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Cell Metabolism
Short Article

Resveratrol Supplementation Does Not Improve Metabolic Function in Nonobese Women with Normal Glucose Tolerance

Jun Yoshino,[1,2,8] Caterina Conte,[1,4,8] Luigi Fontana,[1,5,6] Bettina Mittendorfer,[1] Shin-ichiro Imai,[2] Kenneth B. Schechtman,[1,3] Charles Gu,[1,3] Iris Kunz,[7] Filippo Rossi Fanelli,[4] Bruce W. Patterson,[1] and Samuel Klein[1,*]
[1]Center for Human Nutrition and Atkins Center of Excellence in Obesity Medicine
[2]Department of Developmental Biology
[3]Division of Biostatistics
Washington University School of Medicine, St. Louis, MO 63110, USA
[4]Department of Clinical Medicine, Sapienza University of Rome, 00185 Rome, Italy
[5]Department of Medicine, Salerno University Medical School, 84081 Baronissi (SA), Italy
[6]CEINGE Biotecnologie Avanzate, 80145 Napoli, Italy
[7]DSM Nutritional Products, R&D Human Nutrition and Health, 4303 Kaiseraugst Switzerland
[8]These authors contributed equally to this work
*Correspondence: sklein@dom.wustl.edu
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SUMMARY

Resveratrol has been reported to improve metabolic function in metabolically abnormal rodents and humans, but it has not been studied in nonobese people with normal glucose tolerance. We conducted a randomized, double-blind, placebo-controlled trial to evaluate the metabolic effects of 12 weeks of resveratrol supplementation (75 mg/day) in nonobese, postmenopausal women with normal glucose tolerance. Although resveratrol supplementation increased plasma resveratrol concentration, it did not change body composition, resting metabolic rate, plasma lipids, or inflammatory markers. A two-stage hyperinsulinemic-euglycemic clamp procedure, in conjunction with stable isotopically labeled tracer infusions, demonstrated that resveratrol did not increase liver, skeletal muscle, or adipose tissue insulin sensitivity. Consistent with the absence of in vivo metabolic effects, resveratrol did not affect its putative molecular targets, including AMPK, *SIRT1*, *NAMPT*, and *PPARGC1A*, in either skeletal muscle or adipose tissue. These findings demonstrate that resveratrol supplementation does not have beneficial metabolic effects in nonobese, postmenopausal women with normal glucose tolerance.

INTRODUCTION

Resveratrol, a naturally occurring polyphenol that is found primarily in the skin of grapes, is purported to mimic the health benefits of calorie restriction (CR) by improving metabolic function, reducing cancer risk, and ameliorating other age-related pathology (Baur and Sinclair, 2006; Mercken et al., 2012). These potential benefits have led to a marked growth in the purchase of resveratrol supplements, with annual sales of $30 million in the United States alone (http://newhope360.com/ingredients/what-will-be-superstar-ingredients-2010/). Data from a series of studies conducted in rodent models of diet-induced obesity have shown that resveratrol increases insulin sensitivity, improves glucose tolerance and plasma lipids, prevents the development of fatty liver, enhances mitochondrial biogenesis, suppresses inflammation and oxidative stress, and extends life span (Baur et al., 2006; Lagouge et al., 2006; Sun et al., 2007; Um et al., 2010). In contrast, resveratrol did not improve glucose tolerance, insulin sensitivity, plasma lipid profile, or life span in normal rodents (Jeon et al., 2012; Juan et al., 2002; Miller et al., 2011; Strong et al., 2012; Turrens et al., 1997), but it did mimic transcriptional changes induced by calorie restriction (Barger et al., 2008a, 2008b; Pearson et al., 2008) and improved several age-associated abnormalities in different organ systems (Pearson et al., 2008).

Recently, it was reported that resveratrol improves insulin sensitivity, postprandial plasma glucose concentration, and mitochondrial function and decreases inflammation in adults who are obese, have type 2 diabetes, or have impaired glucose tolerance (Brasnyó et al., 2011; Crandall et al., 2012; Timmers et al., 2011). However, it is not known whether resveratrol supplementation has similar benefits in nonobese people who have normal oral glucose tolerance, which has important implications for the general population.

