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Effects of Resveratrol Supplementation on Liver Fat Content in overweight and insulin resistant Subjects: A randomized, double-blind and placebo-controlled Clinical Trial

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Abstract

We performed the so far largest randomized and placebo-controlled clinical trial (N=112, 12 week intervention) to investigate effects and safety of resveratrol supplementation on liver fat content and cardiometabolic risk parameters in overweight and obese and insulin resistant subjects.

At baseline the variability in liver fat content was very large, ranging from 0.09% to 37.55% (median 7.12%, interquartile range 3.85%−12.94%). The mean (SD) liver fat content was 9.22 (6.85)% in the placebo group and 9.91 (7.76) in the resveratrol group. During the study liver fat content decreased in the placebo (−0.7%) but not in the resveratrol group (−0.03%; differences between groups: p=0.018 for the intention to treat [ITT, N=54 resveratrol, N=54 placebo] and p=0.0077 for the per protocol [PP] population). No effects of resveratrol supplementation on cardiometabolic risk parameters were observed. Resveratrol supplementation was well tolerated and safe.

In conclusion, these data suggest that resveratrol supplementation is safe and that it does not considerably impact on liver fat content or cardiometabolic risk parameters in humans.

Introduction

Nonalcoholic fatty liver disease (NAFLD) strongly associates with progressive hepatic disease and is also involved in the pathogenesis of cardiometabolic diseases (1,2). Lifestyle intervention is an effective and safe strategy for reducing liver fat content and for improving hepatic inflammation and glucose metabolism. However, while in most subjects liver fat content decreases, e.g. with increased exercise (3), in some individuals hepatic steatosis and insulin resistance do not improve, although an adequate reduction of total- and visceral fat mass is observed (4). Thus, the question is what pharmacological intervention may be both, effective and safe, to treat NAFLD.

In this respect the plant polyphenol resveratrol, which is found in grape skins, red wine, all kinds of berries and peanuts and which was identified as an activator of sirtuin-1 (SIRT-1), is an interesting candidate (5). While data from rodent models of obesity and NAFLD provided support for a favorable effect of resveratrol supplementation on glucose and lipid metabolism (5), such data from studies in humans are inconclusive. This may result from a relatively small number of participants in these studies, the different metabolic characteristics of the subjects and the different doses and durations of the resveratrol supplementation that were used (6-9). Of note in these studies treatment duration ranged from 4 to 12 weeks and the number of subjects included ranged from 11 to 50.

Therefore, the primary aim of this study was to assess whether resveratrol treatment for 12 weeks in the so far largest randomized, double-blind, placebo-controlled clinical trial is effective in reducing hepatic steatosis in insulin resistant subjects with a broad range of liver fat content. Secondary aims were to

investigate whether this treatment reduces insulin resistance, total body- and particularly visceral fat mass and cardiometabolic risk parameters, and whether it is safe and well tolerated.

Research Design and Methods

Study design and participants

This monocentric, 12 weeks, randomized, double-blind, placebo-controlled trial was conducted at the Department of Internal Medicine IV of the University Hospital Tübingen, Germany. All study subjects gave their written informed consent prior to any study procedure and the study protocol was approved by the Ethical Committee of the University of Tübingen. The study was conducted in accordance with the principles of the "Declaration of Helsinki" and Good Clinical Practice.

Men and women were eligible for the study if 1) their age was between 18 and 70 years; 2) they had a BMI >= 27 $kg \cdot m^{-2}$ and 3) a homeostatic model assessment-insulin resistance (HOMA-IR) >=2.0. The other main inclusion and exclusion criteria are shown in the supplementary appendix.

