of the basal, low- and high-insulin state of the clamp. Glucose levels were clamped ~5 mmol/L. Insulin-stimulated glucose disposal, expressed as the R_d (*B*), and EGP were calculated for the last 30 min of the basal, low- and high-insulin state (*C*). *D*: EGP suppression upon low- and high-insulin infusion. Data are presented as mean ± SEM. R_d, rate of disappearance.

In accordance with the lack of effect of resveratrol on insulin sensitivity, no improvement in fasting glucose, insulin, or HbA_{1c} levels were found compared with placebo (Supplementary Table 3). We did observe a time effect for HbA_{1c}; however, this was found in both treatment conditions. Similarly, no resveratrol effect on other markers of metabolic health was found (Supplementary Table 3).

Sleeping Metabolism

Sleeping metabolic rate, measured in a whole-body respiratory chamber during the last night of the intervention, decreased upon resveratrol supplementation in 82% of subjects. However, the difference failed to reach statistical significance (7.81 ± 0.20 MJ/day for placebo vs. 7.55 ± 0.16 MJ/day for resveratrol, P = 0.14). During the night, substrate utilization (reflected by the respiratory quotient) was similar for both treatments, at 0.81 (95% CI 0.81–0.88) for placebo vs. 0.82 (95% CI 0.80–0.85) for resveratrol (P = 0.45).

Ectopic Lipid Storage

Lipid area fraction, measured ex vivo in muscle biopsy specimens by Oil Red O staining, increased significantly in type 2 muscle fibers (P = 0.03) (Fig. 2*A*), and a similar tendency was observed for total lipid content (P = 0.08) (Fig. 2*A*),.

IHL content, determined by ^{1}H-MRS, remained unaffected by 30 days of resveratrol supplementation (Fig. 2*B*). Interestingly, linear regression analysis showed that the difference in IHL content between resveratrol and placebo negatively correlated with the parental plasma resveratrol concentration (R = −0.68, P = 0.03) (Fig. 2*C*), indicating that a decrease in IHL may depend on the plasma resveratrol concentrations achieved.

Mitochondrial Function

Mitochondrial state 3 respiration on a lipid-derived substrate (malate + octanoyl-carnitine) and upon parallel electron input to both complex I and II (malate + octanoyl-carnitine + glutamate + succinate) (Fig. 3*A*), and maximal FCCP-induced uncoupled respiration (P = 0.001) (Fig. 3*B*) were significantly higher after resveratrol supplementation. However, in the absence of a lipid-derived substrate, no resveratrol effect was observed in state 3 respiration upon complex I- and II-linked substrates (Supplementary Fig. 1*A*). In addition, state 4o respiration upon addition of oligomycin (reflecting mitochondrial proton leak) was similar between the resveratrol and the placebo condition (Supplementary Fig.1*B*). Mitochondrial DNA copy number (2,251 ± 162 arbitrary units vs. 2,234 ± 152 arbitrary units, respectively, P = 0.91), mean protein content of the OXPHOS complexes, PGC1-α protein content, and phosphorylated AMPK/AMPK ratio remained unaffected by resveratrol supplementation (Supplementary Fig. 1*C*–*E*). Mean PCr recovery half-time, a measure of in vivo mitochondrial function, was unchanged by resveratrol compared with placebo (Supplementary Fig. 1*F*). In line, VO_{2max}, a measure of cardiorespiratory fitness, was not affected by resveratrol supplementation (23.88 ± 1.10 mL · kg^{-1} · min^{-1} for placebo vs. 23.40 ± 1.34 mL · kg^{-1} · min^{-1} for resveratrol, P = 0.29).

Cardiac Function

Systolic blood pressure, measured in overnight fasted state on day 30, tended to decrease by resveratrol supplementation (P = 0.09), whereas diastolic blood pressure remained unchanged (Supplementary Table 4). Echocardiography revealed a marginal reduction in left ventricular end systolic diameter upon resveratrol supplementation (P = 0.04) (Supplementary Table 4). Because the values remain well within the normal range, they are not interpreted as clinically significant. No change in stroke volume, cardiac output, or left ventricular ejection fraction was found. Parameters of diastolic function of the heart remained unaffected by resveratrol. Furthermore, no structural changes were observed in the heart by resveratrol (Supplementary Table 4).