The purpose of the present study was to conduct a randomized, double-blind, placebo-controlled trial to evaluate the metabolic effects of resveratrol supplementation (75 mg/day, 99% pure *trans*-resveratrol [resVida from DSM Nutritional Products] for 12 weeks) in lean and overweight women. To this end, we determined the effect of resveratrol on insulin sensitivity in vivo by using a two-stage hyperinsulinemic-euglycemic clamp procedure, in conjunction with stable isotopically labeled tracer infusions, and investigated global gene expression and the key molecular events induced by resveratrol in adipose tissue and skeletal muscle.

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Table 1. Body Composition Before and After Placebo and Resveratrol Treatment

	Placebo		Resveratrol	
	Before	After	Before	After
Body mass index (kg/m^2)	24.3 ± 2.7	24.3 ± 2.7	24.2 ± 2.8	24.2 ± 2.9
Fat-free mass (kg)	40.6 ± 3.1	40.8 ± 2.9	42.4 ± 4.2	42.6 ± 3.9
Fat mass (% body weight)	36.0 ± 5.6	35.6 ± 5.8	35.7 ± 6.1	35.3 ± 6.6
Subcutaneous abdominal fat volume (cm^3)	2,080 ± 794	2,065 ± 785	2,269 ± 785	2,287 ± 811
Intra-abdominal fat volume (cm^3)	822 ± 526	811 ± 517	1,031 ± 550	1,077 ± 569
Intrahepatic triglyceride content (%)	2.85 ± 4.55	2.50 ± 3.39	2.61 ± 1.47	3.17 ± 2.41

Values are means ± SD. See also Table S2.

RESULTS

Resveratrol Supplementation Was Well Tolerated

Subjects randomized to the resveratrol supplementation (n = 15; age, 58.2 ± 4.0 years) or placebo (n = 14; age, 59.8 ± 4.3 years) had similar baseline characteristics (Tables 1 and 2). No adverse effects of resveratrol on standard blood tests or electrocardiogram were detected (Table S1 and Supplemental Experimental Procedures available online). Based on the assessment of pill counts, all subjects took at least 80% of the capsules with an average compliance of 92% in the placebo group and 94% in the resveratrol group. To further ensure that subjects were compliant with resveratrol supplementation, we measured plasma resveratrol and dihydroresveratrol concentrations before and after 12 weeks of treatment. Total resveratrol and dihydroresveratrol (free and conjugated forms) were not detected in plasma in the resveratrol or placebo groups at baseline but were present in plasma after intervention in the resveratrol group only (Table 2). Total plasma resveratrol concentration increased to a maximal concentration of 992 ± 258 ng/ml at ~2 hr after dosing and did not reach baseline levels after 6 hr; the estimated half-life of elimination was ~6.5 hr (range: 3.5 hr to 11 hr).

Resveratrol Does Not Affect Body Composition, Basal Metabolic Variables, or Insulin Sensitivity

After 12 weeks of resveratrol supplementation, body weight and body composition (fat mass, fat-free mass [FFM], intra-abdominal fat volume, and intrahepatic triglyceride content) did not change (Table 1). Plasma substrates and hormones (glucose, insulin, and plasma lipids), adipokines (adiponectin and leptin), markers of inflammation (c-reactive protein [CRP] and interleukin-6 [IL-6]), the homeostasis model assessment of insulin resistance (HOMA-IR) score, blood pressure, heart rate, and resting metabolic rate did not change after resveratrol supplementation (Table 2). A hyperinsulinemic-euglycemic clamp procedure was performed to more carefully assess multiorgan insulin action. No effect of resveratrol supplementation was detected in basal glucose or fatty acid kinetics (Table 2) or insulin sensitivity in liver (hepatic insulin sensitivity index and insulin-mediated suppression of glucose rate of appearance [R_a] into plasma), adipose tissue (insulin-mediated suppression of palmitate R_a), and skeletal muscle (insulin-mediated stimulation of glucose rate of disappearance [R_d]) (Figure 1).

