No Beneficial Effects of Resveratrol on the Metabolic Syndrome: A Randomized Placebo-Controlled Clinical Trial

Thomas Nordstrøm Kjær,[1,2] Marie Juul Ornstrup,[1,2] Morten Møller Poulsen,[1,2] Hans Stødkilde-Jørgensen,[2,3] Niels Jessen,[4] Jens Otto Lunde Jørgensen,[1,2] Bjørn Richelsen,[1,2] and Steen Bønløkke Pedersen[1,2]

[1]Department of Endocrinology, Aarhus University Hospital, 8000 Aarhus, Denmark; [2]Department of Clinical Medicine, Aarhus University, 8000 Aarhus, Denmark; [3]The MR Research Centre, Aarhus University Hospital, 8000 Aarhus, Denmark; and [4]Department of Clinical Pharmacology, Aarhus University Hospital, 8000 Aarhus, Denmark

Context: Low-grade inflammation is associated with obesity and the metabolic syndrome (MetS). Preclinical evidence suggests that resveratrol (RSV) has beneficial metabolic and anti-inflammatory effects that could have therapeutic implications.

Objective: To investigate effects of long-term RSV treatment on inflammation and MetS.

Setting and Design: A randomized, placebo-controlled, double-blind, parallel group clinical trial conducted at Aarhus University Hospital.

Participants: Middle-aged community-dwelling men (N = 74) with MetS, 66 of whom completed all visits (mean ± standard error of the mean): age, 49.5 ± 0.796 years; body mass index, 33.8 ± 0.44 kg/m^2; waist circumference, 115 ± 1.14 cm.

Intervention: Daily oral supplementation with 1000 mg RSV (RSV_{high}), 150 mg RSV, or placebo for 16 weeks.

Main outcome measures: Plasma levels of high-sensitivity C-reactive protein (hs-CRP), circulating lipids, and inflammatory markers in circulation and adipose/muscle tissue biopsy specimens; glucose metabolism; and body composition including visceral fat and ectopic fat deposition.

Results: RSV treatment did not lower circulating levels of hs-CRP, interleukin 6, or soluble urokinase plasminogen activator receptor in plasma, and inflammatory gene expression in adipose and muscle tissues also remained unchanged. RSV treatment had no effect on blood pressure, body composition, and lipid deposition in the liver or striated muscle. RSV treatment had no beneficial effect on glucose or lipid metabolism. RSV_{high} treatment significantly increased total cholesterol ($P < 0.002$), low-density lipoprotein (LDL) cholesterol ($P < 0.006$), and fructosamine ($P < 0.013$) levels compared with placebo.

Conclusion: RSV treatment did not improve inflammatory status, glucose homeostasis, blood pressure, or hepatic lipid content in middle-aged men with MetS. On the contrary, RSV_{high} significantly increased total cholesterol, LDL cholesterol, and fructosamine levels compared with placebo. **(*J Clin Endocrinol Metab* 102: 1642–1651, 2017)**

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Abbreviations: ACC, acetyl-CoA carboxylase; acetyl-lysine, acetylation status of lysine residues; ANOVA, analysis of variance; BMI, body mass index; BP, blood pressure; AMPK, adenosine monophosphate-activated protein kinase; CI, confidence interval; DXA, dual-emission X-ray absorptiometry; ELISA, enzyme-linked immunosorbent assay; HOMA-IR, homeostatic model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IHL, intrahepatic lipid; IL-6, interleukin 6; LDL, low-density lipoprotein; MetS, metabolic syndrome; MR, magnetic resonance; RT-PCR, real-time polymerase chain reaction; RSV, resveratrol; SAT, subcutaneous adipose tissue; SEM, standard error of the mean; VAT, visceral adipose tissue.

Obesity is associated with numerous metabolic consequences that increase morbidity and mortality risk (1). The prevention and treatment of obesity have proven difficult and the need for new therapies remains unfulfilled. Preclinical trials provide substantial evidence to support the concept that resveratrol (RSV) might counteract the negative consequences of obesity (2, 3). Bauer *et al.* (4) demonstrated that chronic RSV intake in C57BL/6NIA mice fed a high-calorie diet normalized insulin sensitivity, protected against hepatic lipid deposition, and normalized lifespan despite the rodents remaining obese. We have shown that resveratrol consistently normalizes adipocytokine production and protects against hypoxia-induced inflammation and angiogenesis in cultured human adipose-tissue explants (5, 6). Contrary to the substantial preclinical findings of beneficial metabolic effects of resveratrol in an obesity or inflammation setting, the outcome of human clinical trials of resveratrol effects on obesity-related morbidities have been much more inconsistent. Some studies report significant yet modest beneficial effects of RSV on insulin sensitivity, low-grade inflammation, blood pressure, lipids in plasma, and hepatic fat accumulation (7, 8). In contrast, we and others have been unable to detect any clinically relevant effects (9, 10). The reasons for these discrepancies are unclear but may, in part, reflect differences in design and experimental settings. The study participants differed between the trials and included healthy but obese young men (8, 9), postmenopausal women (10), and men with type 2 diabetes (7), and study duration varied from a few weeks (7–9) to several months (11–13). Furthermore, the range of RSV doses [5 mg to 3 g (7–9, 13)], as well as the formulations of RSV used in the studies are of potential importance. Some trials used formulations of pure *trans*-resveratrol (7–9), whereas others used formulations of various extracts in combination with *trans*-resveratrol (14, 15).

Based on the conflicting outcomes of previous clinical trials, we designed a randomized, double-blind, placebo-controlled parallel group clinical trial in male volunteers with the metabolic syndrome (MetS). To determine possible dose-response connections, we examined two different doses of RSV [1000 mg/d (high-dose RSV, or RSV_{high}) and 150 mg/d (low-dose RSV, or RSV_{low})]. The intervention period was relatively long (16 weeks) to allow sufficient time for any effect to materialize.

