

Resveratrol Increases Bone Mineral Density and Bone Alkaline Phosphatase in Obese Men: A Randomized Placebo-Controlled Trial

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Context: Metabolic syndrome (MetS) is associated with low-grade inflammation, which may harmfully affect bone. Resveratrol (RSV) possesses anti-inflammatory properties, and rodent studies suggest bone protective effects.

Objective: This study sought to evaluate effects of RSV treatment on bone in men with MetS.

Setting and Design: The study was conducted at Aarhus University Hospital as a randomized, double-blinded, placebo-controlled trial assessing changes in bone turnover markers, bone mineral density (BMD), and geometry.

Participants: The study population comprised 74 middle-aged obese men with MetS recruited from the general community, of which 66 completed all visits. Mean age of participants was 49.3 ± 6.3 years and mean body mass index was 33.7 ± 3.6 kg/m².

Intervention: Oral treatment with 1.000 mg RSV (RSV_{high}), 150mg RSV (RSV_{low}), or placebo daily for 16 weeks.

Main Outcome Measure: Prespecified primary endpoint was change in bone alkaline phosphatase (BAP).

Results: BAP increased dose dependently with RSV ($R = 0.471$, $P < .001$), resulting in a significantly greater increase in BAP in the RSV_{high} group compared with placebo at all time-points (week 4, $16.4 \pm 4.2\%$, $P < .001$; week 8, $16.5 \pm 4.1\%$, $P < .001$; week 16, $15.2 \pm 3.7\%$, $P < .001$). Lumbar spine trabecular volumetric bone mineral density (LS vBMD_{trab}) also increased dose dependently with RSV ($R = 0.268$, $P = .036$), with a significant increase of $2.6 \pm 1.3\%$ in the RSV_{high} group compared with placebo ($P = .043$). In addition, changes in BAP and LS vBMD_{trab} were positively correlated ($R = 0.281$, $P = .027$). No consistent changes were detected in bone density at the hip.

Conclusions: Our data suggest that high-dose RSV supplementation positively affects bone, primarily by stimulating formation or mineralization. Future studies of longer duration comprising populations at risk of osteoporosis are needed to confirm these results. (*J Clin Endocrinol Metab* 99: 4720–4729, 2014)

Abbreviations: aBMD, areal bone mineral density; ALT, alanine transaminase; AP, alkaline phosphatase; BAP, bone alkaline phosphatase; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; BV/TV, bone volume/tissue volume; CT, computed tomography; CTx, C-terminal telopeptide of type 1 collagen; CV, coefficient of variation; DXA, dual-energy x-ray absorptiometry; FN, femoral neck; hs-CRP, high-sensitive C-reactive protein; HRpQCT, high-resolution peripheral quantitative computed tomography; IT, intertrochanteric; LS, lumbar spine; MetS, metabolic syndrome; NTx, N-terminal telopeptide of type 1 collagen; OPG, osteoprotegerin; P1NP, procollagen I N-terminal propeptide; QCT, quantitative computed tomography; RSV, resveratrol; RSV_{high}, 1.000 mg resveratrol; RSV_{low}, 150 mg resveratrol; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular space; TH, total hip; TR, trochanter; vBMD, volumetric bone mineral density; vBMD_{integral}, integral volumetric bone mineral density; vBMD_{trab}, trabecular volumetric bone mineral density; VOI, volume of interest; WB, whole body.

Obesity and metabolic syndrome (MetS) are major health problems worldwide, and due to several severe comorbidities, the health care costs related to these conditions are high (1, 2). MetS is associated with low-grade inflammation (3), and inflammation is a major cause of both local and systemic bone loss, caused by excessive bone resorption as well as impaired bone formation (4, 5). This imbalance in bone remodeling is at least in part mediated by cytokines activating osteoclasts and impairing osteoblast function (6), as seen in chronic inflammatory diseases such as inflammatory bowel disease (7) and rheumatoid arthritis (8). In the Swedish Osteoporotic Fractures in Men (*MrOS*) Study, elderly men with the highest tertile of high-sensitive C-reactive protein (hs-CRP) had an increased risk of vertebral fractures, and this association was independent of BMD (9).

Resveratrol (RSV) is a natural polyphenolic compound (3,5,4'-trihydroxy-*trans*-stilbene) present in nuts and fruits such as grapes. RSV has anti-inflammatory properties both in vitro and in vivo (10–13). Also, inflammation-independent effects of RSV have been described in relation to bone. RSV stimulates osteoblast differentiation (14–16), inhibits osteoclast activity (17, 18), and protects against bone loss in ovariectomized rats, immobilized rats, and aged mice (19–22). We previously analyzed the effect of RSV on biochemical markers of bone turnover in a randomized placebo-controlled trial designed to investigate potential effects of high-dose RSV supplementation on substrate metabolism, insulin sensitivity, and body composition (23). Obese men were randomly assigned to either placebo or 1,500 mg RSV daily for 4 weeks. We found a highly significant increase in bone alkaline phosphatase (BAP), without changes in other biochemical markers of bone turnover or calcium homeostasis (24).

To further explore the results from the initial trial we conducted a larger randomized placebo-controlled trial of longer duration and using two doses of RSV, to investigate the effects of RSV treatment on bone turnover markers, bone mass, and bone structure in obese men with MetS.

Materials and Methods

Study design and subjects

The study was a randomized, double-blinded, placebo-controlled trial (single center). Primary endpoint was changes in BAP. Secondary endpoints were changes in BMD assessed by quantitative computed tomography (CT) (QCT) and dual-energy x-ray absorptiometry (DXA), changes in bone geometry and microstructure assessed by QCT and high-resolution peripheral quantitative computed tomography (HRpQCT), and changes in other biochemical markers of bone turnover, as well as markers of calcium homeostasis. Subjects were randomly assigned (1:1:1) to treatment for 16 weeks with tablets containing 500 mg tran-

sresveratrol (RSV_{high}), 75 mg transresveratrol (RSV_{low}), or placebo twice daily. Tablets containing resveratrol were provided by Evolva, and placebo by Robinson Pharma. Randomization, blinding, packaging, and labeling were performed by the pharmacy at Aarhus University Hospital. In detail, the randomization was performed in blocks of six; initially RSV_{high} and placebo 4:2, followed by RSV_{low} and placebo 4:2.