Randomisation, masking and measurements

Study subjects were randomly allocated to two oral doses of 75 mg resveratrol (total daily dose of 150 mg) per day or matching placebo. Resveratrol and placebo capsules were provided by DSM Nutritional Products Ltd., Kaiseraugst, Switzerland as resVida® capsules containing 99.7% trans-resveratrol. To make our clinical trial comparable to the other, smaller, clinical trials that used resveratrol supplemented as resVida® in the dosage of 75 mg/day (7) or 150 mg/day (6,9), we used the 150 mg/day dose. Detailed information about the measurements is given in the supplementary appendix. In brief liver fat content was measured by localized ^{1}H-MR spectroscopy and the distribution of lean and

adipose tissues was measured by whole body MR imaging as previously described (10). The Matsuda insulin sensitivity index was measured during a frequently sampled 2 hours oral glucose tolerance test (OGTT). In addition, anthropometrics, cardiorespiratory fitness (VO_{2max}), carotid intima-media thickness (cIMT), dietary intake were measured and safety assessments were done (supplementary appendix).

Outcomes

The primary efficacy endpoint was the change of liver fat content measured in percent from baseline to the end of the intervention period.

Secondary efficacy endpoints were: 1) the fold change from baseline in absolute liver fat content 2) the status of liver fat content in subjects with NAFLD 3) the changes of total-, visceral- and subcutaneous abdominal fat mass, 4) changes in the metabolic parameters fasting and 120 min glucose, HbA1c, HOMA-IR, Matsuda insulin sensitivity index and, 5) the changes of the cardiovascular parameters, cIMT and cardiorespiratory fitness.

The exploratory endpoints are summarized in the supplementary appendix.

Statistical analysis

For the primary efficacy endpoint an analysis of covariance (ANCOVA) with 'treatment (placebo, resveratrol)' and 'gender (male, female)' as fixed factors and 'baseline liver fat' and 'age' as continuous covariates was pre-specified in a statistical analysis plan. 'Age' and 'gender' were chosen as covariates because they are strong determinants of liver fat content and glucose metabolism. For all

other endpoints with only two measurements (baseline and final visit) the same analysis strategy (linear model on the change between measurements with the baseline measurement, sex and age as adjusting covariate) was planned to be applied, as for the primary endpoint. Furthermore, an additional post-hoc ANCOVA was applied including the interaction of baseline liver fat with treatment as covariate. Details about the statistical analyses in the intention to treat (ITT) and per protocol (PP) populations are given in the appendix.

The trial is registered with ClinicalTrials.gov, number NCT01635114.

Results

This clinical trial was conducted between July 3, 2012, and October 30, 2016. The trial profile is given in the supplementary figure 1.

Baseline demographic and metabolic characteristics were well balanced between the treatment groups (supplementary table 1). During the study the primary endpoint liver fat content somewhat decreased in the placebo group (−0.7%), but did not change in the resveratrol group (−0.03%) (table, figure). The difference between the groups in change of absolute liver fat content was statistically significant, both in the ITT (p=0.018) and in the PP (p=0.0077) populations. These results were not affected when in *post hoc* analyses other parameters possibly influencing liver fat content, such as total body fat mass at baseline and at follow-up, which did not change significantly between both groups (table), were additionally included in the statistical models (p=0.025 for the ITT population and p=0.008 for the PP

population). In respect to the secondary outcome measures no differences in the changes of the metabolic parameters HbA1c, HOMA-IR, ISI_{OGTT}, blood pressure, cIMT and cardiorespiratory fitness were found. In addition, no differences between the changes of the secondary exploratory efficacy results were found (table 1). Finally, the SNPs rs2273773, rs12413112 and rs7069102 of the sirtuin-1 gene did not interact with the treatment on the change of liver fat content (data not shown).