Our study end points included inflammatory markers in plasma and adipose tissue, glycemic status, circulating lipid parameters, blood pressure (BP), and body composition.

Materials and Methods

Study design and ethics

The study was a randomized, double-blind, placebo-controlled parallel group trial. The prespecified primary end point was change in plasma levels of high-sensitivity C-reactive protein (hs-CRP). Secondary end points were changes in glucose metabolism [determined by homeostatic model assessment of insulin resistance (HOMA-IR)]; expression of inflammatory genes and genes involved in mitochondrial biogenesis in adipose tissue and striated muscle [measured by real-time polymerase chain reaction (RT-PCR)]; body composition [determined by whole-body dual-emission X-ray absorptiometry (DXA)]; lipid deposition in liver and muscle [determined by magnetic resonance (MR)] spectroscopy); volume of subcutaneous and visceral adipose tissue (VAT) (determined by MR imaging); plasma levels of inflammatory markers, lipids, and adipokines; and ambulatory BP.

Inclusion criteria were as follows: male sex, 30 to 60 years old, and diagnosed with MetS but otherwise healthy. The International Diabetes Federation criteria for MetS in men were used (16). The participants were randomly assigned to 16 weeks of treatment with tablets containing placebo, 75 mg RSV, or 500 mg RSV twice daily. A total of 74 men were randomly assigned, of whom 66 completed the study (placebo, n = 24; RSV_{low}, n = 21; RSV_{high}, n = 21). Details on recruitment, participant flow, randomization, and blinding are provided in Supplemental Fig. 1. RSV's effects on bone metabolism, prostate size, and androgen synthesis in this study population have been published (17, 18). RSV (purity >98%) was produced by Evolva (Basel, Switzerland). Robinson Pharma (Santa Ana, CA) produced placebo and RSV tablets composed of identical, biologically inert constituents, apart from the RSV fraction.

Compliance was evaluated at each visit by counting tablets. Adverse events were documented at each visit and have been reported previously (18).

The study was conducted in accordance with the Declaration of Helsinki II and the protocol was approved by the Danish Data Protection Agency and the Regional Committee on Health Research Ethics (M-20110111). The protocol was registered at ClinicalTrials.gov (NCT01412645).

General measurements

Standing height and weight were measured on a wall-mounted stadiometer with the participants lightly clothed. Waist circumference was measured at baseline with a tape measure in the horizontal plane midway between the inferior margin of the ribs and the superior border of the iliac crest. At each visit, BP was measured three times using the same electronic sphygmomanometer and with the subject in a resting position. The BP reported is the mean of the two last measurements.

DXA

Total mass, fat mass, lean mass, and fat percentage were measured by DXA using the same Hologic Discovery scanner (Hologic, Waltham, MA) at baseline and after 16 weeks of treatment.

MR imaging and MR spectroscopy

Abdominal subcutaneous adipose tissue (SAT) and VAT volumes were determined with MR imaging using a fast spin-echo sequence (19). Axial slices were obtained from the proximal border of the femoral heads to the upper pole of the most proximal kidney. On the captured MR images, Hippo Fat software (20) automatically generated borders between adipose tissue compartments and other tissues to provide estimates of VAT and SAT volumes. All automatically generated borders used for VAT and SAT volume estimates were visually inspected

for artifacts and manually corrected. The same person performed this quality control and correction step for all scans, and pre- and postscans were corrected in one session.

Intrahepatic lipid (IHL) and intramyocellular lipid content was determined by ^{1}H-MR spectroscopy using point-resolved spectroscopy sequences, as described by Poulsen *et al.* (9). Overall, full width at half maximum was 11.5 ± 0.215 Hz for liver spectra (n = 122) and 11.1 ± 0.893 Hz for muscle spectra (n = 131). The software package LC Model (Steven Provencher, PhD; Ontario, Canada) was used to quantify the spectra in a dedicated muscle and liver spectroscopy fitting model, estimating the lipid-to-water ratio within the tissue (21).

Both MR imaging and MR spectroscopy were performed on the same Signa Excite 1.5 Tesla, Twin Speed, MR scanner (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, United Kingdom) at baseline and after 16 weeks of treatment.

Muscle and adipose tissue biopsy specimens

Skeletal muscle biopsy specimens were obtained from the lateral aspect of the quadriceps femoris muscle using a Bergström cannula. The SAT biopsy specimens were obtained by performing liposuction lateral and distal to the umbilicus. Before procedures, the areas to be biopsied were treated with a local anesthetic (Lidocaine SAD, 10 mg/mL; Amgros, Copenhagen, Denmark) and all procedures were performed under sterile conditions. Immediately after harvesting the muscle and SAT biopsy specimens, they were frozen in liquid nitrogen.

Biochemistry

Blood samples were collected between 07:30 and 11:00 after an overnight fast. Safety biochemistry (*i.e.*, hemoglobin, alanine transaminase, bilirubin, alkaline phosphatase, creatinine, sodium, potassium, and ionized calcium levels) was routinely analyzed after each visit at the Department of Clinical Biochemistry at Aarhus University Hospital using standard methods of analysis. Samples for analysis of pertinent outcomes were centrifuged and serum was immediately frozen at −80°C until the time of analysis. Samples were analyzed in single batches to reduce analytical variation.

