High-Dose Resveratrol Treatment for 2 Weeks Inhibits Intestinal and Hepatic Lipoprotein Production in Overweight/Obese Men

Satya Dash,* Changting Xiao,* Cecilia Morgantini, Linda Szeto, Gary F. Lewis

Objective—Overproduction of hepatic apolipoprotein B (apoB)-100 containing very low-density lipoprotein particles and intestinal apoB-48 containing chylomicrons contributes to hypertriglyceridemia seen in conditions such as obesity and insulin resistance. Some, but not all, preclinical and clinical studies have demonstrated that the polyphenol resveratrol ameliorates insulin resistance and hypertriglyceridemia. Here, we assessed intestinal and hepatic lipoprotein turnover, in humans, after 2 weeks of treatment with resveratrol (1000 mg daily for week 1 followed by 2000 mg daily for week 2) or placebo.

Approach and Results—Eight overweight or obese individuals with mild hypertriglyceridemia were studied on 2 occasions, 4 to 6 weeks apart, after treatment with resveratrol or placebo in a randomized, double-blinded, crossover study. Steady-state lipoprotein kinetics was assessed in a constant fed state with a primed, constant infusion of deuterated leucine. Resveratrol treatment did not significantly affect insulin sensitivity (homeostasis model of assessment of insulin resistance), fasting or fed plasma triglyceride concentration. Resveratrol reduced apoB-48 production rate by 22% (P=0.007) with no significant effect on fractional catabolic rate. Resveratrol reduced apoB-100 production rate by 27% (P=0.02) and fractional catabolic rate by 26% (P=0.04).

Conclusions—These results indicate that 2 weeks of high-dose resveratrol reduces intestinal and hepatic lipoprotein particle production. Long-term studies are needed to evaluate the potential clinical benefits of resveratrol in patients with hypertriglyceridemia, who have increased concentrations of triglyceride-rich lipoprotein apoB-100 and apoB-48.

Clinical Trial Registration—URL: www.clinicaltrials.gov. Unique identifier: NCT01451918.

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Key Words: apolipoproteins B ◼ chylomicrons ◼ intestines ◼ lipoproteins, VLDL ◼ resveratrol

Increased production of triglyceride-rich lipoproteins (TRL), in the form of apolipoprotein B (apoB)-100 containing very low-density lipoprotein (VLDL) from the liver and apoB-48 containing chylomicrons from the gut, in conditions such as overweight/obesity and insulin resistance, may contribute to an increased risk of atherosclerotic disease.1,2 Recently, we have shown that, in healthy human volunteers, intestinal secretion of apoB-48—containing TRL particles is regulated by free fatty acids,3 dietary monosaccharides (glucose and fructose),4 and hormones such as insulin5 and glucagon-like peptide-1.6 Individuals with obesity/overweight,7 insulin resistance,7 and type 2 diabetes mellitus8 have increased production of apoB-48 TRL particles. This, along with the well-established overproduction of apoB-100-containing VLDL by the liver,1 contributes to the elevated TRL seen in these individuals. Identifying novel strategies to lower TRL production by the liver and intestine may have clinical benefits in conditions such as in overweight/obese states. In the present study, we have examined the effect of resveratrol in overweight and obese men with relatively mild metabolic abnormalities. Polyphenol resveratrol, widely available over the counter, has been demonstrated to have numerous beneficial metabolic effects in vitro and in vivo in murine models. In vitro studies in hepatocytes have demonstrated that resveratrol reduces de novo lipogenesis.9 Resveratrol administration in vivo protects mice from diet-induced obesity, insulin resistance, and hepatic steatosis.10,11 Some, but not all,12,13 studies in humans have shown that resveratrol improves insulin sensitivity14–16 and lowers plasma triglyceride.14 The metabolic effects of resveratrol are thought to be mediated at least in part via indirect activation of AMP kinase and the NAD+-dependent deacetylase silent mating type information regulation 2 homolog (sirtuin) 1 (SIRT1).17 Adenine monophosphate kinase (AMPK) has multiple effects on lipid metabolism by...
activating downstream targets such as PGC-1α, inhibiting acetyl-CoA carboxylase, and phosphorylating SREBP-1c, which leads to increased lipid oxidation and reduced lipid synthesis. AMPK also has indirect effects such as activation of SIRT1. SIRT1, thought to mediate many of the beneficial effects of caloric restriction, deacetylates multiple targets including PGC-1α, FoxO1, AMPK itself, and nuclear factor κB, thereby influencing lipid and glucose metabolism as well as inflammation. No studies to date, either in rodents or humans, have assessed the effects of resveratrol on lipoprotein production and clearance. Therefore, we sought to assess the effects of short-term administration of high-dose resveratrol on intestinal and hepatic lipoprotein turnover as well as insulin sensitivity in nondiabetic, overweight, and obese men with mild hypertriglyceridemia. Although in most human studies, resveratrol was administered for ≥4 weeks, many of these studies used low doses (≤150 mg/d) with positive metabolic effects. Dose-dependent increases in plasma concentrations of resveratrol (at doses between 0.5 and 5 g) have been reported previously with higher elimination half-lives of doses between 1 and 2.5 g (>9 hours) compared with the lower dose of 0.5 g (0–4 hours). We hypothesized that higher doses of resveratrol could elicit beneficial effects on TRL metabolism with 2 weeks of administration. Resveratrol was administered at 1000 mg/d for a week and increased to 2000 mg/d in the second week, the lower dose during the first week required by Health Canada regulatory authorities to ensure that there were no adverse effects. These doses of resveratrol were based on a previous study in which resveratrol at a dose of 1000 to 2000 mg/d improved glucose tolerance without eliciting any adverse effects.