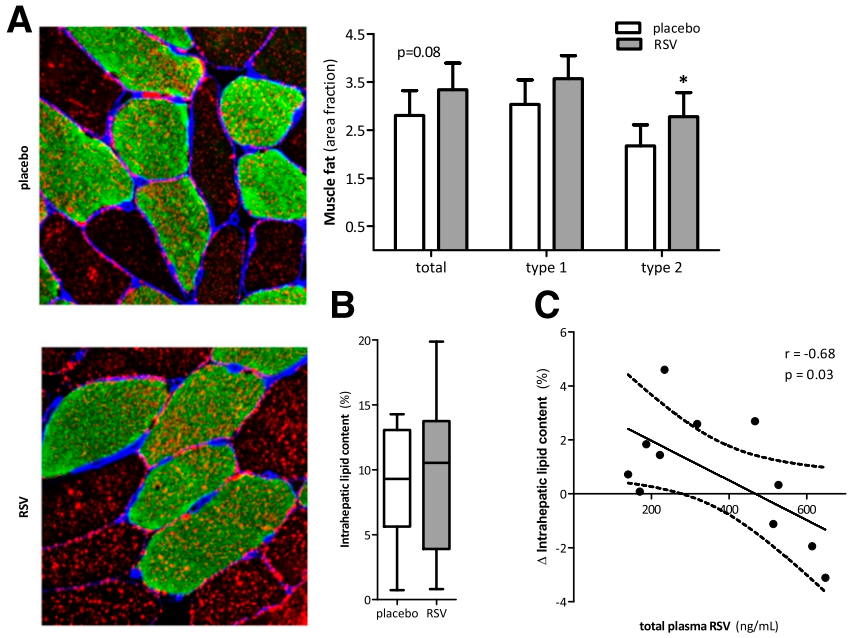

Figure 2—Effect of resveratrol (RSV) on ectopic lipid storage. *A*: Muscle biopsy sections from a representative study participant, stained for IMCL with Oil Red O staining (in red), muscle laminin (in blue), and type 1 muscle fibers (in green). IMCL content is quantified as the percentage area of a muscle fiber that is covered by lipids (n = 17). *B*: IHL content quantified by ^{1}H-MRS after 29 days of resveratrol and placebo supplementation (n = 10). *C*: ΔIHL in relation to total plasma resveratrol concentration (n = 10). Data are presented as means ± SEM. Box plot represents minimum, first quartile, median, third quartile, and maximum. *$P < 0.05$.

Plasma Resveratrol Levels and Metformin Dose

A strong correlation was found between plasma DHR concentration and metformin dose after resveratrol treatment at day 30 (R = 0.66, P = 0.005) (Fig. 4*A*) suggesting an interaction between metformin and the metabolism of resveratrol. Post hoc analysis revealed that total plasma DHR levels were higher during 30 days of resveratrol supplementation in subjects taking a relatively high daily dose of metformin (>1,000 mg/day [n = 9]; average metformin dose, 2,188 mg/day) compared with patients treated with a lower dose (≤1,000 mg/day [n = 8]; average metformin dose, 639 mg/day) (Fig. 4*B*), whereas the daily dose of metformin did not seem to influence plasma levels of parental resveratrol (Fig. 4*C*).

Adverse Events

Resveratrol was well tolerated by the participants, and no adverse events occurred. Measurement of parameters for kidney and liver function (creatinine, aspartate aminotransferase, alanine aminotransferase, γ- glutamyl transferase and bilirubin) indicated that resveratrol was well tolerated (Supplementary Table 3). Creatinine showed a significant, albeit very modest, decline from day 0 to day 30 in both treatment conditions, but no treatment effect over time was found (Supplementary Table 3).