Based on the fact that in our study the variability in liver fat content at baseline was very large, ranging from 0.09% to 37.55% (median 7.12%, interquartile range 3.85%−12.94%), we then tested in a *post hoc* analysis the heterogeneity of treatment effects across the baseline levels of liver fat content. For this we additionally included the interaction term liver fat content at baseline*treatment in the multivariate model that was used for the analysis of the primary endpoint. This interaction term proved to be significant in the PP population (p=0.026), but not in the ITT population (p=0.168). When plotting the adjusted baseline liver fat content vs the adjusted liver fat content at the end of the treatment it became obvious that in subjects with a low or a moderately elevated liver fat content at baseline resveratrol treatment associated with no change of liver fat content. Instead, in subjects with a very high liver fat content at baseline resveratrol treatment associated with a lower liver fat content at the end of the treatment (supplementary figure 2). The estimated regression lines (adjusted for sex and age) for both treatments cross at a liver fat content of 19% (supplementary figure 2), indicating that with an initial liver fat content above that threshold, resveratrol supplementation appears to be associated with a decrease of liver fat content. This threshold for high liver fat content is in agreement

with data from Maximos *et al.*, (11) showing that subjects with NAFLD and a mean liver fat content of 18% did not have elevated ALT levels (20.5% for NASH patients, respectively).

With respect to compliance we found that with a compliance of 97.23% (96.59% for placebo and 97.85% for resveratrol) subjects very well followed the recommendations given during the trial (for details see the supplementary appendix). With respect to safety statistical analyses of longitudinal data of safety end-points did not point to a safety issue for treatment with resveratrol (all p values for differences in blood glucose levels, liver enzymes, blood pressure and heart rate e0.39 between the placebo and the resveratrol groups). For details see the supplementary appendix.

Discussion

We found a small decrease of liver fat content in the subjects supplemented with placebo and no change of liver fat content in the subjects supplemented with resveratrol. The difference between the groups in change of absolute liver fat content was statistically significant, which may indicate that resveratrol supplementation may prevent from an improvement of liver fat content during a placebo-controlled clinical trial. However, when investigating the effects of treatment with the 11²-hydroxysteroid dehydrogenase type 1 inhibitor RO5093151 or placebo on liver fat content in a very similar clinical trial with the same behavioral recommendations we did not observe a decrease of liver fat content in the placebo group (12). Thus, in the present clinical trial a decrease of liver fat content in the placebo group was not expected. Consequently, we cannot conclude that resveratrol supplementation may prevent from a decrease of liver fat content.

Having observed a large variability in the baseline liver fat content in our study, we then asked whether it may be necessary to account for this variability in our analyses. Our *post hoc* analyses indicate that in subjects with a very high liver fat content at baseline resveratrol treatment associated with a lower liver fat content (figure 2) at the end of the treatment in the PP population, but not in the ITT population. Our data appear to be in agreement with recent findings from another study showing that 12 weeks supplementation with a daily dose of 500 mg resveratrol reduced liver fat content, measured by ultrasonography and ALT levels, in 50 subjects with NAFLD (13). On the other hand, in a recent meta-analysis of four studies with a total of 158 patients allocated to resveratrol or placebo no clear benefit

of resveratrol on liver enzymes or parameters of glucose metabolism was observed (14). Thus it remains unclear whether resveratrol may have beneficial effects in patients with NASH and significant fibrosis.

In respect to glucose metabolism, we did not find an effect of resveratrol supplementation on insulin sensitivity.

Furthermore, because resveratrol was shown to improve endothelial function, to inhibit aortic smooth muscle cell proliferation and to improve muscle mitochondrial respiration (15,16), we investigated whether resveratrol supplementation may affect early atherosclerosis and an estimate of mitochondrial function. We did not find an effect of resveratrol supplementation on cIMT, an early marker of atherosclerosis or on cardiorespiratory fitness, which we precisely measured by determining the VO_{2max} on a cycle ergometer.