Resveratrol Supplementation Does Not Induce Beneficial Molecular Adaptations

Data from studies conducted in animal models suggest the beneficial effects of resveratrol are mediated by the pathways involving AMP-activated protein kinase (AMPK), NAD^+ biosynthesis, NAD^+-dependent protein deacetylase SIRT1, and peroxisome proliferator-activated receptor γ coactivator-1α (PPARGC1A), which stimulate mitochondrial biogenesis by increasing key regulators such as uncoupling protein-3 (*Ucp3*) (Baur et al., 2006; Lagouge et al., 2006; Park et al., 2012; Um et al., 2010). Moreover, gene expression of *SIRT1*, *nicotinamide phosphoribosyltransferase* (*NAMPT*; a key NAD^+ biosynthetic enzyme), *PPARGC1A*, and *UCP3* are upregulated by resveratrol (Ajmo et al., 2008; Lagouge et al., 2006; Mukherjee et al., 2009; Um et al., 2010). Therefore, we measured gene expression of these putative resveratrol targets in skeletal muscle and adipose tissue in a subset of subjects in the placebo and resveratrol groups, and we found the expression of these genes in both skeletal muscle and adipose tissue were not affected by resveratrol supplementation (Figure 2A). To further examine global transcriptional changes caused by resveratrol, we performed microarray analyses of both skeletal muscle and adipose tissue samples and conducted a gene set enrichment analysis (GSEA) to identify the biological pathways that might be affected by resveratrol. Resveratrol supplementation was associated with a significant effect on only two pathways (KINESIN_COMPLEX, false discovery rate [FDR] = 0.015; UBIQUITIN_LIGASE_COMPLEX, FDR = 0.216) in skeletal muscle and did not have any significant effects in adipose tissue. In contrast with data from previous reports that found resveratrol affected the biological pathways linked to mitochondrial function and inflammation in obese humans (Timmers et al., 2011) and rodents (Lagouge et al., 2006), we did not detect any effect of resveratrol on these pathways in either skeletal muscle or adipose tissue (Figure 2B). Furthermore, resveratrol had no effect on the biological pathways related to AMPK (Figure 2B) and did not alter the phosphorylation levels of AMPKα (Thr172) in skeletal muscle (Figure 2C).

Moderate Weight Loss Induced by Short-Term CR Changes Body Composition and Tissue Gene Expression

Moderate weight loss induced by short-term CR decreased total body fat mass, intra-abdominal adipose tissue volume, and intrahepatic triglyceride content but did not result in significant

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Table 2. Plasma Resveratrol and Metabolic Variables Before and After Placebo and Resveratrol Treatment

	Placebo		Resveratrol	
	Before	After	Before	After
Total resveratrol (ng/ml)	ND	ND	ND	109.2 ± 185.0
Total dihydroresveratrol (ng/ml)	ND	ND	ND	168.9 ± 106.0
Glucose (mg/dl)	94.2 ± 6.7	91.5 ± 6.2	94.8 ± 5.7	93.1 ± 5.5
Insulin (mU/liter)	4.9 ± 2.7	4.2 ± 2.0	5.9 ± 3.2	5.7 ± 3.1
HOMA-IR	1.17 ± 0.69	0.96 ± 0.48	1.41 ± 0.80	1.32 ± 0.75
Free fatty acids (mmol/liter)	0.62 ± 0.15	0.58 ± 0.10	0.67 ± 0.14	0.58 ± 0.15
Total cholesterol (mg/dl)	187 ± 30	186 ± 37	210 ± 35	197 ± 32
LDL cholesterol (mg/dl)	108 ± 27	109 ± 32	131 ± 33	120 ± 33
Triglyceride (mg/dl)	97 ± 58	93 ± 57	118 ± 42	118 ± 52
HDL cholesterol (mg/dl)	60 ± 13	58 ± 13	56 ± 10	54 ± 12
Adiponectin (μg/ml)	13.3 ± 4.8	13.4 ± 4.6	12.5 ± 5.6	12.0 ± 5.0
Leptin (ng/ml)	24.5 ± 17.4	22.4 ± 16.5	21.1 ± 13.1	20.4 ± 11.8
IL-6 (pg/ml)	2.08 ± 2.39	1.84 ± 1.68	1.58 ± 0.60	2.01 ± 1.98
CRP (ng/ml)	1.68 ± 1.64	1.51 ± 1.72	2.10 ± 2.33	2.78 ± 3.01
Resting metabolic rate (kcal/kg/day)	19.5 ± 2.5	19.0 ± 2.6	18.7 ± 2.5	17.7 ± 1.9
HISI (1,000/μmol/min × μU/ml)	0.41 ± 0.24	0.47 ± 0.23	0.37 ± 0.25	0.38 ± 0.24
Basal glucose *Ra* (μmol/kg FFM/min)	17.1 ± 2.9	15.8 ± 1.9	15.9 ± 2.0	15.5 ± 1.5
Basal palmitate *Ra* (μmol/kg FFM/min)	1.78 ± 0.50	1.75 ± 0.42	1.82 ± 0.41	1.64 ± 0.62
Systolic blood pressure (mm Hg)	123 ± 15	121 ± 14	118 ± 16	119 ± 16
Diastolic blood pressure (mm Hg)	65 ± 10	63 ± 9	67 ± 11	72 ± 10
Heart rate (beats/min)	68 ± 8	65 ± 10	66 ± 8	65 ± 7