Participants were recruited through advertisements in local newspapers. We screened 123 men, enrolled and randomly assigned 76, of whom 66 completed the study (placebo, *n* = 24; RSV_{low}, *n* = 21; RSV_{high}, *n* = 21) (Figure 1). Inclusion criteria were male sex, age 30–60 years, and presence of MetS. The International Diabetes Federation criteria for MetS (25) in men were used, according to which the following features should be present; Central obesity (Waist circumference \geq 94 cm and/or body mass index [BMI] $>$ 30 kg/m²) plus any two of the following: raised triglycerides (\geq 1.7 mmol/l), reduced high-density lipoprotein (\leq 1.03 mmol/l), raised blood pressure (systolic \geq 130 mm Hg or diastolic \geq 85 mm Hg), raised fasting plasma glucose (\geq 5.6 mmol/l). Exclusion criteria were other overt endocrine diseases, renal disease, hepatic disease, heart disease, malignant disease, anemia, alcohol abuse, and planned lifestyle changes.

The participants were instructed to maintain their body weight (BW) and lifestyle (including eating patterns, physical exercise etc.) and to abstain from any changes in intake of nutritional supplements (including calcium and vitamin D) during the study period. Information about adverse events was obtained at each visit, and the compliance was estimated based on tablet counting at each visit.

Ethical aspects

The protocol was approved by the Regional Committee on Health Research Ethics (M-20110111) and the Danish Data Protection Agency, and the study was conducted in agreement with the Declaration of Helsinki II. All participants were given oral and written information before written informed consent was obtained. According to the International Committee of Medical Journal Editors, the protocol was registered at ClinicalTrials.gov (NCT01412645) before recruitment was initiated.

General measurements

Standing height and weight were measured on a wall-mounted stadiometer with the participants lightly clothed. In addition, electrocardiogram, routine biochemistry, and physical examinations were performed at screening to investigate the presence of exclusion criteria.

Dual-energy x-ray absorptiometry

Areal bone mineral density (aBMD) and bone mineral content (BMC) at the lumbar spine (L1–L4), hip, and WB was measured by DXA using the same Hologic Discovery scanner at baseline and after 16 weeks of treatment. Coefficient of variation (CV) of repositioning is 1.5% for lumbar spine bone mineral density (LS aBMD) and 2.1% for femoral neck (26, 27).

Quantitative CT

Volumetric bone mineral density (vBMD) at lumbar vertebra 2 (LS vBMD) and the proximal femur was measured using a Philips Brilliance 40 multidetector helical CT scanner. For this purpose, CT scans were acquired from the distal endplate of L1