The samples were analyzed for hs-CRP [via enzyme-linked immunosorbent assay (ELISA); DRG Diagnostics, Marburg, Germany], interleukin 6 (IL-6; Quantikine HS ELISA; R&D Systems, Minneapolis, MN), soluble urokinase plasminogen activator receptor (suPARnostic ELISA; ViroGates, Copenhagen, Denmark), adiponectin (B-Bridge International, Santa Clara, CA), leptin (Leptin ELISA; Mediagnost, Reutlingen, Germany), and insulin (Insulin ELISA; DAKO, Glostrup, Denmark) according to the respective manufacturer's instructions.

Glucose and fructosamine both were measured photometrically using an enzymatic colorimetric assay (Glucose GOD-PAP) and a fructosamine kit, respectively (both, Roche Diagnostics, Mannheim, Germany). HOMA-IR was calculated from values of fasting glucose (mmol/L) and insulin (mU/L), using the following formula: HOMA-IR = (glucose × insulin) / 22.5. Total cholesterol, triglyceride, and high-density lipoprotein levels were quantified by the Department of Clinical Biochemistry at Aarhus University Hospital using absorption photometry (Cobas 6000c; Roche Diagnostics) and low-density lipoprotein (LDL) was calculated using the Friedewald formula. The intra-assay coefficient of variance ranges of pertinent outcome measures are listed in Supplemental Table 1.

RNA isolation

Total RNA was extracted from SAT and muscle biopsy samples using TriZol reagent (catalog no.15596018; Life Technologies Europe, Naerum, Denmark) according to the manufacturer's protocol. RNA was quantified by measuring absorbance at 260 nm using a NanoDrop 8000 (Thermo Fischer Scientific, Waltham, MA) and quality was checked by inspecting the ribosomal bands on an agarose gel.

Quantitative RT-PCR analysis

Complementary DNA was synthesized using random hexamer primers (Verso cDNA kit; Thermo Fisher Scientific, Waltham, MA) and quantitative RT-PCR performed with mRNA against β2-microglobulin mRNA as housekeeping gene (because its expression was stable in all groups and over time). The reactions were performed in duplicate using the KAPA SYBR FAST qPCR kit (Kapa Biosystems, Woburn, MA) in a LightCycler 480 (Roche Applied Science, Penzberg, Germany) using the following protocol: one step at 95°C for 3 minutes, then 95°C for 10 seconds, 60°C for 20 seconds, and 72°C for 10 seconds. The specificity of the primers was tested by melting curve analysis and agarose gel electrophoresis. All primers had amplification efficiency between 1.9 and 2.0. Primer sequences are listed in Supplemental Table 2.

Western blotting

Approximately 20 mg of muscle tissue was prepared for western blotting using the same protocol and equipment as previously described (22). Control for equal loading was performed using the Stain-Free technology (Bio-Rad Laboratories, Hercules, CA) (23). Antibodies against the following proteins were purchased from Cell Signaling Technology (Danvers, MA): acetyl-CoA carboxylase (ACC) (catalog no. 3662) and phosphorylated ACC (catalog no. 3661), adenosine monophosphate-activated protein kinase (AMPK) (catalog no. 2532), phosphorylated AMPK (catalog no. 2531), and acetylated-lysine (catalog no. 9441). After incubation with primary antibodies, the membranes were incubated for 1 hour with horseradish peroxidase-conjugated secondary antibody (GE Healthcare Life Sciences). Proteins were visualized by chemiluminescence (Pierce Supersignal West Dura; Thermo Fisher Scientific) and quantified with the ChemiDoc MP imaging system (Bio-Rad Laboratories). Protein Plus Precision All Blue standards were used as a molecular weight marker (Bio-Rad Laboratories).

Statistics

For baseline Pearson correlations, normality was checked by Shapiro-Wilk test and linearity was assessed by inspecting scatterplots. Results are presented as Pearson correlation coefficients with a two-tailed significance level. For baseline characteristics and compliance, normality was checked by QQ plots and Shapiro-Wilk test. Equal variance was assessed by Levene test. If appropriate, data were log-transformed before analysis of variance (ANOVA).

For posttreatment analysis, analysis of covariance (ANCOVA) was performed using pretreatment values as covariate. Linearity between pretreatment and posttreatment values for each group was assessed by scatterplots with fitted group lines. Homogeneity of regression slopes was assessed in the SPSS GLM Univariate procedure (IBM, Armonk, NY). Normality

of standardized residuals within groups was assessed by Shapiro-Wilk test. Homoscedasticity was checked by plotting the standardized residuals against the predicted values. Homogeneity of variances was assessed by Levene test. If a significant difference was discovered in an ANOVA or ANCOVA, *post hoc* Sidak multiple comparisons were used to determine which group means were different.

ANOVA results are presented as mean ± standard error of the mean (SEM) or as median with interquartile range (25%, 75%), unless otherwise stated. ANCOVA results are presented as adjusted mean ± SEM along with a *P* value and the covariate value, or as the difference of adjusted means with a 95% confidence interval (CI) and a *P* value from the *post hoc* analysis. For all statistical tests, $P < 0.05$ was considered statistically significant. Power and sample size calculations were based on changes in hs-CRP level. To detect a minimum treatment difference of 0.6 ng/mL, at a two-sided significance level of 0.05, a power of 80%, and standard deviation of 0.6, 66 test subjects would need to complete the study. All end-point analyses were performed using IBM SPSS Statistics version 20.0.02 software (IBM) and power and sample size calculations were performed using STATA/IC version 12.1 software (StataCorp, College Station, TX).

Results

Baseline characteristics

The mean age of participants was 49.5 ± 0.796 years, body mass index (BMI) of 33.8 ± 0.44 kg/m^2, and waist circumference of 115 ± 1.14 cm at baseline. At baseline, systolic BP was significantly higher in the placebo group compared with the RSV_{low} group, lean mass was significantly higher in the placebo group as compared with the RSV_{high} group, and fructosamine levels in the RSV_{low} group were significantly higher than that of the placebo and RSV_{high} groups (Table 1).