**Materials and Methods**

Materials and Methods are available in the online-only Supplement (Table; Figure 1).

**Results**

**Resveratrol Treatment Had No Significant Effects on Homeostasis Model of Assessment of Insulin Resistance**

There was no significant difference between resveratrol and placebo treatments in fasting plasma glucose (Figure 2A; placebo 5.3±0.2 versus resveratrol 5.2±0.2 mmol/L; P=0.4). Fasting plasma insulin (Figure 2B; placebo 53±4.2 versus resveratrol 40.8±8.5 pmol/L; P=0.2) and homeostasis model of assessment of insulin resistance (HOMA-IR; Figure 2C; placebo 1.78±0.16 versus resveratrol 1.35±0.3; P=0.2) tended to be lower with resveratrol. However, neither of these parameters reached statistical significance when assessed by paired t tests. There were no significant differences in plasma glucose (Figure 2D) or insulin (Figure 2E) during constant feeding with resveratrol treatment.

**Table. Demographic Characteristics and Fasting Plasma Concentrations**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean±SEM (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>45.8±3.1 (28–55)</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>91.5±6.4 (70.5–126.0)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.1±1.7 (27.0–40.0)</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.0±0.2 (4.3–6.1)</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>28±6.5 (26.9–82.2)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.8±0.1 (1.2–2.5)</td>
</tr>
<tr>
<td>Plasma TG, mmol/L</td>
<td>2.5±0.3 (1.6–3.9)</td>
</tr>
<tr>
<td>Plasma FFA, mmol/L</td>
<td>0.4±0.1 (0.2–0.6)</td>
</tr>
<tr>
<td>Plasma TC, mmol/L</td>
<td>5.1±0.7 (1.6–6.9)</td>
</tr>
<tr>
<td>TRL-apoB100, mg/L</td>
<td>263.3±23.8 (197.8–280.2)</td>
</tr>
<tr>
<td>TRL-apoB48, mg/L</td>
<td>2.5±0.4 (1.0–3.7)</td>
</tr>
</tbody>
</table>

Data are mean±SEM (range); n=8. apoB indicates apolipoprotein B; BMI, body mass index; FFA, free fatty acids; HOMA-IR, homeostasis model of assessment of insulin resistance; TC, total cholesterol; TG, triglycerides; and TRL, TG-rich lipoprotein.
Resveratrol Did Not Affect Fasting or Fed Triglyceride Concentrations in Plasma or TRL Fractions