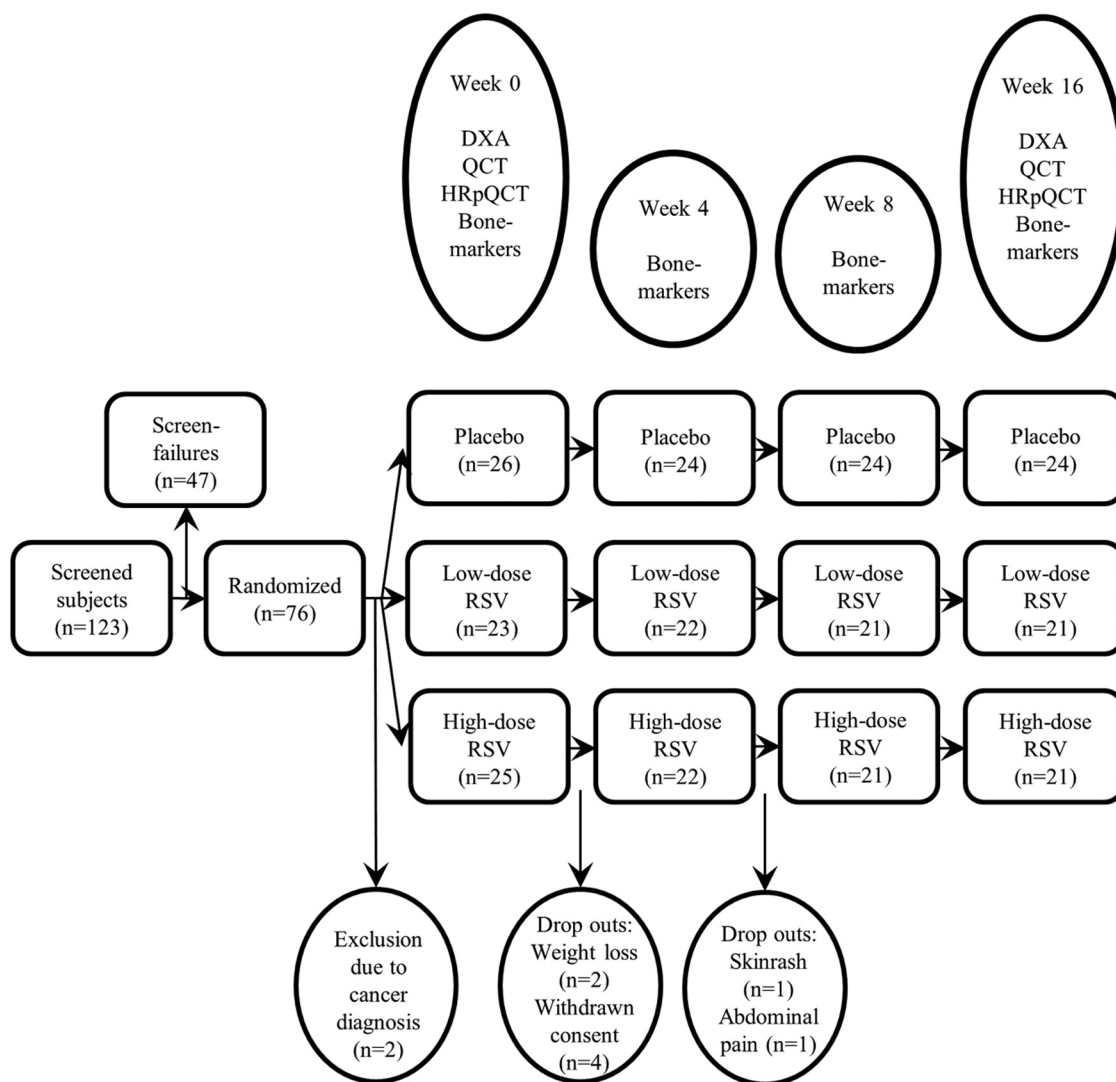


Figure 1. Flowchart.

to the proximal endplate of L3, and from acetabulum directly above the femoral head to 2 cm below the lesser trochanter, with 3-mm slice thickness and spacing. The scans were performed at 120 kV and 50 mAs/slice (LS) or 125 mAs/slice (hip), rotation time, 1 second; field of view, 360 mm; and collimation, 40×0.625 . QCT Pro (version 4.2.3, Mindways Software) was used to determine vBMD, in conjunction with a solid-state CT calibration phantom (Mindways phantom QA 3325), which was scanned simultaneously with the patient. MJ Ornstrup analyzed all patient QCT data, blinded to treatment allocation, with a reanalysis precision of $0.6 \pm 0.6\%$ for LS vBMD_{trab}, and $0.6 \pm 0.7\%$ for total hip integral volumetric bone mineral density (vBMD_{integral}).

L2 was initially rotated manually in 3D, followed by automatic positioning of the volume of interest (VOI) by QCTPro, in the anterior trabecular portion of the vertebral body. VOI was manually adjusted in case of inaccurate positioning. At the nondominant hip, proximal femur was rotated in 3D, followed by automatic positioning of VOI at different hip sites. We determined vBMD_{integral} in standard regions (total hip (TH), femoral neck (FN), trochanter (TR), and intertrochanteric (IT)), along with separate trabecular estimates (vBMD_{trab}). A fixed threshold

for cortical separation of 0.350 g/cm^3 was used (28, 29). CV of repositioning is 1.3% for LS vBMD_{trab} and 1.8% for TH vBMD_{integral} (30, 31).

High-resolution peripheral QCT

Assessment of geometry and microarchitecture of the nondominant distal radius and tibia (or in case of a previous fracture, the nonfractured limb) were obtained using HRpQCT (XtremeCT, Scanco Medical). To immobilize the arm and leg, a standard carbon fiber cast was used. A scout view was used to define the measurement region, using an offset from the endplate of radius and tibia of 9.5 mm and 22.5 mm, respectively. At each skeletal site, 110 slices were obtained, providing a 3D representation of approximately 9 mm in the axial direction. After each scan, image quality was assessed, and a rescans was conducted if necessary. Analyses were performed solely by MJ Ornstrup. CV of repositioning for radius and tibia density measures are 0.5–1.5% and of the structural parameters 1–5% (32, 33). The following parameters were measured or calculated: Trabecular bone volume fraction (BV/TV) is derived from the trabecular volume density, assuming a mineral density of fully mineralized

bone of 1200 mg hydroxyapatite per cm³. Trabecular number (Tb.N) is a direct measure. Trabecular thickness (Tb.Th) and trabecular space (Tb.Sp) are calculated and from Tb.N and BV/TV, using standard stereologic relations and assuming plate model geometry (34).

Biochemistry

Blood and urine samples were collected between 0730 and 1100 hours after an overnight fast. Routine biochemistry (creatinine, sodium, potassium, ionized calcium, alanine transaminase [ALT], bilirubin, total alkaline phosphatase [AP], and hemoglobin) was analyzed continuously throughout the study. Blood and urine for the analysis of biochemical markers of calcium homeostasis (PTH, vitamin D, phosphate, magnesium, and urinary excretion of calcium and bone turnover were frozen at -80°C and -20°C , respectively, until the time of analysis. Samples were analyzed in a single batch to reduce analytical variation. Bone turnover markers were collected at all visits: at ran-

domization (wk 0), after 4 (wk 4) and 8 weeks (wk 8) of treatment, and at the end of the study (wk 16). We analyzed BAP, osteoprotegerin (OPG), intact and N-terminal midfragment osteocalcin, and procollagen I N-terminal propeptide (P1NP) as markers of bone formation. As markers of bone resorption we analyzed C-terminal telopeptide of type 1 collagen (CTX), and cross-linked N-terminal telopeptide of type 1 collagen (NTx). All analyses, except OPG, were performed by standard laboratory methods at the Department of Clinical Biochemistry (Aarhus University Hospital, Aarhus, Denmark) (Supplemental Table 1). OPG was measured with ELISA OPG kit (Biomedica) and CV was 4.2%.