Correlations between body composition and metabolic variables

At baseline, we found the expected correlations between measures of obesity, metabolic disturbances, and inflammatory markers. Adiponectin level was negatively correlated with BMI ($r = -0.449$; $P < 0.0001$), HOMA-IR ($r -0.445$; $P < 0.0001$), waist circumference ($r = -0.033$; $P = 0.006$), hs-CRP ($r = -0.305$; $P = 0.013$), and IHL ($r = -0.268$; $P < 0.03$). Alanine transaminase level was correlated with the amount of IHL ($r = 0.52$; $P < 0.0001$). HOMA-IR was correlated with amount of IHL ($r = 0.25$; $P < 0.05$) and with BMI ($r = 0.56$; $P < 0.0001$; Table 2).

Changes in clinical biochemistry

Inflammation

hs-CRP values were similar in all three groups after 16 weeks of treatment, thus demonstrating no anti-inflammatory biochemical effect of RSV. Similarly, no changes were observed in IL-6, adiponectin, or soluble urokinase plasminogen activator receptor levels after RSV treatment (Table 3).

Insulin sensitivity

RSV treatment did not result in changes in circulating levels of insulin and glucose, or changes in HOMA-IR. However, fructosamine levels changed significantly ($P < 0.011$) after 16 weeks of treatment. *Post hoc* analysis revealed that fructosamine levels in the RSV_{high} group were significantly increased compared with the placebo group (mean difference, 11.8 μmol/L (95% CI, 2.04 to 21.5 μmol/L; $P < 0.013$; Fig. 1).

Lipids and leptin

RSV treatment did not result in any change in high-density lipoprotein or triglyceride levels. Total cholesterol was significantly increased after treatment ($P < 0.002$). *Post hoc* analysis revealed that total cholesterol levels increased significantly in the RSV_{high} group compared with placebo (mean difference, 0.69 mmol/L (95% CI, 0.22 to 1.16 mmol/L; $P < 0.002$). LDL levels were significantly changed by treatment ($P < 0.007$). *Post hoc* analysis revealed that LDL level increased in the RSV_{high} group compared with placebo (mean difference, 0.61 mmol/L (95% CI, 0.15 to 1.08 mmol/L; $P < 0.006$). Leptin was unchanged by RSV (Fig. 2).

Routine clinical biochemistry

Alanine transaminase, bilirubin, creatinine, sodium, and potassium concentrations were unaffected by RSV treatment. Hemoglobin values were significantly higher in the RSV_{low} group than in the placebo group, but there was no difference between the RSV_{high} and RSV_{low} groups. Calcium levels were significantly higher in the RSV_{low} group as compared with the RSV_{high} group, but there was no difference when compared with the placebo group (Supplemental Table 3). Alkaline phosphatase, measured as percentage change from baseline, increased dose dependently in the intervention groups. This finding has been reported previously (18).

Ectopic lipid accumulation and body composition

Using MR spectroscopy, we detected no changes in IHL or intramyocellular lipid within or among the three groups. Multiple-slice MR imaging revealed no volumetric changes in SAT or VAT in the region between the proximal part of femoral heads and the upper pole of the most proximal kidney. Groups were significantly different in lean mass values by ANCOVA ($P < 0.046$). However, *post hoc* Sidak multiple comparisons could not identify any significant differences in the group means. No changes were detected in total mass, fat mass, or fat percentage by DXA scan (Table 3).

Gene expression in muscle and SAT

SAT

No changes were detected in the gene expression of *TNFα, IL-6, IGF-1, IGF-2, SIRT-1*, or *TFAM*. In

Table 1. Baseline Characteristics

Characteristics	Placebo	RSV_{low}	RSV_{high}	*P* Value
Patients, n	24	21	21	
Age, y	47.8 ± 1.30	49.1 ± 1.46	51.9 ± 1.28	0.092
Weight, kg	113 ± 2.66	110 ± 3.22	106 ± 2.33	0.222
BMI, kg/m^2	34.1 ± 0.770	33.4 ± 0.858	33.8 ± 0.668	0.815
Waist circumference, cm	116 ± 2.1	116 ± 1.9	114 ± 2.1	0.835
Systolic BP, mm Hg	150 ± 3.44[a]	140 ± 2.34[a]	146 ± 2.34	0.050
Diastolic BP, mm Hg	91.3 ± 2.10	86.9 ± 1.54	89.3 ± 1.68	0.236
VAT, cm^3[b]	2564 ± 113	2888 ± 182	2823 ± 217	0.371
SAT, cm^3[b]	6486 ± 565	5799 ± 304	6135 ± 508	0.597
VAT/SAT, %[b]	45.1 ± 3.88	52.5 ± 4.33	50.4 ± 4.88	0.459
Fasting glucose, mmol/L	5.79 ± 0.108	5.76 ± 0.210	5.74 ± 0.125	0.966
Insulin, pmol/L	100 ± 9.33	100 ± 13.3	89.4 ± 10.0	0.737
HOMA-IR	4.36 ± 0.450	4.42 ± 0.662	3.87 ± 0.497	0.735
Fructosamine, μmol/L	241 ± 3.10	261 ± 5.38[c]	241 ± 3.11	0.001
Total cholesterol, mmol/L	5.81 ± 0.233	5.38 ± 0.204	5.78 ± 0.232	0.336
HDL, mmol/L	1.19 ± 0.043	1.20 ± 0.055	1.27 ± 0.073	0.563
LDL, mmol/L	3.71 ± 0.208	3.30 ± 0.156	3.53 ± 0.249	0.367
Triglyceride, mmol/L	1.7 (1.25, 2.40)	1.7 (1.10, 2.10)	1.8 (1.30, 2.30)	0.577
IHL[d]	0.230 ± 0.0350	0.231 ± 0.0380	0.254 ± 0.0395	0.875
ALT, U/L	40.2 ± 3.53	39.5 ± 2.84	42.1 ± 2.88	0.838
IMCL	6.84 ± 1.00	5.66 ± 0.726	5.81 ± 0.855	0.577
Lean mass, g	76,736 ± 1420[e]	74,284 ± 1406	71,503 ± 1608[e]	0.048
Fat mass, g	35,433 ± 1673	35,612 ± 2038	33,827 ± 1209	0.718
Fat, %	31.3 ± 0.881	32.0 ± 0.957	32.0 ± 0.732	0.746
Leptin, ng/mL	13.7 (9.75, 21.1)	15.3 (11.8, 17.2)	16.0 (12.6, 20.0)	0.806
hs-CRP, mg/L	3.55 (2.05, 6.25)	2.70 (1.90, 4.70)	2.30 (1.30, 3.90)	0.586
Adiponectin, ng/mL	6766 ± 580	7844 ± 532	6323 ± 533	0.155
IL-6, pg/mL	1.39 (0.733, 2.05)	1.44 (1.06, 2.20)	1.32 (0.704, 2.15)	0.503
suPAR, ng/mL	2.74 ± 0.082	3.00 ± 0.166	2.71 ± 0.137	0.236

