No Effect of Resveratrol on VLDL-TG Kinetics and Insulin Sensitivity in Obese Men with Nonalcoholic Fatty Liver Disease

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ABSTRACT

The present study assess long-term effects of high-dose Resveratrol (RSV) on basal and insulin-mediated very low-desity lipoprotein triglyceride (VLDL-TG), palmitate and glucose kinetics, and liver fat content in men with nonalcoholic fatty liver disease (NAFLD). Participants (n=16) were non-diabetic, upper-body obese (BMI >28 kg/m^2, WHR >0.9) men with NAFLD randomized (1:1) in a double-blinded, placebo-controlled clinical trial to either RSV or placebo (500 mg 3 times daily) for 6 months. Magnetic resonance (MR) spectroscopy, dual-X-ray absorptiometry and MR imaging assessed liver fat content and body composition, respectively. ^{14}C-labeled VLDL-TG and ^{3}H-labeled glucose and palmitate tracers in combination with indirect calorimetry and breath samples were used to assess kinetics and substrate oxidations during basal and hyperinsulinemic euglycaemic clamp conditions. RSV did not improve neither basal nor insulin-mediated VLDL-TG secretion, oxidation or clearance rates, nor did it affect palmitate or glucose turnover. Likewise, no changes in body composition or liver fat content occurred following RSV compared with placebo treatment. RSV can therefore not be recommended for treatment of metabolic abnormalities in NAFLD. NCT01446276, ClinicalTrials.gov.

INTRODUCTION:

Obesity has escalated with a concomitant rise in nonalcoholic fatty liver disease (NAFLD) and cardiovascular disease regardless of diabetes status [1]. NAFLD is associated with glucose and lipid metabolic abnormalities including increased fasting and reduced insulin suppression of hepatic VLDL-TG and apolipoprotein B-100 (apoB-100) secretion [2, 3]. Moreover, fasting VLDL-TG secretion rate increases linearly with intrahepatic TG (IHTG) content in subjects without NAFLD but plateaus already at modest increases in IHTG [2]. Likewise, we reported that obese men with NAFLD exhibit reduced insulin suppression of VLDL-TG and free fatty acid (FFA) kinetics as well as impaired insulin-stimulated glucose disposal (Rd) compared with men without NAFLD [3].

Resveratrol (RSV) is a naturally occurring polyphenol with potential anti-oxidative, anti-inflammatory, and anti-fibrotic properties, as well as favorable metabolic effects suggested to protect against hepatic steatosis in animals [4]. However, limited human, short-term (≤3 months) studies have been performed in different phenotypes (normal weight, obese) with different metabolic conditions (impaired glucose tolerance, NAFLD and type 2 diabetes), with ambiguous results. A recent trial showed 2 weeks of RSV treatment inhibits hepatic VLDL-Apo-B100 secretion during constant feeding (hyperinsulinemia and chylomicronemia) in obese men [5]. However, no studies have investigated whether RSV affects basal and insulin-mediated suppression of VLDL-TG kinetics in subjects with NAFLD.

The purpose of this study was to determine long-term effects of high-dose RSV on basal and insulin-stimulated VLDL-TG, palmitate and glucose kinetics, and IHTG content in non-diabetic, upper-body obese (UBO, BMI >28 kg/m^2, WHR >0.9) men with NAFLD. We hypothesized that RSV treatment improves insulin suppression of VLDL-TG secretion and oxidation leading to lower plasma VLDL-TG concentrations. Furthermore, that RSV

increases insulin suppression of lipolysis and improves hepatic and peripheral insulin-mediated glucose metabolism and, possibly, reduces IHTG content.

METHODS

See supplementary appendix for description of methods, statistics and kinetic parameters.

RESULTS

Subject characteristics are shown in Table S1, and hormones and metabolites are presented in Figure S3.

VLDL-TG kinetics

Basal VLDL-TG secretion and oxidation rates remained unchanged between groups with intervention (Figure 1*A* and *C*). Basal VLDL-TG clearance rate decreased ($P<0.0001$) (Figure 1*B*), whereas VLDL-TG concentration increased ($P=0.02$) (Figure 1*D*), but not significantly different between groups ($P=0.13$ and $P=0.75$). Hyperinsulinemia exerted no difference between groups on VLDL-TG secretion, oxidation and clearance rates or VLDL-TG concentration with intervention (all $P>0.45$). However, hyperinsulinemia exerted a significant, but similar suppressive effect on VLDL-TG secretion and clearance rates as well as on VLDL-TG concentration (all $P<0.005$), whereas no significant effect on VLDL-TG oxidation was noted ($P=0.16$).