Values are means ± SD. ND, not detectable; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HISI, hepatic insulin sensitivity index; FFM, fat-free mass. See also Table S2.

improvements in in vivo metabolic outcomes (Table S2), which is consistent with the results obtained in previous weight loss studies conducted in metabolically healthy obese people and in postmenopausal women (Janiszewski and Ross, 2010; Joseph et al., 2001; Karelis et al., 2008; Shin et al., 2006). Nonetheless, CR-induced moderate weight loss altered adipose tissue gene expression profiles in several CR targets identified previously in human subjects, including upregulation of *CTEP* (Johansson et al., 2012) and downregulation of *LEP* (Viguerie et al., 2005), *ALDOC* (Capel et al., 2008; Johansson et al., 2012; Ong et al., 2009), *ABCC6* (Ong et al., 2009), and *CCND2* (Kolehmainen et al., 2008) (Figure S1).

DISCUSSION

The use of resveratrol supplements to promote health has become increasingly popular (Mercken et al., 2012). Data from a series of studies conducted in obese, metabolically abnormal rodent models have demonstrated that resveratrol improves metabolic outcomes, particularly insulin sensitivity, glucose tolerance, and plasma lipids (Baur et al., 2006; Lagouge et al., 2006; Sun et al., 2007; Um et al., 2010). Furthermore, it was recently reported that resveratrol improves metabolic outcomes in people who were either obese, had impaired glucose tolerance or had type 2 diabetes (Brasnyó et al., 2011; Crandall et al., 2012; Timmers et al., 2011). The present study provides a comprehensive evaluation of the use of resveratrol in nonobese women with normal glucose tolerance. Our findings demonstrate that 12 weeks of resveratrol supplementation (75 mg/day) increased plasma total resveratrol and total dihydroresveratrol concentrations but did not alter liver, skeletal muscle, or adipose tissue insulin sensitivity and did not have any effects on other key metabolic variables, such as body composition, plasma lipids, plasma markers of inflammation, or resting metabolic rate. Furthermore, resveratrol supplementation did not affect its major putative molecular targets in either adipose tissue or skeletal muscle, including *SIRT1*, *NAMPT*, *PPARGC1A*, and *UCP3* expression, AMPK phosphorylation, and biological pathways linked to mitochondrial function or inflammation. These data show that resveratrol supplementation (equivalent to the amount of resveratrol ingested by consuming ~8 liters of red wine per day [Stark et al., 2011]) in nonobese women with normal glucose tolerance does not affect cellular signaling or result in metabolic benefits.

Three previous studies, conducted in different cohorts of metabolically abnormal subjects, found 4 weeks of resveratrol therapy, given at doses ranging from 10 mg to 2,000 mg per day, resulted in several metabolic benefits, including an improvement in insulin sensitivity (Brasnyó et al., 2011; Crandall et al., 2012; Timmers et al., 2011), postprandial plasma glucose concentrations (Crandall et al., 2012), and plasma lipid profile (Timmers et al., 2011). However, the overall conclusions from these studies are limited because the beneficial effects were not consistent across studies and were not proportional to resveratrol dose. For example, both low and moderate doses (10 mg/day and 150 mg/day) (Brasnyó et al., 2011; Timmers