Results are expressed as mean ± SEM or as median (interquartile range) with a *P* value from the overall ANOVA. In case of a significant finding in the ANOVA, *post hoc* Sidak multiple comparisons were used to identify which group means were significantly different.

Abbreviations: ALT, alanine aminotransferase; HDL, high-density lipoprotein; IMCL, intramyocellular lipid content; suPAR, soluble urokinase plasminogen activator receptor.

[a]Values for placebo and RSV_{low} groups in this row are significantly different at $P < 0.05$.

[b]Placebo group (n = 22), RSV_{low} (n = 20), and RSV_{high} (n = 20).

[c]Value for RSV_{low} group differs significantly, at $P < 0.05$, from the values for the placebo and RSV_{high} groups in this row.

[d]Placebo group (n = 24), RSV_{low} (n = 20), and RSV_{high} (n = 21).

[e]Values for placebo and RSV_{high} groups in this row are significantly different at $P < 0.05$.

terms of *NRF1*, groups were significantly different in the ANCOVA ($P < 0.050$). However, *post hoc* Sidak multiple comparisons could not identify any significant differences in the group means (Supplemental Table 4).

Muscle

No changes were detected in the gene expression of *TNFα*, *IL-6*, *IGF-1*, *IGF-2*, *SIRT1*, *NRF1*, or *TFAM* (Supplemental Table 4).

Western blotting: protein phosphorylation and deacetylation in muscle

No changes in the phosphorylation of AMPK and ACC were detected after RSV treatment. The total acetylation status of lysine residues (acetyl-lysine) was unchanged by RSV treatment (Fig. 3; Supplemental Fig. 2).

Compliance

Compliance in the placebo, RSV_{low}, and RSV_{high} groups was 94.1% ± 1.75%, 92.4% ± 1.73%, and 96.5% ± 1.03%, respectively, and there was no significant difference among the groups ($P = 0.204$).

Discussion

In this placebo-controlled, double-blind, randomized parallel group clinical trial, we investigated the metabolic effects of high-dose (1000 mg daily) and low-dose (150 mg daily) RSV in men with the MetS. We demonstrated that RSV treatment does not affect inflammation, glucose, and lipid metabolism in a positive manner. On the contrary, we found an increase in total cholesterol, LDL cholesterol, and fructosamine levels in the RSV_{high} group compared with the placebo group, indicating an exacerbation of the lipid and glucose metabolism by high-dose RSV treatment.

Table 2. Baseline Correlations

	ADPN	ALT	HOMA	VAT	hs-CRP	BMI	Waist Circumference	IHL
ADPN								
ALT	−0.223							
HOMA	−0.445[a]	0.115						
VAT	−0.216	0.244	−0.009					
hs-CRP	−0.305[b]	−0.032	0.048	0.081				
BMI	−0.449[a]	−0.007	0.561[a]	0.008	0.182			
Waist circumference	−0.333[a]	0.082	0.410[a]	0.133	0.210	0.860[a]		
IHL	−0.268[b]	0.517[a]	0.251[b]	0.143	0.243	0.356[a]	0.364[a]	

Pearson product-moment correlations between baseline measures of body composition, insulin sensitivity, inflammation, and liver fat or enzymes. Tabulated values are the Pearson correlation coefficients.

Abbreviation: ADPN, adiponectin.

[a]Pearson correlation coefficient is significantly different from 0 at the 0.01 level (two-tailed).

[b]Pearson correlation coefficient is significantly different from zero at the 0.05 level (two-tailed).

We detected significant posttreatment differences between groups in lean mass and *NRF1* in SAT, which is probably chance findings, because there were outliers in the data and *post hoc* testing could not identify differences of the group means in either parameter. Thus, RSV treatment did not alter the expression of the mitochondrial biogenesis-related genes *SIRT-1*, *NRF1*, or *TFAM* in SAT or striated muscle. Furthermore, we did not detect any effects of RSV on the levels of total lysine acetylation or the levels of phosphorylated AMPK or phosphorylated ACC in striated muscle.