There were no significant differences between the 2 treatments in terms of mean fasting plasma triglyceride (placebo 1.9±0.3 versus resveratrol 2.0±0.2 mmol/L; \( P = 0.5 \); Figure 3A) and TRL triglyceride (placebo 1.4±0.2 versus resveratrol 1.5±0.2 mmol/L; \( P = 0.6 \); Figure 3B). There were no differences in mean triglyceride during constant feeding between treatments in either plasma (placebo 3.3±0.3 versus resveratrol 3.2±0.4 mmol/L; \( P = 0.5 \); Figure 3C) or TRL fraction (placebo 2.7±0.3 versus resveratrol 2.6±0.3 mmol/L; \( P = 0.6 \); Figure 3D). Resveratrol did not affect fasting TRL cholesterol (placebo 0.9±0.3 versus resveratrol 1.1±0.4 mmol/L; \( P = 0.2 \)), plasma cholesterol (placebo 4.8±1.7 versus resveratrol 5.2±1.8 mmol/L; \( P = 0.6 \)), or high-density lipoprotein cholesterol (placebo 1.2±0.4 versus resveratrol 1.3±0.4 mmol/L; \( P = 0.8 \); Figure I in the online-only Data Supplement).

Resveratrol Reduced TRL ApoB-48 Production Rate With No Significant Change in the Fractional Catabolic Rate

Resveratrol treatment significantly reduced apoB-48 production rate (PR; placebo 1.4±0.2 versus resveratrol 1.1±0.3 mg/kg per day; \( P = 0.007 \)) with no change in apoB-48 fractional catabolic rate (FCR; placebo 2.4±0.2 versus resveratrol 2.1±0.3 pools/d; \( P = 0.4 \); Figure 4A). There was no significant difference in fasting TRL apoB-48 concentration (placebo 2.5±0.4 versus resveratrol 2.9±0.8 mg/L; \( P = 0.6 \); Figure 4B). TRL apoB-48 concentration during the kinetic study was not significantly lower with resveratrol treatment, although there was a trend toward a slight reduction (placebo 13.0±2.1 versus resveratrol 10.6±1.3 mg/L; \( P = 0.15 \); Figure 4B). Model fits to the apoB-48 TTR are shown in the online-only Data Supplement (Figure II in the online-only Data Supplement).

Resveratrol Reduced Both PR and FCR of TRL ApoB-100

Resveratrol treatment significantly decreased apoB-100 FCR (placebo 4.2±0.9 versus resveratrol 3.3±0.6 pools/d; \( P = 0.04 \); Figure 5A) and apoB-100 PR (placebo 46.9±8.5 versus resveratrol 34.3±5.9 mg/kg per day; \( P = 0.02 \)). There was no significant difference in fasting TRL apoB-100 concentration (placebo 263.5±23.8 versus resveratrol 276.5±45.7 mg/L; \( P = 0.7 \); Figure 5B). There was no net difference in TRL apoB-100 concentration during the kinetic study (placebo 251.5±26.2 versus resveratrol 245.1±28.7 mg/L; \( P = 0.8 \); Figure 5C). Model fits to the apoB-100 TTR are shown in the online-only Data Supplement (Figure II in the online-only Data Supplement).

Discussion

Elevated TRL particle production by the liver and intestine contributes to the highly prevalent postprandial dyslipidemia seen in conditions such as obesity/overweight, insulin resistance, and type 2 diabetes mellitus.\(^1\,2\,22\,23\) This may play...
a role in the increased risk of atherosclerosis seen in these individuals. A better understanding of the regulation of TRL particle production could potentially be of therapeutic benefit. Here, we report that high-dose resveratrol significantly reduced apoB-48 (by 22%) and apoB-100 (by 27%) PRs by the intestine and the liver, respectively, in overweight and obese men with relatively mild hypertriglyceridemia. This is the first study in humans to demonstrate beneficial effects of resveratrol on triglyceride-rich lipoprotein metabolism.