Our study has limitations. We did not perform tissue biopsies and, thereby, may have missed effects of resveratrol supplementation on the molecular level. However, we chose to focus on precise clinical phenotyping of glucose and lipid metabolism and in the same study on early markers of atherosclerosis and cardiorespiratory fitness. Furthermore, we were able to include a relatively large number of subjects in our study. We only studied relatively healthy Caucasians and cannot conclude about effects of a 12 week resveratrol treatment in other ethnic groups or in patients with advanced cardiometabolic diseases. Finally, our 12 week duration of resveratrol supplementation was rather short and we have no

information whether a longer period of resveratrol supplementation may reveal different effects. Nevertheless, we found that resveratrol treatment was very well tolerated, that the subjects were very compliant with the supplementation of the products and that resveratrol supplementation had a good safety profile.

In conclusion, these data suggest that resveratrol supplementation is safe and that it does not impact on liver fat content or cardiometabolic risk parameters in humans.

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Conflict of interest

Iris Kunz and Rotraut Schoop are employees of DSM Nutritional Products, Ltd. All other authors declare that they have no conflicts of interest.

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Table Primary, secondary and exploratory outcomes (intention to treat population).

	Placebo		Resveratrol		p
	Baseline (N=54)	**Week 12 (N=52)**	**Baseline (N=54)**	**Week 12 (N=53)**	
Primary endpoint					
Liver fat content (%)	9.22 (6.85) N=54	8.61 (6.61) N=52	9.91 (7.76) N=53	9.83 (6.91) N=53	0.018
Secondary endpoints					
NAFLD (N)	31	30	33	35	1*
TAT (mL)	42.85 (12.69) N=54	43.15 (12.46) N=51	42.71 (9.94) N=54	42.95 (9.75) N=53	0.57
VAT (mL)	5.61 (2.34) N=54	5.81 (2.5) N=52	5.22 (2.64) N=52	5.29 (2.77) N=53	0.52
SCAT (mL)	15.85 (5.58) N=54	15.92 (5.54) N=51	16.32 (5.21) N=54	16.22 (4.82) N=53	0.831
HbA_{1c} (%)	5.57 (0.44)	5.61 (0.44)	5.58 (0.4)	5.65 (0.42)	0.62
HbA_{1c} (mmol/mol)	37.28 (4.8)	37.81 (4.79)	37.43 (4.46)	38.11 (4.65)	0.62
HOMA-IR	3.97 (2.5–6.07)	4.05 (3.22– 6.01)	3.46 (2.56–4.37)	3.84 (2.85–4.81)	0.98
ISI_{OGTT} (arb. units)	5.44 (3.54–8.41)	5.66 (3.49–8.23)	6.05 (4.5 –8.7)	5.34 (4.02–7.98)	0.36
Fasting glucose (mmol/L)	5.51 (0.54)	5.51 (0.64)	5.31 (0.6)	5.4 (0.61)	0.63

120 min glucose (mmol/L)	7.16 (1.99)	6.89 (2.29)	6.78 (1.82)	6.81 (1.92)	0.4
cIMT (mm)	0.67 (0.11)	0.65 (0.11)	0.69 (0.13)	0.65 (0.13)	0.61
VO_{2max} (mL/min/kg)	1.71 (0.5) N=54	1.67 (0.52) N=49	1.69 (0.43) N=54	1.68 (0.41) N=53	0.15
Exploratory efficacy endpoints					
Systolic blood pressure (mmHg)	139 (16)	139 (15)	136 (16)	135 (11)	0.40
Diastolic blood pressure (mmHg)	88 (12)	88 (9)	88 (10)	86 (10)	0.42
Total cholesterol (mg/dL)	200 (38)	198 (41)	201 (40)	200 (41)	0.14
HDL cholesterol (mg/dL)	46 (7)	48 (9)	50 (13)	51 (13)	0.54
LDL cholesterol (mg/dL)	116 (31)	115 (31)	110 (30)	112 (33)	0.34
...iglycerides (mg/dL)	152 (104)	132 (64)	176 (249)	153 (94)	0.057
AUC of FFA (µmol/L)	549 (216)	598 (290)	546 (177)	585 (234)	0.95
AST (U/L)	23.91 (9.68)	25.65 (10.76)	23.72 (7.01)	25.34 (7.7)	0.83