From preclinical studies in cell models and animal models it has repeatedly been shown that RSV possesses beneficial effects on several of the biochemical pathways

Table 3. Posttreatment Analysis

Characteristics	Placebo	RSV_{low}	RSV_{high}	*P* Value	Covariate
Patients, n	24	21	21		
Weight, kg	109 ± 0.574	110 ± 0.608	110 ± 0.617	0.334	110
BMI, kg/m^2	33.4 ± 0.170	33.7 ± 0.182	33.7 ± 0.182	0.257	33.8
Systolic BP, mm Hg[a]	142 ± 2.53	145 ± 2.58	140 ± 2.64	0.355	144
Diastolic BP, mm Hg[a]	86.0 ± 1.34	87.7 ± 1.35	87.8 ± 1.41	0.581	88.9
VAT, cm^3[b]	2669 ± 80.3	2924 ± 83.8	2776 ± 83.5	0.100	2752
SAT, cm^3[b]	6083 ± 69.0	6243 ± 72.4	6083 ± 72.0	0.200	6151
VAT/SAT, %[b]	47.8 ± 1.18	50.9 ± 1.23	50.6 ± 1.23	0.145	49.2
Glucose, mmol/L	5.62 ± 0.0962	5.90 ± 0.103	5.89 ± 0.103	0.073	5.76
Insulin, pmol/L	99.9 ± 5.48	93.2 ± 5.85	101 ± 5.87	0.569	96.7
HOMA-IR	4.19 ± 0.260	4.17 ± 0.278	4.50 ± 0.278	0.643	4.22
ALT, U/L	41.7 ± 3.05	47.3 ± 3.26	45.4 ± 3.26	0.443	40.6
IHL[c]	0.236 ± 0.0194	0.234 ± 0.0203	0.273 ± 0.0209	0.326	0.237
IMCL[d]	6.00 ± 0.682	5.50 ± 0.745	7.32 ± 0.744	0.209	6.20
Lean mass, g	74,327 ± 507	75,993 ± 530	74,404 ± 544	0.046[e]	74,291
Fat mass, g	34,525 ± 508	34,062 ± 544	35,104 ± 545	0.406	34,979
Fat, %	31.5 ± 0.414	30.7 ± 0.441	31.7 ± 0.441	0.222	31.8
Leptin, ng/mL	17.5 ± 0.884	17.3 ± 0.945	17.2 ± 0.945	0.966	17.2
hs-CRP, mg/L	4.56 ± 0.868	4.53 ± 0.925	3.45 ± 0.925	0.623	5.63
Adiponectin, ng/mL	6956 ± 316	6869 ± 344	7190 ± 341	0.793	6968
IL-6, pg/mL	1.49 ± 0.292	2.29 ± 0.313	1.39 ± 0.311	0.087	1.61
suPAR, ng/mL	2.87 ± 0.103	2.92 ± 0.112	2.99 ± 0.110	0.750	2.82

Posttreatment results are expressed as adjusted mean ± SEM of the posttreatment values with a *P* value and covariate value (*i.e.*, an adjusted pretreatment mean common for all groups) from the overall ANCOVA. *Post hoc* Sidak multiple comparisons were used to identify which group means were significantly different.

Abbreviation: IMCL, intramyocellular lipid content; suPAR, soluble urokinase plasminogen activator receptor.

[a]Placebo group (n = 21), RSV_{low} (n = 21), and RSV_{high} (n = 19).

[b]Placebo group (n = 22), RSV_{low} (n = 20), and RSV_{high} (n = 20).

[c]Placebo group (n = 22), RSV_{low} (n = 20), and RSV_{high} (n = 19).

[d]Placebo group (n = 24), RSV_{low} (n = 20), and RSV_{high} (n = 20).

[e]ANCOVA *P* value was statistically significant, but *post hoc* Sidak multiple comparisons could not identify which groups were different.

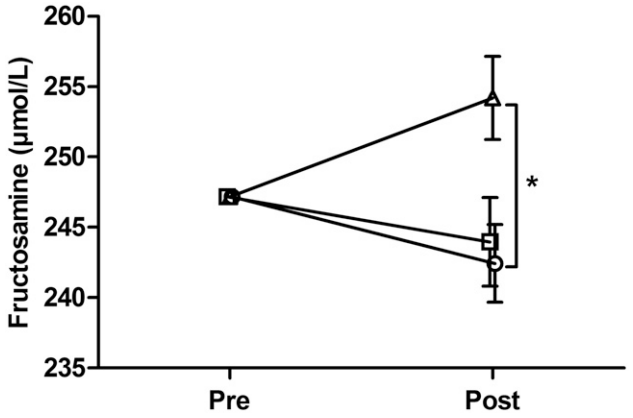

Figure 1. Change in fructosamine. Changes in posttreatment values (adjusted mean ± SEM) from the common pretreatment value of 247 μmol/L (the covariate). Posttreatment adjusted mean ± SEM for the placebo (n = 24), RSV_{low} (n = 21), and RSV_{high} (n = 21) groups were 242 ± 2.76 μmol/L, 244 ± 3.14 μmol/L, and 254 ± 2.95 μmol/L (ANCOVA $P < 0.011$). *Post hoc* Sidak multiple comparisons showed fructosamine level was significantly increased in the RSV_{high} group compared with that of the placebo group [mean difference, 11.8 μmol/L (95% CI, 2.04 to 21.5 μmol/L)]. $*P < 0.013$. Symbols represent the study groups: placebo (○), RSV_{low} (□), and RSV_{high} (Δ). Pre, pretreatment; Post, posttreatment.

involved in obesity-associated morbidity (3). RSV inhibits inflammation (24), improves insulin sensitivity (25), inhibits hepatic fat accumulation (26), possesses anticancer activity (27), and improves endothelial function (28). However, as reviewed by Poulsen *et al.* (2, 29), translation of the preclinical findings into human clinical practice has been cumbersome and inconclusive. A too-short study period, too-healthy participants, and differences in RSV dosage might be some of the reasons why differing results have been obtained in the various clinical settings. The current study was designed to accommodate some of these shortcomings to determine the potential clinical relevance of daily intake of RSV in people with MetS.