CONCLUSIONS

Preclinical research has suggested that resveratrol could be used to prevent the metabolic consequences of high-fat feeding, including insulin resistance, by improving mitochondrial function. We previously observed in obese, normoglycemic subjects that resveratrol supplementation exerted beneficial metabolic effects accompanied by an improvement in mitochondrial function. The current study did not find a stimulatory effect of resveratrol on peripheral or hepatic insulin sensitivity in subjects with well-controlled T2D on top of standard diabetes care. Other markers of metabolic health also largely remained unaffected by resveratrol, although improvements in ex vivo muscle oxidative capacity were detected. Overall, our results suggest that resveratrol may not be of added value as adjunct therapy for subjects with T2D receiving the oral glucose-lowering medication metformin.

The metabolic actions of resveratrol are mainly ascribed to activation of the energy-sensing pathway AMPK-SIRT1-PGC1-α (1). Several downstream targets of this pathway (e.g., mitochondrial biogenesis and glucose and lipid homeostasis) are interesting targets for treatment of T2D. No positive effect of 30 days of resveratrol supplementation on hepatic EGP, (non)oxidative skeletal muscle glucose disposal, circulating insulin levels, and substrate utilization was detected. Also, fasting glucose, insulin, and HbA_{1c} values remained unaffected by resveratrol. It should be noted that we used an add-on approach, in which resveratrol was supplemented on top of the

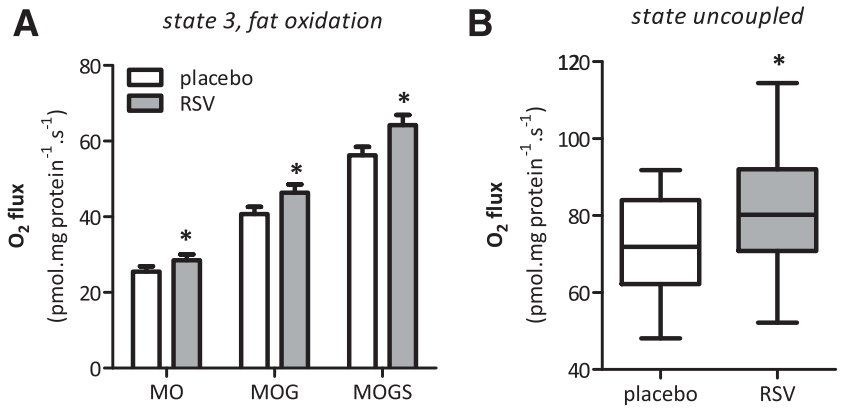

Figure 3—Effect of resveratrol (RSV) on ex vivo mitochondrial function. After 30 days of resveratrol and placebo, a muscle biopsy specimen was obtained from the vastus lateralis muscle. Part of the specimen was used for evaluation of ex vivo mitochondrial function (n = 17). *A*: ADP-stimulated respiration (state 3) upon a lipid-like substrate and upon parallel electron input into complex I and II. *B*: Maximally uncoupled respiration upon FCCP. Data are presented as means ± SEM. Box plot represents minimum, first quartile, median, third quartile, and maximum. *$P <$ 0.05. G, glutamate; M, malate; O, octanoyl-carnitine; S, succinate.