Figure 3. Fasting plasma triglyceride (TG; A; placebo: white bars, resveratrol: black bars) and TG-rich lipoprotein (TRL) TG (B; placebo: white bars, resveratrol: black bars) was measured the day before the lipoprotein kinetic study. Plasma (C; placebo: ○, resveratrol: ■) and TRL (D; placebo: ○, resveratrol: ■) TG were measured during constant feeding on the day of the lipoprotein kinetic study. There were no differences between resveratrol and placebo treatments in the fasted or constant fed states.

Figure 4. Resveratrol did not affect triglyceride-rich lipoprotein (TRL)–apoB-48 fractional catabolic rates (FCR; P=NS), but reduced TRL–apoB-48 production rate (PR; A; *P=0.007; placebo: white bars, resveratrol: black bars). It did not affect fasting TRL–apoB-48 concentration (P=NS; placebo: white bars, resveratrol: black bars; B) with a nonsignificant trend toward reducing TRL–apoB-48 concentration during the kinetics study (P=0.15; placebo: ○, resveratrol: ■). apoB indicates apolipoprotein B.

Figure 5. Resveratrol significantly reduced triglyceride-rich lipoprotein (TRL)–apoB-100 fractional catabolic rates (FCR; A; **P=0.04) and production rate (PR; A; †P=0.03; placebo: white bars, resveratrol: black bars). It did not affect fasting TRL–apoB-100 concentration (P=NS; placebo: white bars, resveratrol: black bars; B). There was no significant change in TRL–apoB-100 concentration during the kinetic study (C; placebo: ○, resveratrol: ■). apoB indicates apolipoprotein B.
Resveratrol administration on intestinal and hepatic TRL particle production.

There has been tremendous interest in harnessing the beneficial effects of resveratrol, a widely available over-the-counter supplement, seen in murine studies. These effects, including protection from insulin resistance and hepatic steatosis in diet-induced obesity,10,11 are thought to be mediated via indirect activation of AMPK and SIRT1. This has been postulated to mimic some of the beneficial effects of caloric restriction, seen in preclinical12 and some human studies,25,26 on glucose and lipid metabolism. The therapeutic effects of resveratrol in humans are incompletely understood. Some, but not all,1,2,3 short-term human studies in small cohorts have demonstrated the efficacy of resveratrol on improving insulin sensitivity, fasting triglycerides, and skeletal muscle mitochondrial function.4-6 No human studies to date have assessed effect of resveratrol on TRL production. Therefore, we performed this double-blinded crossover study, with 2 weeks of treatment with resveratrol or placebo, to investigate the effects of resveratrol on lipoprotein turnover. Resveratrol was administered at 1000 mg/d for a week and increased to 2000 mg/d in the second week, the lower dose during the first week required by Health Canada regulatory authorities to ensure that there were no adverse effects. These doses of resveratrol have previously been shown to improve glucose tolerance without eliciting any adverse effects.7,8 Published studies reporting a beneficial metabolic effect of resveratrol have been performed in participants with either impaired insulin sensitivity, glucose intolerance, type 2 diabetes mellitus, and dyslipidemia,9,10,11,12,13,14,15 with no effect seen in a study in lean healthy women.16 Therefore, we studied TRL production in overweight and obese men with mild hypertriglyceridemia (TG>1.6 mmol/L), a group more likely to respond to resveratrol.

To elucidate the mechanism(s) underlying the reduced PR of apoB-100 and apoB-48, we assessed the effects of resveratrol treatment on known regulators of apoB particle production. Plasma concentrations of free fatty acids (Figure III in the online-only Data Supplement) and insulin, both of which are known to affect apoB-100 and apoB-48 production,3,5 were not significantly different between treatments. Although unlikely to be the case, it is not possible to completely rule out reduced free fatty acids flux from reduced lipolysis, as any adverse effects. These doses of resveratrol have previously been shown to improve glucose tolerance without eliciting any adverse effects.9,10 Published studies reporting a beneficial metabolic effect of resveratrol have been performed in participants with either impaired insulin sensitivity, glucose intolerance, type 2 diabetes mellitus, and dyslipidemia,9,10,11,12,13,14,15 with no effect seen in a study in lean healthy women.16 Therefore, we studied TRL production in overweight and obese men with mild hypertriglyceridemia (TG>1.6 mmol/L), a group more likely to respond to resveratrol.