Statistics

Normality of data was checked by Q-Q plots, and equal variance between groups was assessed by Levene's test for equal variances. Baseline comparisons were assessed by ANOVA, or ANOVA on ranks if data were not normally

Table 1. Baseline Characteristics

Characteristic	Placebo (n = 26)	RSV _{low} (n = 23)	RSV _{high} (n = 25)	P Value
Age, y	48.2 \pm 6.4	48.9 \pm 6.5	50.9 \pm 5.9	.31
Body mass index, kg/m ²	34.3 \pm 3.8	33.4 \pm 4.0	33.3 \pm 3.0	.59
Smoking (yes/no)	(3/23)	(2/21)	(3/22)	N/A
Biochemistry				
AP, U/L	76.0 \pm 16.5	68.5 \pm 15.3	69.9 \pm 16.1	.22
ALT, U/L	39.8 \pm 16.6	41.1 \pm 13.6	44.8 \pm 17.0	.51
U-calcium, mmol/L	3.4 \pm 2.2	4.0 \pm 3.0	3.2 \pm 1.9	.48
Plasma Ca ²⁺ , mmol/L	1.22 \pm 0.03	1.21 \pm 0.03	1.22 \pm 0.03	.39
PTH, pmol/L	5.39 (4.28; 7.04)	5.06 (3.98; 6.39)	4.91 (4.17; 6.07)	.62
25-hydroxy vit D, nmol/L	36.4 (28.8; 65.0)	48.2 (31.3; 79.2)	45.3 (30.7; 74.2)	.41
BAP, U/L	29.9 (23.9; 36.4)	27.5 (24.1; 31.9)	26.9 (21.6; 38.1)	.76
Osteocalcin, $\mu\text{g/L}$	22.2 (17.6; 24.7)	21.1 (17.0; 26.1)	18.6 (17.1; 22.7)	.55
P1NP, $\mu\text{g/L}$	48.7 \pm 14.1	45.2 \pm 14.2	42.6 \pm 12.3	.28
OPG, pmol/L	4.83 \pm 1.32	4.45 \pm 1.42	4.90 \pm 1.74	.56
CTX, ng/ml	0.299 (0.25; 0.45)	0.308 (0.23; 0.40)	0.292 (0.25; 0.42)	.94
NTx, nmol/L	18.2 (16.9; 21.9)	17.8 (15.0; 20.8)	19.2 (15.0; 23.2)	.58
DXA, g/cm ²				
Spine aBMD	1.057 \pm 0.096	1.095 \pm 0.148	1.047 \pm 0.139	.41
Total hip aBMD ^a	1.095 \pm 0.106	1.084 \pm 0.122	1.048 \pm 0.125	.34
WB aBMD	1.176 \pm 0.081	1.182 \pm 0.088	1.155 \pm 0.089	.52
QCT, g/cm ³				
Spine vBMD _{trab}	0.149 \pm 0.033	0.147 \pm 0.034	0.134 \pm 0.029	.19
TH vBMD _{integral} ^a	0.323 \pm 0.052	0.321 \pm 0.038	0.308 \pm 0.049	.47
FN vBMD _{integral} ^a	0.300 \pm 0.042	0.303 \pm 0.048	0.295 \pm 0.048	.84
TR vBMD _{integral} ^a	0.246 \pm 0.040	0.239 \pm 0.030	0.238 \pm 0.042	.72
IT vBMD _{integral} ^a	0.378 \pm 0.063	0.379 \pm 0.045	0.355 \pm 0.058	.24
HRpQCT				
Ultra-distal radius				
BV/TV, (1)	0.156 \pm 0.028	0.158 \pm 0.032	0.151 \pm 0.029	.68
Tb.N, mm ⁻¹	2.194 \pm 0.190	2.177 \pm 0.260	2.185 \pm 0.227	.97
Tb.Th, mm	0.072 \pm 0.015	0.073 \pm 0.012	0.069 \pm 0.010	.55
Tb.Sp, mm	0.39 (0.36; 0.41)	0.39 (0.34; 0.41)	0.40 (0.36; 0.41)	.87
Ultra-distal tibia ^b				
BV/TV, (1)	0.163 \pm 0.022	0.157 \pm 0.027	0.163 \pm 0.025	.66
Tb.N, mm ⁻¹	2.164 \pm 0.228	2.063 \pm 0.282	2.103 \pm 0.266	.43
Tb.Th, mm	0.076 \pm 0.009	0.076 \pm 0.008	0.078 \pm 0.008	.68
Tb.Sp, mm	0.39 (0.35; 0.43)	0.40 (0.37; 0.45)	0.40 (0.35; 0.45)	.55

Data are expressed as mean \pm SD or median with interquartile range (25%; 75%).

The P values were calculated by ANOVA or ANOVA on Ranks where appropriate.

^a Data available on 25/26 (placebo).

^b Data available on 24/26 (placebo), 21/23 (RSV_{low}), and 24/25 (RSV_{high}).

distributed. Baseline results are presented as mean \pm SD or median with interquartile (25%; 75%) range. To evaluate possible dose-dependent responses to RSV treatment, linear regression analysis was performed and dependence between two variables was evaluated by Pearson Product Moment Correlation. Absolute changes from baseline were calculated for each time point, and differences between study groups were assessed by unpaired Student *t* test and within groups by paired *t* test. Changes are presented as mean \pm SEM. The primary endpoint was changes in BAP. To detect a treatment difference in BAP of 5 U/L at a two-sided 0.05 significance level with a power of 0.80, 24 participants should be included in each group, assuming a SD of 6U/L.

Results

Baseline characteristics

A total of 74 middle-age obese men with MetS were included in this study, of whom 66 completed all study visits. Average age was 49.3 ± 6.3 years and average BMI was 33.7 ± 3.6 kg/m² at baseline. Baseline characteristics of the participants in the three intervention groups are comparable (Table 1).

Effect of resveratrol on bone mass and density

LS vBMD_{trab} increased $+2.6 \pm 1.3\%$ in the RSV_{high} group after 16 weeks of treatment compared with placebo ($P = .043$) and $+2.6 \pm 0.9\%$ compared with baseline ($P = .009$). LS vBMD_{trab} in the RSV_{low} group increased non-significantly compared with placebo ($+1.0 \pm 1.1\%$, $P = .305$) (Figure 2). A linear regression analysis suggested a dose-dependent increase in LS vBMD_{trab} with increasing RSV dose ($R = 0.268$, $P = .036$; and $R = 0.273$, $P = .047$ after adjusting for changes in vitamin D during the study). In addition, both aBMD and BMC at the spine increased significantly within the RSV_{high} group ($P = .017$ and $P =$

$.014$, respectively). However, the changes were not different from the placebo group (Table 2).