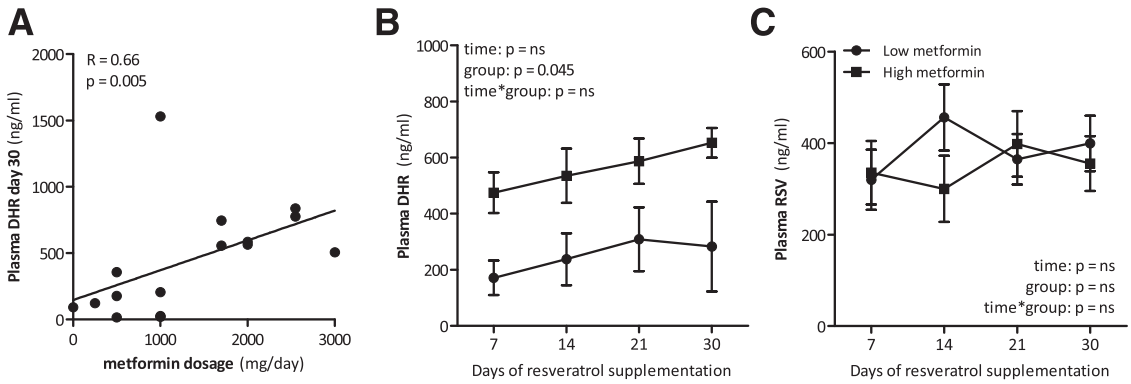

Figure 4—Possible interaction of resveratrol with metformin. Post hoc analysis revealed a possible interaction of metformin with the metabolism of resveratrol. *A*: Correlation of the daily metformin dose used by the patients with T2D and plasma DHR levels. A subsequent subanalysis was performed in which patients with T2D were separated in a group using high daily doses of metformin (>1,000 mg/day [*n* = 9]; average metformin dose, 2,188 mg/day) vs. a group using low daily doses of metformin (≤1,000 mg/day [*n* = 8]; average metformin dose, 639 mg/day). Total plasma DHR levels (*B*) and total resveratrol levels (*C*) for the high- vs. low-dose metformin groups. Data are presented as mean ± SEM.

patients' normal blood glucose–lowering treatment, mainly metformin. It is well known that treatment with the oral glucose-lowering medication metformin leads to phosphorylation of the threonine residue 172 in the α-subunit of AMPK (30). Accordingly, measurement of phosphorylated AMPK by Western blot revealed no significant differences between the resveratrol and placebo condition. Potentially, a daily dose of 150 mg of resveratrol may not have been able to further activate AMPK in subjects with T2D receiving metformin, thereby explaining the lack of effect of resveratrol on insulin sensitivity. In addition to this explanation, our data indicate that metformin may interact with the metabolism of resveratrol. This potential interaction could have influenced the efficacy of the active component, thereby accounting for the lack of a resveratrol-induced improvement of insulin sensitivity.

Interestingly, Movahed et al. (17) recently reported that subjects with T2D treated with metformin only did not show an improvement in circulating insulin or HbA_{1c} levels upon resveratrol administration, whereas patients receiving SUDs only or metformin in combination with SUDs demonstrated a resveratrol-induced improvement in both plasma makers of insulin sensitivity. Together with our study results, these findings reinforce the importance of investigating the relationship between resveratrol and metformin in future research.

A potential mechanism that may partly explain the interaction of metformin with resveratrol can be sought in the recently appreciated influence of metformin on gut microbiota composition (31,32). The gut microbiota is one of the major sites of metabolism of *trans*-resveratrol to the metabolite DHR (33). Although beyond the scope of this research, alterations in gut microbiota composition by metformin could have affected the metabolism of our *trans*-resveratrol. It would therefore be worthwhile to examine if resveratrol could improve insulin sensitivity in drug-naive subjects with T2D or subjects with prediabetes.

Despite the lack of effect of resveratrol on AMPK activity and insulin sensitivity, we did observe an improvement of ex vivo mitochondrial function. These results are in line with Price et al. (34), who recently demonstrated that the effects of resveratrol on mitochondrial metabolism are not always paralleled by an effect on AMPK. Furthermore, we found that resveratrol increased IMCL content, consistent with our previous findings in healthy, obese subjects (10). These findings may suggest that the beneficial effects of resveratrol on insulin sensitivity, as found by others, do not depend entirely on the effects on skeletal muscle mitochondrial function. Alternatively, these results may suggest that the beneficial effects of resveratrol on muscle mitochondrial function may be too small to affect insulin sensitivity or that the duration of the treatment may have been too short to affect glycemic control. In that respect, recent work of Thazhath et al. (35) in subjects with T2D controlled by diet only also failed to improve glycemic control when treated with 500 mg of resveratrol twice daily for 2 weeks.

IHL content also remained unaffected by resveratrol in subjects with T2D, which was in contrast to our previous study in which we noted a significant decrease in healthy, obese subjects (10). However, we did observe a correlation between plasma parental resveratrol levels and changes in IHL content, suggesting that higher doses of resveratrol may be able to reduce IHL content also in subjects with T2D. Further clinical studies are needed to test this hypothesis.