VLDL-TG-to-VLDL-apoB-100 ratio

Basal and insulin-mediated VLDL-apoB-100 concentration and VLDL particle size (VLDL-TG–to-VLDL-apoB-100 ratio) was similar between groups with intervention (all $P>0.30$). Hyperinsulinemia tended to suppress apoB-100 concentration and VLDL particle size (both

P=0.06) with a larger VLDL particle size after intervention compared with before (*P*<0.0001) but the change was not significantly different between groups (*P*=0.93) (Figure S4).

Glucose and palmitate kinetics

Basal and insulin-mediated glucose rate of disposal (Rd) did not differ statistically between groups with intervention (all *P*>0.75) (Figure 2*B*). However, GIR was significantly different in the groups with intervention (*P*=0.04) because of a decrease in GIR in the RSV group (*P*=0.01). Endogeous glucose production (EGP) changed significantly different in the groups with intervention (*P*=0.02) due to a change in the insulin suppression, which was significant at baseline but not after treatment in the RSV compared with the placebo (PL) group (Figure 2*A*). Basal and insulin-suppressed palmitate flux was not significantly different between groups with intervention (*P*=0.19 and *P*=0.97) (Figure 2*C*). Palmitate concentration was less suppressed by insulin after intervention (*P*=0.02) compared with before, but the change was not significantly different between groups (*P*=0.10).

Energy expenditure and substrate oxidation rates

See supplementary appendix for results.

IHTG content and body composition

IHTG content, BMI, percent fat and fat mass did not change statistically significant between groups with intervention (all *P>0.15*). However, FFM changed differently between groups with intervention (PL: 70.6 (65.4; 75.9) to 72.2 (67.0; 77.4) vs. RSV: 64.8 (59.6; 70.0) to 64.7 (59.5; 69.9), *P*=0.03) (Figure S5*A*-*F*).

DISCUSSION

This is the first long-term, placebo-controlled trial of high-dose RSV on basal and insulin-mediated VLDL-TG, palmitate and glucose kinetics in combination with changes in IHTG content in obese, non-diabetic men with NAFLD. We demonstrate the novel finding that both basal and insulin-mediated VLDL-TG secretion, clearance and oxidation rates remain unchanged following 6 months treatment with RSV (500 mg t.i.d.) compared with PL. Moreover, we report that basal and insulin-mediated palmitate and glucose turnover are also unaffected by long-term RSV treatment, and confirm that IHTG content remains unchanged. In animals, RSV is shown to protect against development of hepatic steatosis and to improve insulin sensitivity [4]. However, human studies of RSV effects on substrate kinetics in NAFLD subjects are sparse. Only one previous study by using deuterated leucine in combination with frequent feeding and mathematical modeling showed disminished hepatic and intestinal production as well as hepatic clearance, but not plasma concentration, of apoB-48 and apoB-100 in overweight/obese men with normal glucose tolerance and mild hypertriglyceridemia [5]. However, these changes are most likely of limited clinical significance. Likewise, the study was designed to assess VLDL particle, not VLDL-TG turnover, and was (by design) conducted under constant and partly dynamic insulinemia. In the present study, we used a gold-standard tracer infusion approach in combination with steady-state equations, to measure basal and insulin regulated VLDL-TG fatty acid kinetics and found no effect of RSV on neither VLDL-TG secretion, oxidation nor clearance rates. Moreover, plasma VLDL-TG and total TG concentrations were not affected. Unchanged plasma TG was also reported by others using RSV doses comparable (1500-2000 mg daily) to our study for 2-4 weeks in obese individuals [5, 6], for 6 months in NAFLD men [7] as well as in NAFLD men using even higher doses (3000 mg daily) for 8 weeks [8], while Timmers et al. found a reduction in fasting plasma TG using a lower dose (150 mg o.d.) for 4

weeks in obese, glucose tolerant men [9]. It has been speculated that the presence of reduced insulin sensitivity associated with NAFLD and obesity as compared with normal glucose tolerance may abate the effect of RSV on VLDL-TG kinetics and concentration [5]. Therefore, studies in subjects with less severe insulin resistance, such as moderate overweight are therefore warranted to unravel the potential effects on both VLDL particle and VLDL-TG kinetics.

The effect of RSV on lipolysis has been sparsely studied. We observed no effect of RSV on basal or insulin-suppressed palmitate flux. Our findings therefore extend previous short-term (8 weeks) findings of an unchanged adipose tissue insulin resistance index (AT-IR) in overweight/obese men with NAFLD [8] to also include long-term neutral effects. We found only one other publication using fatty acid tracer to measure lipolysis during RSV treatment showing similar palmitate flux in non-obese, glucose tolerant women [10] treated with RSV (75 mg daily) vs. PL. Using microdialysis and the same RSV dose for 4 weeks in obese/overweight men, Timmers et al. found greater postprandial suppression of adipose tissue glycerol concentration after RSV treatment. Both fasting adipose glycerol and plasma FFA concentrations were, however, similar [9]. Collectively, the results of the present and previous studies strongly indicate that RSV has limited or even no effect on systemic lipolysis. Whether subtle effects on regional lipolysis occur remains, however, to be fully elucidated.