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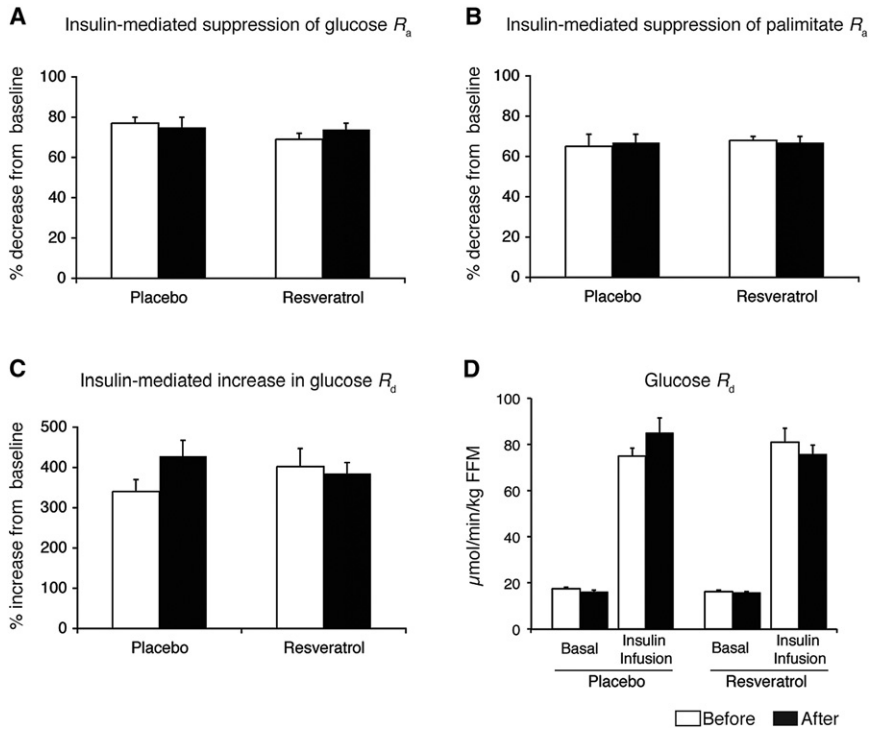

Figure 1. Liver, Adipose Tissue, and Skeletal Muscle Insulin Sensitivity

Insulin-mediated suppression of glucose rate of appearance (R_a) (A), insulin-mediated suppression of palmitate R_a (B), insulin-mediated increase in glucose rate of disappearance (R_d) (C), and absolute glucose R_d values (D) before (white bars) and after (black bars) placebo (n = 14) or resveratrol (n = 15) supplementation. Values are means ± SE. See also Table S2.

et al., 2011), but not high doses (1,000–2,000 mg/day) (Crandall et al., 2012), of resveratrol reduced insulin resistance as measured by HOMA-IR score, and 150 mg/day (Timmers et al., 2011), but not 1,000–2,000 mg/day (Crandall et al., 2012), of resveratrol decreased plasma triglyceride concentration.

If resveratrol supplementation is beneficial as reported, why did we not detect any metabolic effects of resveratrol in our subjects? It is unlikely that the lack of metabolic benefits is simply due to differences in the dose or duration of resveratrol supplementation. The dose of resveratrol given to our subjects (75 mg/day for 12 weeks) was lower than the dose given in the previous study involving obese subjects (150 mg/day for 30 days) (Timmers et al., 2011) or older subjects with impaired glucose tolerance (1,000–2,000 mg/day for 4 weeks) (Crandall et al., 2012) but much higher than the dose given in the study involving subjects with diabetes (10 mg/day for 4 weeks) (Brasnyó et al., 2011). We are not able to determine the bioavailability of resveratrol in some of these studies because different resveratrol compounds were used and plasma concentrations were not reported (Brasnyó et al., 2011; Crandall et al., 2012). The plasma concentrations of total resveratrol and total dihydroresveratrol in our subjects were 40%–50% lower than the plasma concentrations reported in the study conducted in obese subjects supplemented with 150 mg/day (Timmers et al., 2011), which provided the same resveratrol compound used in our study, but our plasma levels were likely higher than the concentrations achieved in the study conducted in diabetic subjects supplemented with 10 mg/day (Brasnyó et al., 2011). Furthermore, the duration of resveratrol supplementation in our subjects (12 weeks) was longer than the duration of supplementation in the previous three studies (4 weeks). Nonetheless, we cannot exclude the possibility that we were unable to detect modest metabolic benefits of resveratrol supplementation due to the number of subjects in our study. However, it seems unlikely that clinically meaningful effects were missed, because the values for the key metabolic outcomes after resveratrol supplementation were nearly identical to values obtained before supplementation.