Neither aBMD nor BMC at total hip changed during the 16 weeks of treatment (Table 2). Results from QCT at the hip were ambiguous. TR vBMD_{integral} increased significantly from baseline within the RSV_{high} group ($P = .008$), and likewise did FN vBMD_{trab} ($P = .053$), without reaching significance when compared with placebo. There were no dose-dependent effects at the hip. Actually, low-dose RSV had negative effects compared with placebo at TH vBMD_{trab} ($P = .035$), but was not significantly different from baseline ($P = .063$). Likewise, vBMD_{trab} at FN was borderline significantly lower in the RSV_{low} group compared with placebo ($P = .064$) (Table 2).

Changes in WB aBMD and WB BMC did not differ between groups; however, WB BMC increased significantly within the RSV_{low} group ($P = .002$) (Table 2).

HRpQCT-derived estimates of microarchitecture at distal radius and distal tibia did not change significantly between or within groups (Table 2), and neither did strength estimates from finite element modeling (data not shown).

Effect of resveratrol on biochemical markers of bone turnover

We found a highly significant dose-dependent increase in BAP ($R = 0.471$, $P < .001$; and $R = 0.471$, $P < .001$ after adjusting for changes in vitamin D during the study). At all time-points the RSV_{high} group had significantly greater increase in BAP from baseline compared with the placebo group (wk 4: $16.4 \pm 4.2\%$, $P < .001$; wk 8: $16.5 \pm 4.1\%$, $P < .001$; wk 16: $15.2 \pm 3.7\%$, $P < .001$). The changes seen in the RSV_{low} group followed the same pattern but were not significantly different from placebo (wk 4: $5.3 \pm 3.2\%$, $P = .20$; wk 8: $4.7 \pm 2.8\%$, $P = .12$; wk 16: $5.2 \pm 3.5\%$, $P = .14$) (Figure 3A). Within the entire population changes in BAP and LS vBMD were positively correlated ($R = 0.281$, $P = .027$).

Other markers of bone formation did not change consistently. Changes in P1NP were significantly different in the RSV_{high} group compared with placebo at week 8 ($+8.0 \pm 4.5\%$, $P = .049$) (Figure 3B). Osteocalcin increased in the RSV_{high} group, but not significantly different from the placebo group (wk 4: $6.2 \pm 4.2\%$, $P = .10$; wk 8: $6.9 \pm 4.9\%$, $P = .12$; wk 16: $3.7 \pm 4.6\%$, $P = .42$) (Figure 3C). Changes in OPG and the two bone resorption markers CTx and S-NTx were comparable between groups at all time points (Supplemental Table 2 and Figure 3D).

25-hydroxy vitamin D increased similarly throughout the study in the placebo group and RSV_{high} group. However, changes in the RSV_{low} group were different from

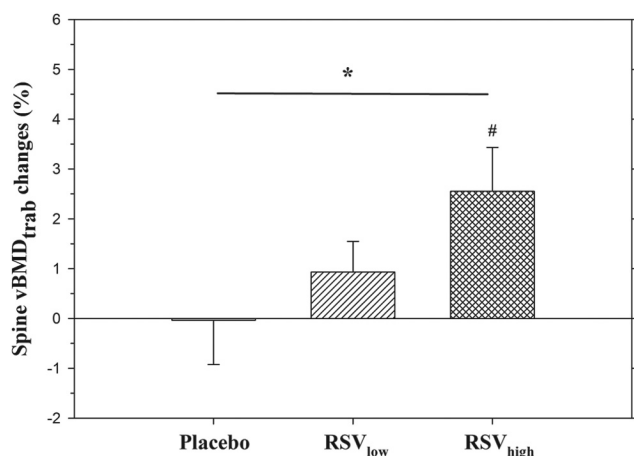


Figure 2. Change in lumbar spine trabecular vBMD after 16 wk intervention (mean \pm SEM). *, $P = .043$ (between-group difference by unpaired *t* test). #, $P = .009$ (within-group difference by paired *t* test).

Table 2. Percent Changes From Baseline

	Placebo (n = 24)	RSV _{low} (n = 21)	RSV _{high} (n = 21)	P Value RSV _{low} vs PLC	P Value RSV _{high} vs PLC
DXA					
L1–L4 aBMD	0.91 ± 0.57	0.62 ± 0.53	1.02 ± 0.38^a	.70	.88
L1–L4 BMC	0.95 ± 0.84	1.20 ± 0.63	1.68 ± 0.61^a	.97	.63
Total hip aBMD	0.58 ± 0.31	0.52 ± 0.30	0.30 ± 0.36	.94	.50
Total hip BMC	0.87 ± 0.61	0.96 ± 0.69	0.40 ± 0.60	.75	.58
WB aBMD	0.44 ± 0.40	0.23 ± 0.29	−0.16 ± 0.30	.62	.20
WB BMC	0.45 ± 0.41	1.05 ± 0.29^b	−0.02 ± 0.26	.28	.29
QCT					
Spine ^c					
L2 vBMD _{trab}	−0.04 ± 0.89	0.93 ± 0.62	2.55 ± 0.88^b	.31	.043
Total hip ^d					
vBMD _{integral}	0.99 ± 0.44	0.35 ± 0.47	0.70 ± 0.61	.29	.62
vBMD _{trab}	0.40 ± 0.32	−0.42 ± 0.20	0.58 ± 0.35	.039	.68
Femoral neck ^d					
vBMD _{integral}	0.92 ± 0.71	0.25 ± 0.48	−0.77 ± 0.61	.43	.08
vBMD _{trab}	0.23 ± 0.45	−1.12 ± 0.60	1.55 ± 0.77	.06	.14
Trochanteric ^d					
vBMD _{integral}	0.53 ± 0.39	0.36 ± 0.29	1.14 ± 0.38^b	.74	.34
vBMD _{trab}	0.50 ± 0.28	0.05 ± 0.23	0.75 ± 0.42	.17	.71
Intertrochanteric ^d					
vBMD _{integral}	0.87 ± 0.55	0.43 ± 0.67	1.32 ± 0.92	.59	.86
vBMD _{trab}	0.29 ± 0.38	−0.57 ± 0.35	−0.04 ± 0.49	.11	.70
HRpQCT					
Ultra-distal radius					
BV/TV	−0.13 ± 0.44	0.17 ± 0.31	−0.47 ± 0.31	.27	.88
Tb.N.	1.54 ± 1.93	1.84 ± 2.16	−0.10 ± 1.81	.92	.53
Tb.Th.	−0.86 ± 1.86	−0.83 ± 1.92	0.23 ± 1.66	.89	.51
Tb.Sp.	−0.68 ± 1.87	−0.96 ± 2.10	0.85 ± 1.89	.98	.57
Ultra-distal tibia ^e					
BV/TV	−0.43 ± 0.29	−0.31 ± 0.27	−0.27 ± 0.35	.70	.84
Tb.N.	−0.97 ± 1.32	−0.68 ± 0.77	−1.76 ± 1.91	.82	.77
Tb.Th.	0.83 ± 1.38	0.72 ± 0.78	2.15 ± 2.20	.95	.67
Tb.Sp.	1.46 ± 1.33	0.83 ± 0.75	2.56 ± 2.06	.77	.58