Resveratrol supplementation did not affect systolic and diastolic function of the heart but tended to decrease systolic blood pressure. A decrease in systolic blood pressure has previously been observed with resveratrol. For example, a recent meta-analysis of six randomized controlled trials investigated the effect of resveratrol on blood pressure and reported that high doses of resveratrol (≥150 mg/day) significantly reduced systolic blood pressure but that lower doses had no effect on blood pressure (36).

In conclusion, 30 days of resveratrol supplementation did not improve hepatic or peripheral insulin sensitivity in subjects with T2D treated with oral glucose-lowering medication. Intriguingly, levels of a metabolite of resveratrol were affected by the dose of metformin used, suggesting that the lack of effect of resveratrol on insulin sensitivity may have been affected by metformin use in these patients. These results question

the generalized value of resveratrol as an add-on therapy for treatment of T2D and emphasize the need to explore the possible interaction between resveratrol and metformin. Studies in subjects with prediabetes are needed to examine whether resveratrol can improve insulin sensitivity when not combined with oral glucose-lowering drug therapy.

Acknowledgments. The authors thank DSM Nutritional Products Ltd. for providing the resVida and placebo capsules and for performing the resveratrol and DHR analysis.

Funding. This study was funded by a European Federation for the Study of Diabetes Clinical Research Grant.