The effect of RSV on glucose turnover has been addressed in a number of studies, however only a few in established NAFLD subjects. Moreover, most studies used the HOMA index, an indirect assessment of insulin resistance. We found no significant effect on basal or insulin-stimulated glucose Rd in our NAFLD male subjects following RSV. Insulin-stimulated glucose Rd was also similar in the study by Chachay et al. following 8 weeks treatment of overweight/obese men with confirmed NAFLD [8]. Our results extend these

findings to also include EGP both basally and during insulin suppression. A slightly greater effect of RSV compared with PL on insulin suppression of EGP was noted in our study. The difference is, however, most likely explained by the "regression to the mean" phenomenon, as the baseline difference was abated after the intervention period. Moreover, both fasting EGP and plasma glucose were similar both before and after 6 months intervention. Basal and insulin-stimulated EGP and glucose Rd were also similar in non-obese women with normal glucose tolerance [10] and in obese men [6] following 4 weeks RSV vs. PL. Other studies using the HOMA model have shown both unchanged [5, 7, 11] and improved [9, 12, 13] insulin sensitivity in type 2 diabetic [12], and overweight/obese [5, 7, 9, 11, 13] subjects. However, only two of these studies were performed in subjects with confirmed NAFLD, with both studies showing no effect on HOMA [7, 11].

Finally, we found no effect of RSV on IHTG content, which supports recent data from our group showing the same results in non-diabetic subjects with biopsy proven NAFLD also treated with RSV for 6 months [7]. In addition, similar findings on IHTG content were reported by Chachay et al. after 8 weeks treatment of men with confirmed NAFLD [8]. Conversely, in subjects with IHTG content well within the normal range a reduction of IHTG content was reported by Timmers et al [9].

Our study has limitations. The relatively low number of participants may introduce type 2 errors. However, power analysis was performed before the study to ensure inclusion of a sufficient number of volunteers. Moreover, since numerous studies have shown clear sex differences in lipid and FFA metabolism we studied only men [14]. Thus, the results cannot be extended to women. Likewise, tracer prepared from fasting VLDL-TG particles may not be representative to VLDL-TG particle composition during hyperinsulinemia. However, Lewis et al. [15] found no difference in VLDL-apoB turnover with this approach. Whether the RSV dose is optimal is difficult to determine. Doses ranging from 10-3000 mg per day

have been published and, as outlined above, studies using low, medium and high doses have demonstrated both positive and no effects on insulin sensitivity. We used a high-dose RSV compound, which has previously been shown to give relevant absorption and plasma concentrations [6]. In addition, the dose was similar to that used by Dash et al. to demonstrate effects on VLDL-apoB-100 [5]. Whether a more optimal dose exists is not clear and it is possible that severity of disease status (insulin sensitive vs. insulin resistance) also impacts on the potential RSV effects. Finally, NAFLD was determined by MRS. Therefore, we do not know whether any of the participants had nonalcoholic steatohepatitis, which could impact our results.

In conclusion, long-term, high-dose RSV treatment of obese, non-diabetic men with NAFLD does not affect basal or insulin-mediated VLDL-TG, palmitate and glucose kinetics or IHTG content. Whether RSV offers protective effects against development of NAFLD if initiated at stages of less severe insulin resistance deserves further investigation.

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FIGURE LEGENDS

Fig. 1: VLDL-TG metabolism

VLDL-TG secretions (*A*), clearances (*B*), oxidations (*C*), and concentrations (*D*) in the basal and clamp steady-state periods (150-180 min and 390-420 min) before and after intervention with either PL or RSV. Data are presented as mean with 95% CI. Three-way RM-ANOVA tested the insulin-suppressive effect on the different parametres. Post-hoc analysis were made on the simplest model.

Fig. 2: Glucose and free fatty acid metabolism

Endogenous glucose production (EGP) (*A*), glucose disposal rate (Rd) (*B*), and palmitate flux(*C*) in the basal and clamp steady-state periods (150-180 min and 390-420 min) before and after intervention with either PL or RSV. Data are presented as mean with 95% CI. Three-way RM-ANOVA tested the insulin-stimulated effect on the different parametres. Post-hoc analysis were made on the simplest model.