Abbreviation: PLC, placebo.

Data are expressed as percentage change from baseline (mean ± SEM).

The *P* values for between-group differences were calculated by unpaired Student *t* test. Significant results are marked in bold.Changes from baseline within groups were calculated by paired *t* test (superscript notation if significant within-group changes: ^a *P* < .05 and ^b *P* < .01).^c Data available on 22/24 (PLC), 20/21 (RSV_{low}), and 20/21 (RSV_{high}).^d Data available on 21/24 (PLC), 21/21 (RSV_{low}), and 19/21 (RSV_{high}).^e Data available on 22/24 (PLC), 19/21 (RSV_{low}), and 19/21 (RSV_{high}).

changes in the placebo group at all time points (*P* < .05) (Figure 4A). PTH levels decreased more in the placebo group compared with RSV_{high} group at week 4 and week 8 (Figure 4B), whereas ionized calcium was decreased at week 8 in the RSV_{high} group compared with placebo (Figure 4C). We found no differences in urinary excretion of calcium, ALT, bilirubin, or creatinine between groups (Supplemental Table 2). AP increased significantly in the RSV_{low} and RSV_{high} group compared with placebo (Figure 4D).

Compliance and tolerability

Compliance rates were 97% (94; 99)%, 93% (87; 98)%, and 96% (93; 99)% in the placebo group, the

RSV_{low} group, and the RSV_{high} group, respectively. Generally, treatments were well tolerated and there were no serious adverse events. Complaints of the gastrointestinal tract were the most common; (7/25) in RSV_{high}, (3/23) in RSV_{low}, and (4/26) in the placebo group. The gastrointestinal complaints were mild, and primarily in relation to increased frequency and/or softer stools, especially the first 3–4 weeks of treatment. Other adverse effects were rare and unlikely to be related to resveratrol treatment. However, one trial subject from the high-dose RSV treated group developed a transient pruritic skin rash after 1 month of treatment, which was resolved 14 days after stopping the treatment.