We did not detect an effect of resveratrol supplementation on the gene expression profiles that are affected by resveratrol in normal mice (Barger et al., 2008a, 2008b; Pearson et al., 2008). In contrast, we found that moderate weight loss induced significant changes in gene expression profiles in adipose tissue and skeletal muscle, particularly genes that are known as CR targets in adipose tissue, identified in previous microarray studies conducted in human subjects (Capel et al., 2008; Johansson et al., 2012; Kolehmainen et al., 2008; Ong et al., 2009; Viguerie et al., 2005). However, data from several studies conducted in rodent models have found many putative resveratrol targets, such as *SIRT1*, *NAMPT*, and *PPARGC1A*, are affected by fasting (Hayashida et al., 2010; Yang et al., 2007; Yoon et al., 2001), so it is possible that collecting tissue samples from our subjects after they fasted overnight (~12 hr) masked an effect induced by resveratrol on tissue gene expression.

An important difference between the present study and those conducted previously is that our subjects were nonobese women with normal glucose tolerance, whereas the subjects in the other studies had more severe pre-existing metabolic dysfunction, such as obesity, type 2 diabetes, and impaired glucose tolerance. Studies conducted in rodent models of diet-induced obesity have shown that resveratrol improves insulin sensitivity, lipids, and mitochondrial function (Baur et al., 2006; Lagouge et al., 2006; Sun et al., 2007; Um et al., 2010) but does not show beneficial metabolic effects in normal rodents (Jeon et al., 2012; Juan et al., 2002; Miller et al., 2011; Strong et al., 2012; Turrens et al., 1997). Therefore, it is possible that resveratrol only improves metabolic outcomes in obese and metabolically abnormal people, and not in nonobese glucose-tolerant women.

In conclusion, we found that 12 weeks of resveratrol supplementation (75 mg/day) does not affect its putative molecular targets in skeletal muscle and adipose tissue or improve metabolic function, including insulin sensitivity and plasma

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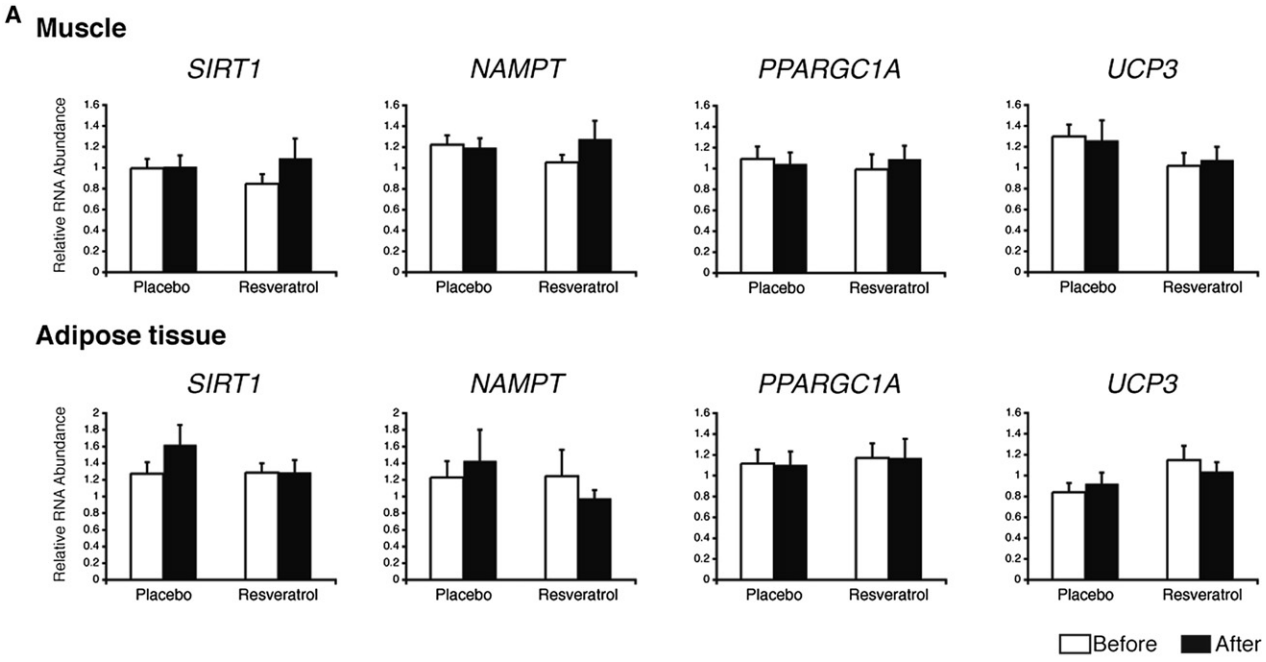