We have previously demonstrated that hyperglycagomina can reduce both the PR and FCR of apoB-100 without net changes in TRL–apoB-100 concentration,27 an effect akin to that seen in this study. However, plasma glucagon concentrations were similar in both treatment groups (data not shown), ruling out hyperglycagomina as a cause for reduced apoB-100 PR and FCR. An alternative possibility is that resveratrol reduces apoC-III concentration, which could in turn reduce lipoprotein lipase activity. However, we saw no differences in plasma or TRL–apoC-III concentrations between treatment groups (data not shown). Another plausible mechanism for the decrease in FCR of apoB-100, but not apoB-48, could be a reduced conversion of VLDL to intermediate-density lipoprotein and LDL with resveratrol treatment. Because the current lipoprotein turnover was only of 10 hours duration, it was not possible to assess the conversion rates of VLDL to intermediate-density lipoprotein and subsequently to LDL. Future studies with longer stable isotope infusion and sampling are needed to examine the effects of resveratrol on apoB-100 kinetics in the intermediate-density lipoprotein and LDL fractions. Altered apoB-48 composition could also affect clearance of apoB-48 because they share saturable common clearance pathways.2 Because there was reduced apoB-48 production (with a trend toward reduced concentration) with no change in TRL–TG, it is possible that the apoB-48 particles are enriched with triglyceride with resveratrol treatment. Because we did not separate the chylomicron fraction with our ultracentrifugation method, it was not possible to measure triglyceride:apoB-48 ratios in chylomicrons, per se. However, there was a significant difference in the mean TG:apoB-48 ratio in the entire TRL fraction (placebo 0.24±0.02 versus resveratrol 0.31±0.04; P=0.02; Figure ID in the online-only Data Supplement), with no differences in TG:B100 ratio (data not shown). Therefore, the exact mechanism(s) by which resveratrol reduces apoB-100 FCR remains unknown and warrants further study.

Our results might indicate that the effects of resveratrol on apoB-100 and apoB-48 production were independent of plasma triglyceride concentration and possibly change in insulin sensitivity (as measured by HOMA-IR, a surrogate measure of insulin resistance). Although we, like others,1,2,3,10 have seen no significant effects of resveratrol treatment on insulin sensitivity, it is not possible to conclusively rule out an effect of resveratrol on insulin sensitivity as reported in murine5,17 and some human studies for several reasons. First, these human studies, including ours, have had small sample sizes, which may have limited the power to detect a statistical significance in HOMA-IR/insulin sensitivity. Second, our treatment duration was shorter (2 weeks) than those of Brasnyó et al13 (4 weeks) and Timmers et al14 (30 days). In addition, our participants were relatively insulin sensitive (based on HOMA-IR measurement) compared with those of Timmers et al14 and had normal glucose tolerance in contrast to the participants with type 2 diabetes mellitus in the study by Brasnyó et al.13 Finally, the pharmacodynamics of resveratrol is not well understood. Beneficial effects of resveratrol on insulin sensitivity have been reported with much lower doses of resveratrol ranging from 103 to 150 mg daily.18 A recent murine study also reported greater effects of resveratrol on hepatic steatosis and adiposity at lower doses compared with higher doses.24 Interestingly, Timmers et al14 reported an improvement in liver function tests with a daily dose of 150 mg/d of resveratrol administered during 30 days. We did not see any significant improvement in liver function tests (data not shown) with higher doses of resveratrol, although this may be explained by the shorter duration of the study. Whether this apparent inverse dose–response relationship holds true in humans remains to be seen. In keeping with our findings, Poulsen et al,2 who also used a high dose of resveratrol (1500 mg daily) but for a longer duration (4 weeks) in a more insulin-resistant cohort, found no effects on insulin sensitivity as measured by hyperinsulinemic-euglycemic clamps.
a more direct measure of insulin sensitivity compared with HOMA-IR measurement. Therefore, the reduction in TRL apoB-48 and apoB-100 PR in our study might be attributed to mechanisms that are not directly because of modulation of insulin sensitivity. Our study also suggests that the effects of resveratrol on apoB-100 and apoB-48 particle production are independent of changes in plasma and TRL triglyceride concentration. Consistent with our findings, Poulsen et al.\textsuperscript{12} using a similar dose of resveratrol (1500 mg daily) administered for 4 weeks did not detect a change in plasma triglyceride concentration in obese insulin-resistant men. In contrast Timmers et al.\textsuperscript{14} reported that resveratrol, administered at a lower dose of 150 mg daily to obese insulin-resistant men for 4 weeks, lowered plasma triglyceride concentration modestly. The precise reasons(s) for this discrepancy between these studies is unclear but, as outlined earlier, possible explanations are the small sample sizes, differing duration, and doses of resveratrol administered in these studies. In addition, these studies have not assessed apoB-100 and apoB-48 particle production or TRL apoB-100 and TRL apoB-48 concentration. Although decreased PRs of TRL apoB-100 and apoB-48 were observed in our study despite a shorter duration of treatment compared with other studies,\textsuperscript{14} it is possible that significant effects of treatment on HOMA-IR and TRL apoB-48 concentration may have been revealed with prolonged treatment.