In the study by Timmers *et al.* (8), 150 mg of RSV daily for 1 month was used in a group of obese but healthy younger men. A modest but significant effect was demonstrated on insulin sensitivity, lipids, inflammation, BP, and hepatic fat accumulation. In a similar study using 1500 mg daily for 1 month, we were unable to demonstrate any effects of RSV in healthy obese younger men (9). Both studies used highly sophisticated methods, like MR spectroscopy, for determination of hepatic fat, so it seems unlikely that it is a methodological problem that gave rise to the different outcome. In the current study, we compared a RSV dose of 150 mg daily [the dose used by Timmers *et al.* (8)] with 1000 mg RSV of daily. We have previously documented that 1500 mg of RSV per day did not have beneficial metabolic effects yet produced mild and temporary adverse effects (9). The findings by Timmers *et al.* (8) indicated that the metabolic effects of RSV might materialize at a lower dose level and, because Brown *et al.* (30) documented that 1000 mg of RSV per day for 29 days is safe and relatively devoid of adverse effects , we decided to use the 1000 mg/d dose as the high-dose treatment in the current study, which is comparable to the dose used in our previous study (9) and the recent study by Thazhath *et al.* (31). In the latter study, a dose of 1000 mg of RSV daily for 5 weeks in patients with type 2 diabetes did not improve glycemic regulation or body composition (31), which is in accordance with the findings in the current study. Moreover, in the current study, we found no indications of a change in hepatic fat accumulation in the RSV_{low} or the RSV_{high} groups assessed by MR spectroscopy.

The participants in the current study were moderately insulin resistant, with a HOMA-IR index of 4.2 ± 0.31. This is more pronounced than the HOMA-IR of 2.8 of the participants included in the study by Timmers *et al.* (8) and our own previous study (HOMA-IR, 3.0) (9). However, despite investigating more insulin-resistant subjects, neither of the RSV doses used in our trial improved the HOMA-IR index. Fructosamine level is a measure of glycated plasma proteins (mainly albumin) and reflects changes in blood glucose levels within the last 2 to 4 weeks, whereas hemoglobin A1c reflects changes in blood glucose over the last 3 to 4 months (32), and both are validated measures of glucose homeostasis (33). Our results clearly demonstrate that RSV has no beneficial effects on glucose homeostasis, because the HOMA-IR index was unchanged; in fact, we even detected a significant increase in fructosamine level in the RSV_{high} group compared with the placebo group, indicating a deterioration of glucose handling. Similarly, we did not detect any improvement in BP, inflammatory status, ectopic lipid deposition (in liver or skeletal muscle), lipids, or gene expression in skeletal muscle or SAT.

Overall, the current study supports our previous finding that RSV does not significantly improve insulin sensitivity, lipid metabolism, or accumulation of hepatic fat, and does not possess anti-inflammatory effects in obese, insulin-resistant patients.

Interestingly, the effects of RSV on glucose and lipid metabolism in clinical trials are inconsistent, whereas the effects on bone metabolism seem more robust. We have previously demonstrated a significant increase in bone-specific alkaline phosphatase in volunteers treated with an even higher RSV dose (1500 mg daily) (34). Recently, we have consolidated the initial finding in the current study population, because RSV dose dependently increased bone-specific alkaline phosphatase and increased bone mineral density (18). In addition, we found that RSV lowered the concentration of circulating androgen precursors (dehydroepiandrosterone and dehydroepiandrosterone sulfate) dose dependently in these middle-aged men (17). Thus, based on our experience, there is no doubt that RSV is