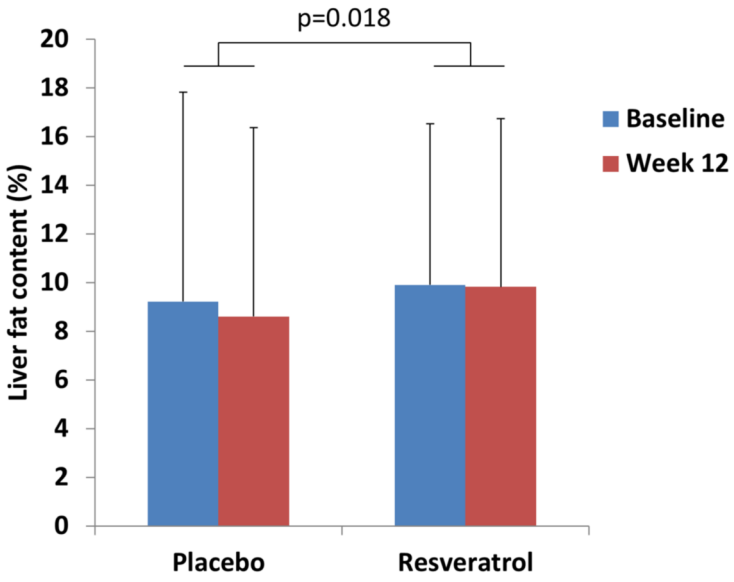
ALT (U/L)	35.59 (17.35)	36.54 (23.49)	32.98 (14.14)	36.09 (15.83)	0.64
GGT (U/L)	38.13 (29.59)	35.19 (32)	32.48 (24.82)	31.28 (22.15)	0.25
hsCRP (mg/dL)	0.28 (0.37)	0.29 (0.4)	0.32 (0.32)	0.34 (0.36)	0.62
Adiponectin (ng/mL)	8202 (6035) N=53	8689 (5951) N=52	8278 (4573) N=54	8328 (4787) N=53	0.34
IL-6 (pg/mL)	2.3 (0.72)	2.44 (1.27)	2.15 (0.41)	2.13 (0.39)	0.44
Fetuin-A (µg/mL)	522 (129)	525 (157)	493 (131)	498 (134)	0.75
SHBG (nmol/L)	30 (22 – 39) N=48	31 (23–39) N=52	33 (23–43) N=47	33 (24–45) N=52	0.75
CK-18 M30 (U/L)	127 (112) N=53	148 (182) N=52	120 (93) N=54	133 (103) N=53	0.34

Data are unadjusted (raw) mean (SD) or median (interquartile range) depending on the skewness of the data. ANCOVA models were applied with the 12 week measurement as dependent variable and with the baseline measurement, sex and age as adjusting covariate and treatment group as explaining variable. P value is based on (e.g. 1 = log10(x+1) – Transformation, 2 = log10(x) Transformation, 3 = Rank transformation). * P value Fisher's Exact Test for favorable responders (NAFLD to No-NAFLD or no change in NAFLD status). TAT, total adipose tissue; VAT, visceral adipose tissue; SCAT, subcutaneous adipose tissue; HbA1c, hemoglobin A1c; HOMA-IR, homeostatic model assessment-insulin resistance; ISI_{OGTT}, insulin sensitivity index measured during the oral glucose tolerance test; cIMT, carotid intima-media thickness; VO_{2max}, cardiorespiratory fitness; FFA, free fatty acids; AST,

aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; hsCRP; high-sensitivity C-reactive protein; IL-6, interleukin 6; SHBG, sex hormone-binding globuline; CK-18, cytokeratin-18

Figure legend

Liver fat content at baseline and after 12 weeks of supplementation. Data are unadjusted means and SD in the intention to treat population. The p-value (adjusted for age, sex, liver fat content at baseline) is shown for the difference between the groups in change of absolute liver fat content between baseline and follow-up.



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