The exact molecular mechanism(s) of action of resveratrol in reducing apoB production remains unclear. One possible mechanism may be related to the capability of resveratrol to activate AMPK and SIRT1. In vitro and murine in vivo studies have reported that resveratrol activates AMPK, which can in turn activate SIRT1.\textsuperscript{17,18} AMPK can phosphorylate PGC-1α and reduce the expression of acetyl-CoA carboxylase with an increase in lipid oxidation in liver and reduction in hepatic steatosis.\textsuperscript{19} This would result in reduced substrate for VLDL TG synthesis and VLDL apoB secretion. More recently, AMPK activation has also been reported to reduce intestinal apoB-48 production.\textsuperscript{19} AMPK can also affect lipogenesis by phosphorylating SREBP-1c reducing its transcriptional activity.\textsuperscript{18} SIRT1 activation is also implicated in amelioration of dyslipidemia.\textsuperscript{15} Resveratrol can reduce SREBP-1c expression in HepG2 cells by activating SIRT1-FoxO1 signaling,\textsuperscript{9} which could also potentially reduce VLDL production. This signaling pathway also plays a role in chylomicron production.\textsuperscript{32} However, some human studies, including the study by Poulsen et al.\textsuperscript{12} in which a similar dose of resveratrol was administered, found no effect of resveratrol on AMPK expression or phosphorylation and SIRT1 expression or activity in skeletal muscle and adipose tissue.\textsuperscript{12,13} Therefore, it is possible that these effects of resveratrol may be mediated by as yet unidentified mechanism(s).

The clinical relevance of these effects of high-dose resveratrol on apoB production, in a small cohort of mildly hypertriglyceridemic individuals, remains to be established. Because there was no net change in apoB-100 TRL concentration attributable to the reduced apoB-100 FCR, the reduced apoB-100 PR is unlikely to be clinically significant. However, a recent study performed in hypercholesterolemic patients on statins demonstrated that 6 months of daily treatment with grape extract containing a low dose of resveratrol (8 mg) resulted in a modest reduction in fasting plasma apoB concentration.\textsuperscript{13} In our study, there was a trend toward a lower apoB-48 TRL concentration with resveratrol treatment. The absence of a statistically significant reduction in apoB-48 TRL concentration may be because of the small sample size, short duration of treatment, and the mild hypertriglyceridemia seen in our study participants. Because of the small sample size, one cannot rule out the possibility of resveratrol significantly reducing apoB-48 FCR, with no net effect on apoB-48 concentration. A longer study, in a larger cohort with moderate hypertriglyceridemia (although resveratrol does not affect plasma triglyceride concentration, these individuals also have moderately increased apoB-100 and 48 concentration), is needed to establish whether high-dose resveratrol treatment lowers apoB-48 TRL concentration. Elevated apoB-48 TRL concentrations are seen in postprandial hypertriglyceridemia, which is associated with an increased risk of atherosclerosis.\textsuperscript{2} Lowering of apoB-48 TRL concentration may be beneficial in lowering atherosclerotic risk. Direct benefits of resveratrol on atherosclerotic risk reduction have yet to be established.