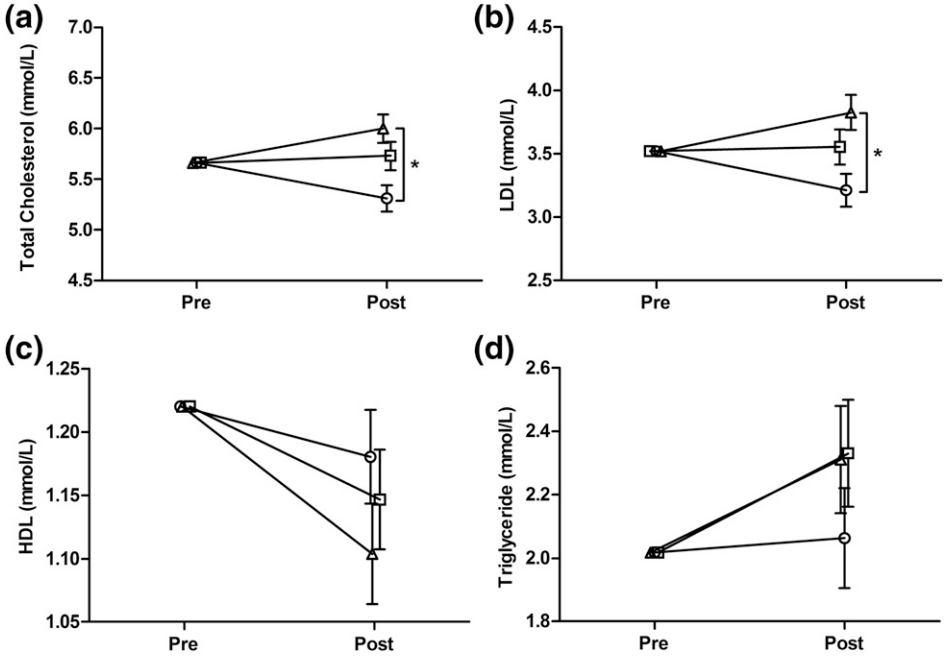

Figure 2. Change in cholesterol and triglyceride levels. The changes in the posttreatment values (adjusted mean ± SEM) from the common pretreatment value (the covariate). The vertical clamped bar with asterisk (*) indicates group means that were significantly different. (a) The mean total cholesterol pretreatment level was 5.66 mmol/L; the posttreatment values for the placebo, RSV_{low}, and RSV_{high} groups were 5.31 ± 0.130 mmol/L, 5.73 ± 0.141 mmol/L, and 6.00 ± 0.139 mmol/L, respectively (ANCOVA $P < 0.002$). *Post hoc* Sidak multiple comparisons showed the RSV_{high} group mean was significantly increased compared with that of the placebo group [mean difference, 0.689 mmol/L (95% CI, 0.222 to 1.16 mmol/L); $P < 0.002$]. (b) The mean LDL cholesterol pretreatment level was 3.52 mmol/L; posttreatment placebo, RSV_{low}, and RSV_{high} values were 3.21 ± 0.130 mmol/L, 3.55 ± 0.139 mmol/L, and 3.83 ± 0.137 mmol/L, respectively (ANCOVA $P < 0.007$). *Post hoc* Sidak multiple comparisons showed the RSV_{high} group mean was significantly increased compared with placebo[(mean difference, 0.614 mmol/L (95% CI, 0.151 to 1.08 mmol/L); $P < 0.006$]. (c) The mean pretreatment high-density lipoprotein cholesterol level was 1.22 mmol/L; posttreatment placebo, RSV_{low}, and RSV_{high} values were 1.18 ± 0.0369 mmol/L, 1.15 ± 0.0394 mmol/L, and 1.10 ± 0.0396 mmol/L, respectively (ANCOVA $P<0.375$). (d) The mean pretreatment triglyceride level was 2.02 mmol/L; posttreatment placebo, RSV_{low}, and RSV_{high} values were 2.06 ± 0.158 mmol/L, 2.33 ± 0.169 mmol/L, and 2.31 ± 0.169 mmol/L, respectively (ANCOVA $P<0.433$). Symbols represent the study groups: placebo (○), RSV_{low} (□), and RSV_{high} (Δ). For all cholesterol and triglyceride values, the number of patients per group was 24, 21, and 21 for the placebo, RSV_{low}, and RSV_{high} groups, respectively.

biologically active and exerts effects on tissues, but it does not seem to have substantial beneficial effects on BP, systemic inflammation, lipid or glucose homeostasis, or change the inflammatory gene expression in SAT or skeletal muscle.

An interesting observation is the significant increase in fructosamine as well as total cholesterol and LDL cholesterol in the RSV_{high} group. This may suggest that high doses of RSV can have detrimental effects on glucose and cholesterol metabolism, whereas data from our recent publication on RSV effects in bone revealed that the highest RSV dose induced the largest increase in bone mineral density. Consequently, these data suggest that the optimal dosage of RSV depends on the target tissue.

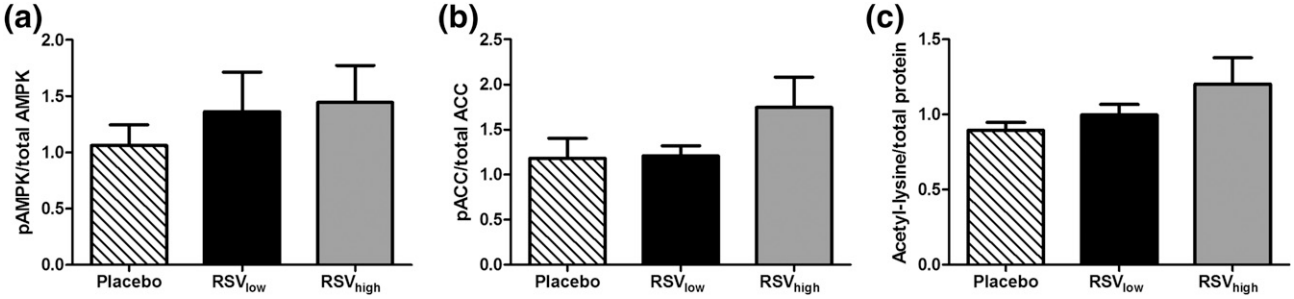

Figure 3. Western blot findings: posttreatment protein phosphorylation and lysine acetylation in muscle tissue. (a) Ratio of pAMPK to total AMPK. (b) Ratio of pACC to total ACC. (c) Ratio of acetyl-lysine to total protein. Stain-Free technology was used to control for equal loading. Data were analyzed using one-way ANOVA and all three parameters were log-transformed before the ANOVA to achieve a normal distribution. There were no significant differences in protein phosphorylation (pAMPK, $P < 0.506$; pACC, $P < 0.299$) or acetyl-lysine ($P < 0.203$) between groups after 4 months of treatment. pACC, phosphorylated acetyl-CoA carboxylase; pAMPK, phosphorylated adenosine monophosphate-activated protein kinase.

In conclusion, prolonged RSV treatment in middle-aged men with the MetS did not confer clinical benefits, as determined by an array of pertinent outcomes. On the contrary, high-dose RSV (1000 mg daily) was associated with an increase in the circulating levels of fructosamine, total cholesterol, and LDL cholesterol.

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Address all correspondence and requests for reprints to: Thomas Nordstrøm Kjær, MD, Department of Endocrinology and Internal Medicine MEA, Aarhus University Hospital, Tage-Hansensgade 2, Entrance 3C, 8000 Aarhus, Denmark. E-mail: thomas.kjaer@clin.au.dk.

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