B

Pathway Name	FDR (q-value) Muscle	Adipose tissue
Mitochondrial function		
BIOCARTA_MITOCHONDRIA_PATHWAY	0.88	0.92
FATTY_ACID_BETA_OXIDATION	1.00	1.00
FATTY_ACID_OXIDATION	1.00	1.00
MITOCHONDRIAL_RESPIRATORY_CHAIN	1.00	1.00
MITOCHONDRIAL_TRANSPORT	1.00	0.79
ELECTRON_TRANSPORT_GO_0006118	1.00	1.00
REACTOME_ELECTRON_TRANSPORT_CHAIN	1.00	1.00
Inflammation		
BIOCARTA_CYTOKINE_PATHWAY	0.98	1.00
CYTOKINE_ACTIVITY	1.00	0.94
CYTOKINE_AND_CHEMOKINE_MEDIATED_SIGNALING_PATHWAY	1.00	1.00
REGULATION_OF_IMMUNE_RESPONSE	0.97	1.00
ACUTE_INFLAMMATORY_RESPONSE	1.00	1.00
AMPK Signaling		
REACTOME_ACTIVATED_AMPK_STIMULATES_FATTY_ACID_OXIDATION_IN_MUSCLE	0.74	1.00
REACTOME_REGULATION_OF_AMPK_ACTIVITY_VIA_LKB1	0.76	1.00
REACTOME_REGULATION_OF_RHEB_GTPASE_ACTIVITY_BY_AMPK	0.96	0.82

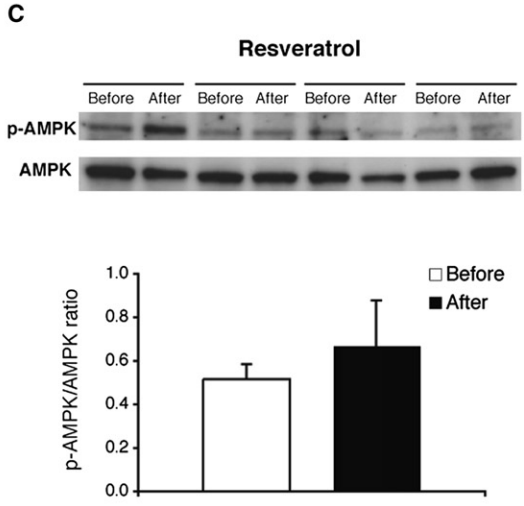

Figure 2. Assessment of Putative Resveratrol Molecular Targets

Relative gene expressions of *SIRTt1*, *nicotinamide phosphoribosyltransferase* (*NAMPT*), *PPARGC1A*, and *UCP3* (means ± SE) determined by quantitative PCR in skeletal muscle (upper panel) and adipose tissue (lower panel) before (white bars) and after (black bars) placebo or resveratrol supplementation (n = 8–12 per group) (A). Microarray analyses were performed with skeletal muscle and adipose tissue biopsy samples. GSEA was used to identify potential biological pathways affected by resveratrol. Representative resveratrol target pathways related to mitochondrial function, inflammation, and AMPK are shown (B). The levels of phosphorylated AMPKα (Thr172) and total AMPKα in skeletal muscle from four subjects before (white bars) and after (black bars) resveratrol supplementation were determined by using western blotting (C). Values are means ± SE. See also Figure S1.

lipids, in nonobese women with normal glucose tolerance. These findings are consistent with data from studies conducted in lean metabolically normal rodents. Additional randomized controlled studies are still needed to assess the potential benefits of resveratrol supplementation in metabolically abnormal individuals.