In summary, this proof-of-principle study has demonstrated that short-term high-dose resveratrol administration in overweight and obese men with mild hypertriglyceridemia significantly reduces apoB-100 and apoB-48 production. Long-term studies in larger cohorts are needed to establish whether resveratrol treatment is beneficial in the treatment of hypertriglyceridemia.

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Disclosures
None.

References


**Significance**

This to our knowledge is the first study assessing the effects of resveratrol on lipoprotein kinetics. High-dose resveratrol reduces hepatic and intestinal lipoprotein production in healthy individuals with mild hypertriglyceridemia. Intriguingly, this effect is independent of changes in triglyceride concentration, plasma glucose levels, and possibly insulin sensitivity. This suggests that the potential effects of resveratrol on human metabolism may extend beyond alterations in glucose tolerance and possibly insulin sensitivity.
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Fasting TRL-cholesterol (A), plasma cholesterol (B) and HDL cholesterol (C). Mean TRL-TG: apoB-48 ratio during the kinetic study (D). Placebo, white bars; resveratrol, black bars. * p=0.02
Model fits (solid lines) to the apoB-100 and apoB-48 TTR using SAAM II software (version 1.2, University of Washington, Seattle, WA). Placebo, square; Resveratrol, triangle.
Supplementary Figure III

Plasma free fatty acids during the lipoprotein kinetic study. Placebo, open symbol and dotted line; resveratrol, solid symbol and solid line.
Research Design and Methods

Participants

8 overweight or obese individuals (BMI range 27-40) with mild to moderate hypertriglyceridemia (TG >1.5, range 1.6-3.9 mmol/l) were recruited via advertisements in the local press. Their demographic and biochemical parameters are shown in Table 1. Participants had no prior medical illnesses and were taking no medications. They underwent a 2-hour, 75 gram oral glucose tolerance test, routine screening blood tests and urinalysis. Those with evidence of diabetes, anemia, coagulopathy, renal dysfunction or impaired liver function tests were excluded. The Human Research Ethics Board of the University Health Network, University of Toronto, approved the study and all subjects gave written, informed consent prior to their participation.

Each participant was studied on 2 occasions, 4-6 weeks apart, in a randomized, double blind, crossover trial. They were randomized to receive two weeks of placebo or resveratrol (Transmax, Biotivia Longevity Biologicals, New York, NY) [1g/d (500mg twice per day) for one week, followed by 2g/d (1g twice per day) for the second week] preceding the lipoprotein kinetics study. Each participant was reviewed after one week of treatment to ensure there were no adverse effects and to ensure compliance by pill counting.

Lipoprotein kinetic studies

Participants were admitted to the Metabolic Test Centre of the Toronto General Hospital after two weeks of treatment. Fasting blood samples were taken for measurement of plasma glucose, insulin and lipids. They had a standardized meal comprising rice, chicken, vegetables and jell-o at 5pm on both occasions. The following day a lipoprotein kinetics
study was carried out (Figure 1a). As apoB-48 concentrations in the fasting state are too low to allow accurate assessment of isotopic TTR (tracer to trace ratio), volunteers were studied in the constant fed state with hourly ingestion of a liquid formula (Great Shake, Hormel Health Labs, Austin, MN; 13% protein, 38% carbohydrate and 49% fat by caloric content) from 5am until the end of the study. The amount of formula ingested by each volunteer was determined by calculating the total daily caloric requirement, as determined by the Harris- Benedict equation, and dividing that into equal hourly aliquots as described previously\(^1\). At 8am, a primed constant infusion (10 μmol/kg bolus followed by 10 μmol/kg/h) of L-[5,5,5\(^2\)H\(_3\)]-leucine (d3-leucine; Cambridge Isotope Laboratories, Andover, MA) was started and continued for 10 hours for assessment of lipoprotein kinetics. Blood samples were collected at 0.5, 1, 2, 3, 4, 5, 7, 8, 9 and 10 h thereafter for isolation of TRL, stable isotope TTR and kinetic analysis. Blood samples for TG, FFA and hormone analysis were collected at regular intervals.