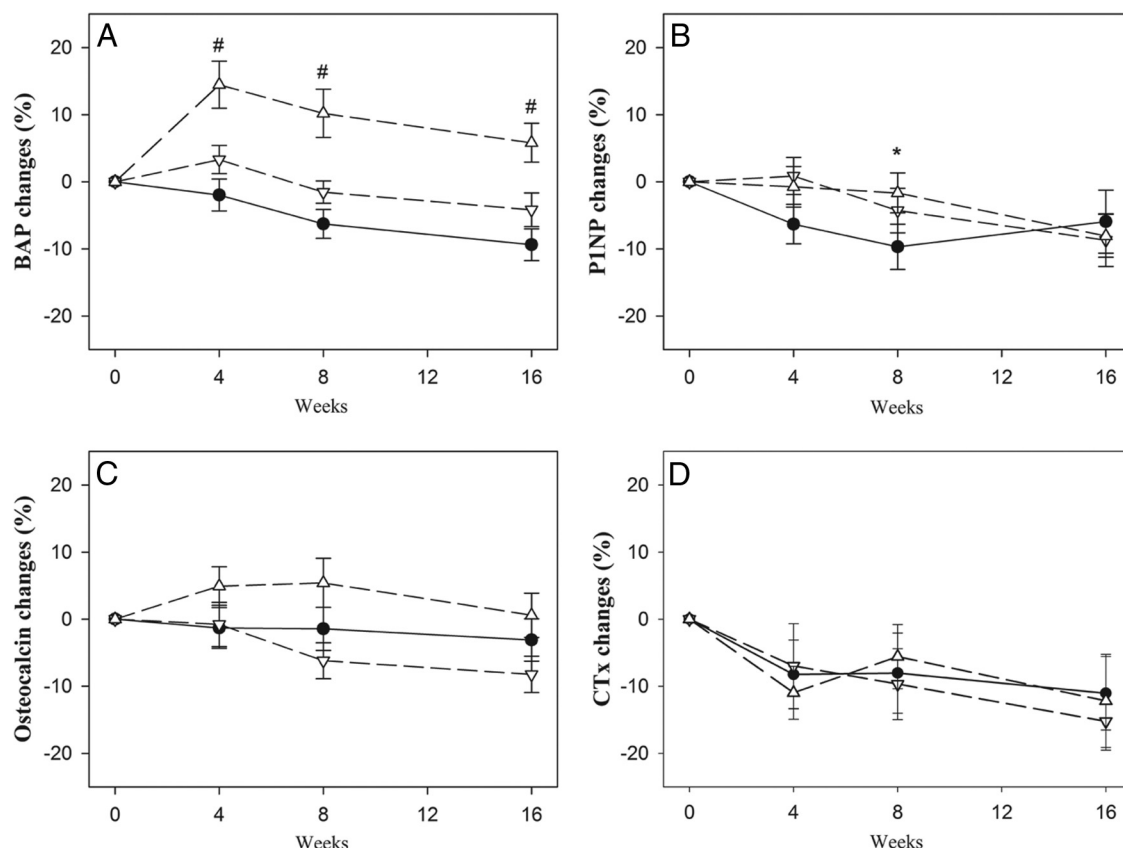


Figure 3. Changes in bone turnover markers over the study period (mean \pm SEM). Change in A, BAP; B, P1NP; C, Osteocalcin; and D, CTx are shown for the placebo group (● with solid line), RSV_{low} group (▽ with dashed line), and RSV_{high} group (Δ with dashed line). #, $P < .001$ (placebo vs RSV_{high}, unpaired t test). *, $P < .05$ (placebo vs RSV_{high}, unpaired t test).

Discussion

In this randomized, placebo-controlled, single-center study of the effects on RSV on bone, we found a significant dose-dependent increase in the trabecular vBMD at the spine, reaching +2.6% in the group receiving high-dose RSV supplementation. The bone formation marker BAP increased by 16% after 4 weeks in the high-dose RSV group compared with placebo and remained elevated throughout the study. Furthermore, the changes in LS vBMD_{trab} and BAP were significantly correlated, supporting a causal relationship. This increase in LS vBMD is impressive considering the short intervention period of 16 weeks, and the age and phenotype of the trial subjects. For comparison, McClung et al (35) reported increases in trabecular vBMD at the spine, in postmenopausal osteoporotic women treated for 24 weeks with either 20 μ g teriparatide or 10 mg alendronate daily, of 12.2% and 5.1%, respectively. Although the effect of RSV was inferior to these recognized antiosteoporotic drugs, we believe that a 2.6% increase over a shorter intervention period in a nonosteoporotic population of obese men makes it worth the effort to further investigate the antiosteoporotic potential of RSV.

DXA-derived aBMD and BMC at the spine increased significantly within the RSV_{high} group, however not sig-

nificantly different from placebo. Although aBMD by DXA is an integrated measurement of the trabecular and cortical bone, vBMD measured by QCT only comprises trabecular bone at the spine. Using vBMD measures offers advantages, especially in our population of obese men. The lumbar vertebrae consist primarily of trabeculae, enclosed by a very thin layer of cortex. The trabecular compartment is the most metabolic active compartment and therefore reveals the earliest and most dramatic changes (30). The precision error of DXA measures increases with increasing BMI, due to increased tissue thickness and fat inhomogeneity (36). The QCT technique reduces noise from excess soft tissue to a minimum, and QCT is less susceptible to disparities in patient positioning from one scan to the next, because postscan rotation of the vertebrae and hip is possible within the analysis software. QCT-derived vBMD may therefore be a better estimate of bone strength and quality in this category of patients (37, 38). No consistent changes were detected in bone density at the hip.

The increase seen in BAP is a confirmation of the findings of our previous study on short-term effects of resveratrol (24). That study lasted only 4 weeks and in the present study we have shown that the initial increase in