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EXPERIMENTAL PROCEDURES

Study Subjects

A total of 45 lean and overweight, Caucasian, postmenopausal women were randomly assigned to one of three groups: (1) placebo treatment for 12 weeks (n = 15), (2) resveratrol supplementation (75 mg/day) for 12 weeks (n = 15), or (3) calorie restriction targeted to achieve a 5% weight loss within 12 weeks (n = 15). One subject who was randomized to the placebo group was dropped from the study because of self-dieting and an 8.7% weight loss. All subjects completed a comprehensive medical evaluation, including a detailed history, physical examination, blood tests, a 12-lead electrocardiogram, and a 2 hr oral glucose tolerance test. No subject had any history or evidence of type 2 diabetes or cardiovascular disease, and no subject had a diagnosis or was being treated for abnormal plasma lipids or hypertension. However, ten subjects within the placebo and resveratrol groups had at least one feature of the metabolic syndrome (HDL-cholesterol < 50 mg/dl, triglyceride > 150 mg/dl, or increased blood pressure [systolic blood pressure $\geq$ 135 mmHg or diastolic blood pressure $\geq$ 85 mmHg]). Subjects provided written informed consent before participating in this study (ClinicalTrials.gov Identifier NCT00823381), which was approved by the Institutional Review Board of Washington University in St. Louis, MO.

Study Protocol

Body Composition

Body fat mass and FFM were determined by dual-energy X-ray absorptiometry, intra-abdominal and subcutaneous adipose tissue volumes were quantified by using magnetic resonance imaging, and intrahepatic triglyceride content was determined by using magnetic resonance spectroscopy (Frimel et al., 2007).

Hyperinsulinemic-Euglycemic Clamp Procedure and Tissue Biopsies

Subjects were admitted to the Clinical Research Unit at Washington University School of Medicine in the afternoon on the day before the clamp procedure. After subjects fasted for 12 hr overnight, a 9.5 hr, two-stage hyperinsulinemic-euglycemic clamp procedure, in conjunction with stable isotopically labeled tracer infusion, was performed as previously described (Fabbrini et al., 2009). Subcutaneous abdominal adipose tissue and skeletal muscle (vastus lateralis) biopsies were obtained during the basal period of the clamp procedure to investigate the molecular events induced by resveratrol treatment. Resting metabolic rate was measured, via indirect calorimetry, during the basal period of the clamp procedure.

Intervention and Postintervention Studies

After the baseline studies were completed, each subject was randomized to 12 weeks of treatment with resveratrol (75 mg/day; resVida 99.7% *trans*-resveratrol, provided by DSM Nutritional Products, Kaiseraugst, Switzerland), placebo, or calorie restriction with a three-block computer-generated randomization scheme with a stratification of subjects based on a BMI value <25 kg/m^2 and $\geq$25 kg/m^2. Subjects were instructed to take one capsule (75 mg resveratrol or placebo) in the morning with breakfast. After 12 weeks of supplementation, all studies performed at baseline were repeated. On the day of the final clamp procedure, resveratrol was given in the morning and blood samples were obtained before and at 30, 60, 90, 120, 240, 360 min after resveratrol administration (before insulin infusion) to evaluate resveratrol pharmacokinetics.

Sample Processing and Analyses

Details of analyses and calculations used to evaluate metabolic variables, substrate kinetics, real-time PCR, microarray analyses, and western blot are available in the Supplemental Experimental Procedures.

Statistical Analyses

The statistical significance of differences in postintervention outcome measures between resveratrol and placebo treatment were evaluated by analysis of covariance (ANCOVA) with the pretreatment values as the covariates. Addition of the CR weight loss group to the analysis of outcome measures did not change the comparisons between the placebo and resveratrol groups. Results are presented as means ± SD, except in the figures, which report data as means ± SE.

ACCESSION NUMBERS

All microarray data used in this study have been deposited into the NCBI GEO database under accession number GSE41168.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, one figure, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2012.09.015.

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