**Laboratory Methods**

Plasma was separated from blood samples, within 2 hours, in a refrigerated centrifuge at 3000 rpm for 15 min at 4º C. Tetrahydrolipstatin (THL, Roche), sodium azide (Sigma Aldrich) and aprotinin(Sigma Aldrich) were added to the plasma to prevent hydrolysis and protein degradation at the following concentrations: THL (0.55 mg/l blood), sodium azide (70 mg/l blood) and aprotinin (1.94 mg/l blood). Triglyceride-rich lipoproteins (TRL) were isolated at each time point at d=1.006 for 16 hrs, 39,000 rpm at 12º C. The TRL fraction thus corresponded to an Sf of >20, which includes lipoprotein fractions of chylomicrons, VLDL and IDL.

Aliquots of TRL fractions (approx. 1mg protein) were delipidated and separated by
preparative 3.3% SDS-PAGE. Gel bands corresponding to apoB-48 and apoB-100 were excised, hydrolyzed and amino acids derivatized to allow for the determination of TTR as described \(^1\). Plasma free amino acids were extracted, dried, derivatized and stable isotope TTR determined as previously described \(^1\). Derivatized samples were analyzed with GC/MS (Agilent 5975/6890N, Agilent Technologies Canada Inc, Mississauga, ON, Canada) with electron impact ionization using helium as the carrier gas. Selective ion monitoring at m/z =200 and 203 was performed and TTR were calculated from isotopic ratios for each sample according to a standard curve of isotopic TTR.

Commercial kits were used to measure cholesterol (Roche Diagnostics, Mannheim, Germany), TG (Roche Diagnostics), FFA (Wako Industrials, Osaka, Japan), insulin (Millipore, Billerica, MA, USA) and glucagon (Millipore). TRL apoB-100 and apoB-48 mass were measured with ELISA kits specific for human apoB-100 (Mabtech Inc, Mariemont, OH, USA; intra-assay CV = 2%, inter-assay CV = 10%) and apoB-48 (Shibayagi Co. Ltd., Shibukawa, Gunma, Japan; intra-assay CV = 3.5%, inter-assay CV = 5.6%).

HOMA-IR was calculated as HOMA-IR = fasting glucose (mmol/L) x fasting insulin (mU/L)/22.5, where fasting plasma glucose and insulin concentrations were obtained the day prior to each lipoprotein kinetics study.

**Kinetic analysis**

A multi-compartmental model using SAAM II software (version 1.2, University of Washington, Seattle, WA) was fitted to stable isotope TTR curves for apoB-48 and apoB-100 to derive the fractional catabolic rates (FCR), as previously described \(^1\) (Figure 1B). The model consisted of synthesis of TRL apoB from the precursor pool via a delay compartment.
Plasma free leucine TTR, determined for each visit of each subject, was used as a forcing function and individual TTR time course curves were used to derive kinetic rate constants. Production rates (PR) of each apolipoprotein were calculated as \( PR = FCR \times \text{pool size} \), where pool size = average plasma concentration (mg/L) over the 10 hrs of the kinetic study \( \times \) plasma volume (estimated as 0.045 liter/kg body weight).

**Statistics**

Results are presented as mean ± SEM. Repeated measures ANOVA was used to compare the time course of parameters during the kinetic experiments. Paired t-test was used to compare TG, FFA, apoB-100 and apoB-48 concentrations, and FCR and PR between the two treatments. All statistics were performed with SAS (version 9, Cary, NC). A \( p \) value < 0.05 was considered significant. Assuming a power of 80% and \( \alpha \) of 0.05, the number of volunteers needed to detect a significant difference in apoB production rates is 8. This is based on the mean apoB-100 PR for placebo (47.7 SD 26.3) and resveratrol (34.27, SD 16.6), as well as the mean apoB-48 PR for placebo (1.34, SD.69) and resveratrol (1.09, SD.75).

**Reference**