Duality of Interest. I.K. is employed at DSM Nutritional Products Ltd., Kaiseraugst, Switzerland. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. S.T. and M.d.L. designed and performed the experiments, analyzed the data, and wrote the manuscript. E.P., T.v.d.W., J.H., E.M.-K., and G.S. assisted during the experiments and reviewed and edited the manuscript. I.K. delivered the resVida and placebo capsules and performed the analysis of plasma resveratrol and DHR levels. M.K.C.H., V.B.S.H., and P.S. contributed to the design of the study, analyzed and interpreted the data, and reviewed and edited the manuscript. All authors approved the final version of the manuscript. P.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Cantó C, Auwerx J. PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. Curr Opin Lipidol 2009;20:98–105
2. Fernandez-Marcos PJ, Auwerx J. Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis. Am J Clin Nutr 2011;93:884S–890S
3. Lagouge M, Argmann C, Gerhart-Hines Z, et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 2006;127:1109–1122
4. Howitz KT, Bitterman KJ, Cohen HY, et al. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature 2003;425:191–196
5. Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov 2006;5:493–506
6. Timmers S, Hesselink MK, Schrauwen P. Therapeutic potential of resveratrol in obesity and type 2 diabetes: new avenues for health benefits? Ann N Y Acad Sci 2013;1290:83–89
7. Baur JA, Pearson KJ, Price NL, et al. Resveratrol improves health and survival of mice on a high-calorie diet. Nature 2006;444:337–342
8. Burgess TA, Robich MP, Chu LM, Bianchi C, Sellke FW. Improving glucose metabolism with resveratrol in a swine model of metabolic syndrome through alteration of signaling pathways in the liver and skeletal muscle. Arch Surg 2011;146:556–564
9. Marchal J, Blanc S, Epelbaum J, Aujard F, Pifferi F. Effects of chronic calorie restriction or dietary resveratrol supplementation on insulin sensitivity markers in a primate, Microcebus murinus. PLoS One 2012;7:e34289
10. Timmers S, Konings E, Bilet L, et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metab 2011;14:612–622
11. Crandall JP, Oram V, Trandafirescu G, et al. Pilot study of resveratrol in older adults with impaired glucose tolerance. J Gerontol A Biol Sci Med Sci 2012;67:1307–1312
12. Chachay VS, Macdonald GA, Martin JH, et al. Resveratrol does not benefit patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2014;12:2092–2103.e1, 6
13. Dash S, Xiao C, Morgantini C, Szeto L, Lewis GF. High-dose resveratrol treatment for 2 weeks inhibits intestinal and hepatic lipoprotein production in overweight/obese men. Arterioscler Thromb Vasc Biol 2013;33:2895–2901
14. Poulsen MM, Vestergaard PF, Clasen BF, et al. High-dose resveratrol supplementation in obese men: an investigator-initiated, randomized, placebo-controlled clinical trial of substrate metabolism, insulin sensitivity, and body composition. Diabetes 2013;62:1186–1195
15. Brasnyó P, Molnár GA, Mohás M, et al. Resveratrol improves insulin sensitivity, reduces oxidative stress and activates the Akt pathway in type 2 diabetic patients. Br J Nutr 2011;106:383–389
16. Elliott PJ, Walpole S, Morelli L, et al. Resveratrol/SRT-501. Drugs Future 2009;34:291–295
17. Movahed A, Nabipour I, Lieben Louis X, et al. Antihyperglycemic effects of short term resveratrol supplementation in type 2 diabetic patients. Evid Based Complement Alternat Med 2013;2013:851267
18. Bhatt JK, Thomas S, Nanjan MJ. Resveratrol supplementation improves glycemic control in type 2 diabetes mellitus. Nutr Res 2012;32:537–541
19. Snedecor GW, Cochran WG. *Statistical Methods*. Ames, Iowa State University Press, 1980
20. Bilet L, Brouwers B, van Ewijk PA, et al. Acute exercise does not decrease liver fat in men with overweight or NAFLD. Sci Rep 2015;5:9709
21. Lindeboom L, Nabuurs CI, Hesselink MK, Wildberger JE, Schrauwen P, Schrauwen-Hinderling VB. Proton magnetic resonance spectroscopy reveals increased hepatic lipid content after a single high-fat meal with no additional modulation by added protein. Am J Clin Nutr 2015;101:65–71
22. Guiu B, Petit JM, Loffroy R, et al. Quantification of liver fat content: comparison of triple-echo chemical shift gradient-echo imaging and in vivo proton MR spectroscopy. Radiology 2009;250:95–102
23. Schoffelen PF, Westerterp KR, Saris WH, Ten Hoor F. A dual-respiration chamber system with automated calibration. J Appl Physiol (1985) 1997;83:2064–2072
24. Phielix E, Schrauwen-Hinderling VB, Mensink M, et al. Lower intrinsic ADP-stimulated mitochondrial respiration underlies in vivo mitochondrial dysfunction in muscle of male type 2 diabetic patients. Diabetes 2008;57:2943–2949
25. Koopman R, Schaart G, Hesselink MK. Optimisation of oil red O staining permits combination with immunofluorescence and automated quantification of lipids. Histochem Cell Biol 2001;116:63–68
26. van de Weijer T, Phielix E, Bilet L, et al. Evidence for a direct effect of the NAD+ precursor acipimox on muscle mitochondrial function in humans. Diabetes 2015;64:1193–1201
27. Steele R. Influences of glucose loading and of injected insulin on hepatic glucose output. Ann N Y Acad Sci 1959;82:420–430
28. Meex RC, Schrauwen-Hinderling VB, Moonen-Kornips E, et al. Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. Diabetes 2010;59:572–579
29. Pocock S. *Clinical Trials: A Practical Approach*. Chichester, John Wiley and Sons, 1987
30. Fryer LG, Parbu-Patel A, Carling D. The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. J Biol Chem 2002;277:25226–25232
31. Maratos-Flier E. Metabolic disease puts up a fight: microbes, metabolism and medications. Nat Med 2013;19:1218–1219
32. Lee H, Ko G. Effect of metformin on metabolic improvement and gut microbiota. Appl Environ Microbiol 2014;80:5935–5943
33. Bode LM, Bunzel D, Huch M, et al. In vivo and in vitro metabolism of trans-resveratrol by human gut microbiota. Am J Clin Nutr 2013;97:295–309
34. Price NL, Gomes AP, Ling AJ, et al. SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. Cell Metab 2012;15:675–690
35. Thazhath SS, Wu T, Bound MJ, et al. Administration of resveratrol for 5 wk has no effect on glucagon-like peptide 1 secretion, gastric emptying, or glycemic control in type 2 diabetes: a randomized controlled trial. Am J Clin Nutr 2016;103:66–70
36. Liu Y, Ma W, Zhang P, He S, Huang D. Effect of resveratrol on blood pressure: a meta-analysis of randomized controlled trials. Clin Nutr 2015;34:27–34