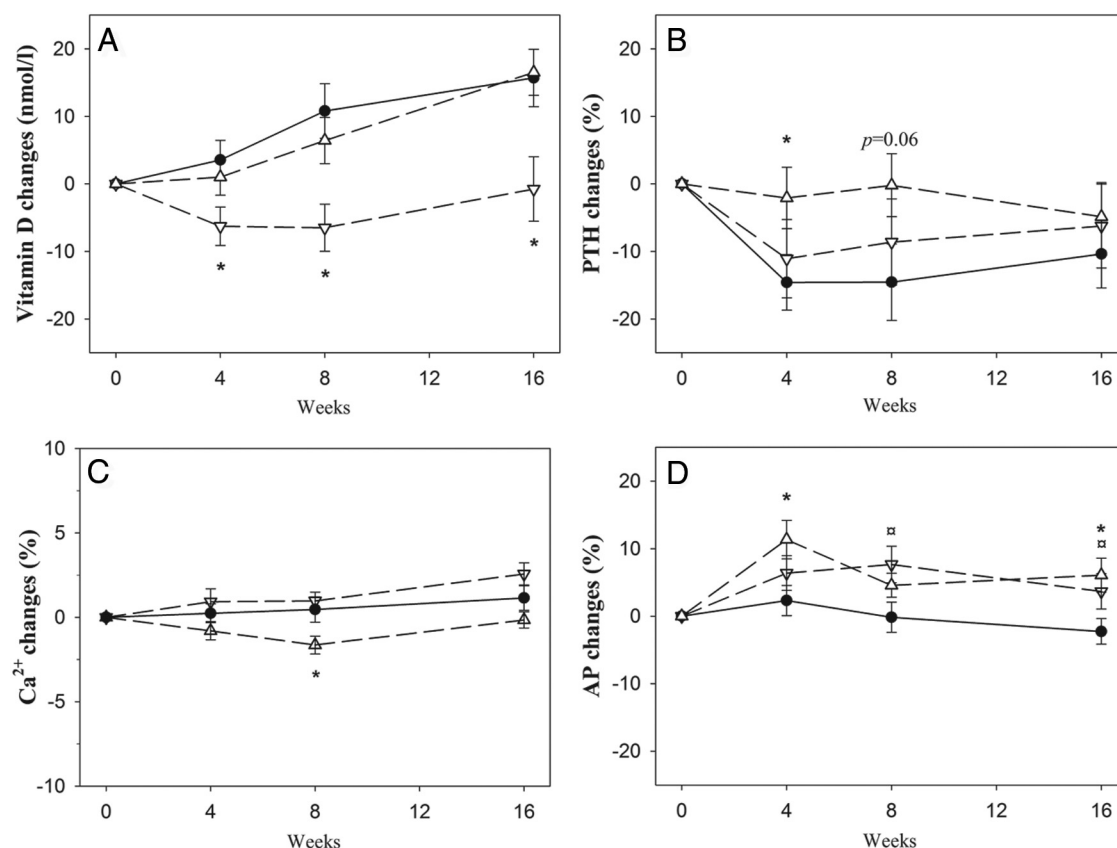


Figure 4. Changes in other biochemical markers over the study period (mean \pm SEM). Absolute change in A, 25-hydroxy vitamin D; and B, percent change in PTH; C, ionized calcium; and D, AP are shown for the placebo group (● with solid line), RSV_{low} group (▽ with dashed line), and RSV_{high} group (Δ with dashed line). *, $P < .05$ (placebo vs RSV_{high}, unpaired t test). □, $P < .05$ (placebo vs RSV_{low}, unpaired t test). In panel B, $P = .06$ indicates a borderline significant difference between the placebo group and the RSV_{high} group at wk 8 (unpaired t test).

BAP is maintained for at least 16 weeks. Also, AP increased in the RSV groups compared with placebo, but to a lesser extent. The AP measurement includes all iso-enzymes of alkaline phosphatase. In adults, AP primarily represents bone and liver iso-enzymes, with an intestinal fraction of less than 10%. Cross-reactivity between bone and liver iso-enzymes is modest (around 5%). The differences in AP changes between groups are probably driven by differences in BAP.

None of the markers of bone resorption changed differently between groups during the study, whereas the formation marker osteocalcin followed a pattern similar (nonsignificant) to the changes seen in BAP, and P1NP was significantly increased in the RSV_{high} group at wk 8.

Baseline vitamin D levels were low and comparable between groups. Changes in vitamin D during the 16 weeks of intervention were modest; however, significantly different between groups, as levels increased in the RSV_{high} group and the placebo group, but not in the RSV_{low} group. There was a tendency toward more participants being included in the late fall/early winter in the RSV_{low} group and late winter/early spring in the other two groups. We speculate if time of year at inclusion could explain the different changes in vitamin D levels, because a nadir of vitamin D

is present in late winter (39). Because differences in vitamin D could have potentially affected the changes found in vBMD and BAP, we included changes in vitamin D in a multiple linear regression analysis. However, increasing doses of RSV still predicted an increasing LS vBMD_{trab} and increasing BAP independently of changes in vitamin D. The increase in serum levels of vitamin D and the accompanying decrease in serum levels of PTH in the placebo group is probably the explanation to why bone resorption and formation decline during the course of the study. Although we did see similar changes in serum levels of vitamin D in the RSV_{high} group, PTH surprisingly remained at baseline level. The increase in bone formation and/or mineralization could explain the temporary decrease in serum levels of ionized calcium, due to calcium deposition in the bone and this decrease in calcium would tend to stimulate PTH secretion and thereby counteract the expected decrease in PTH with increasing vitamin D. Surprisingly, the higher serum levels of PTH in the RSV_{high} group did not seem to induce osteoclast activity, as bone resorption markers were comparable to placebo. Thus, our data suggest that high-dose RSV supplementation affects bone primarily by stimulating formation or mineralization; however, we speculate that RSV may inhibit

osteoclast activity, as shown in cell culture studies (17, 18), but the expected decrease in bone resorption is counteracted by the higher serum levels of PTH. Regardless, RSV seems to uncouple bone formation and resorption, possibly due to direct effects on both osteoblasts and osteoclasts. To gain better insight, future studies should include double-labeled bone biopsies useful to determine for example mineral apposition rate and to quantify osteoclasts by tartrate-resistant acid phosphatase (TRAP) staining. We measured urine calcium levels to ensure that calcium was not simply excreted, and thereby causing the temporary decrease in ionized calcium in the RSV_{high} group. There were no differences between groups, which support calcium deposition in the bone as a possible explanation, and ties in well with the increase in LS vBMD_{trab}.

Changes in level of low-grade inflammation could also have an effect on bone turnover in these men with MetS. However, no effect on inflammation markers after 16 weeks of RSV treatment could be demonstrated (40). Therefore, the positive effect of RSV on bone is not explained by anti-inflammatory effects. Our previous study supports this claim given that we found increasing BAP after 4 weeks of RSV treatment with no simultaneous reduction in inflammatory markers (23, 24).

The strengths of this study include the design; a randomized, placebo-controlled, double-blind, single-center study with a high adherence rate, and that the effect of RSV on bone have been examined using several methods, including DXA, QCT, HRpQCT, and biochemical markers of bone turnover. The study also has limitations. The short intervention period prevents us from drawing any conclusions about the long-term effect of RSV on bone and calcium metabolism. The different changes in vitamin D makes the interpretation of some of the findings more difficult; however, the changes were very modest and regression analyses demonstrated that the differences found in BAP and vBMD in response to RSV treatment were not explained by changes in vitamin D. Finally, recognizing that effects of RSV are not mediated through reduced inflammation, the chosen study population is not the best suited to investigate effects on bone, and generalizability is limited.

In conclusion, we have investigated the effects of resveratrol on bone, and found dose-dependent and correlated increases in bone alkaline phosphatase and lumbar spine volumetric BMD after only 16 weeks of treatment. The effects of RSV were not mediated by changes in inflammation, and we speculate that RSV stimulates bone formation or mineralization directly. Future studies of longer duration comprising populations at risk of osteo-

porosis are needed to confirm these positive effects of resveratrol.

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