Digestive and Liver Disease xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Liver, Pancreas and Biliary Tract

Resveratrol improves insulin resistance, glucose and lipid metabolism in patients with non-alcoholic fatty liver disease: A randomized controlled trial

Shihui Chen[a], Xiaolan Zhao[b], Li Ran[a], Jing Wan[a], Xiaofang Wang[b], Yu Qin[a], Furong Shu[a], Yanxiang Gao[a], Lijia Yuan[a], Qianyong Zhang[a,*], Mantian Mi[a,*]

[a] Research Center of Nutrition and Food Safety, The Third Military Medical University, Chongqing Key Laboratory of Nutrition and Food Safety, Chongqing, China
[b] Health Care Center of Southwest Hospital, The First Affiliated Hospital of The Third Military Medical University, Chongqing, China

ARTICLE INFO

Article history:
Received 23 July 2014
Accepted 30 November 2014
Available online xxx

Keywords:
Insulin sensitivity
Metabolic syndrome
Nutrition

ABSTRACT

Background: Non-alcoholic fatty liver disease is a major health problem worldwide. Resveratrol is a natural polyphenol found in edible plants that has a variety of biochemical and physiological effects.
Aims: To evaluate the effect of resveratrol on insulin resistance, glucose and lipid metabolism in non-alcoholic fatty liver disease.
Methods: Double-blind, randomized, placebo-controlled trial: 60 subjects with non-alcoholic fatty liver disease were given 2 placebo capsules (placebo group) or 2 150 mg resveratrol capsules (resveratrol group) twice daily for three months. Liver ultrasound imaging, anthropometric profile, serum liver enzymes, insulin, glucose, C-peptide, lipid profile, and inflammation-related cytokines were compared pre and post-treatment.
Results: Compared with the placebo group, resveratrol significantly decreased aspartate aminotransferase, glucose and low-density lipoprotein cholesterol [−6.00 (−9.00, −3.00) IU/L, −0.64 ± 0.31 mmol/L, and −0.41 ± 0.35 mmol/L, respectively, $P \leq 0.001$] alanine aminotransferase, total cholesterol [−7.00 (−11.0, −2.50) IU/L and −0.67 ± 0.50 mmol/L, respectively, $P = 0.002$], and homeostasis model assessment insulin resistance index (−0.60 ± 1.15, $P = 0.016$). In the resveratrol group significant reductions of the levels of tumour necrosis factor-alpha, cytokeratin 18 fragment, and fibroblast growth factor 21 [−0.53 ± 1.30 pg/mL, −26.9 (−70.3, 5.12) IU/L and −23.3 (−43.0, 0.31) pg/mL, respectively, $P < 0.05$] and elevation of adiponectin level [1.22 (−0.37, 1.60) ng/mL, $P = 0.025$] were observed.
Conclusion: Resveratrol supplementation may benefit patients with non-alcoholic fatty liver disease.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease that encompasses a spectrum of liver injuries ranging from simple steatosis (SS) to non-alcoholic steato-hepatitis (NASH) [1]. Observational studies of the natural history of NAFLD have shown that NAFLD increases not only all-cause and liver-related mortality but also the risk of certain malignancies, type 2 diabetes, postoperative complications after liver surgery, cardiovascular disease (CVD), and chronic kidney disease [2]. NASH can progress to liver failure, and it is projected to be the leading cause of liver transplantation in 2020 [3]. Therefore, there is an essential clinical need for an effective treatment for NAFLD. The first-line NAFLD therapy consists in the correction of central obesity, which is achieved by combining dietary measures with increased physical activity to obtain modest weight reduction [4]. However, more than 50% of patients fail to achieve their target weight loss [3]. Given the poor compliance with lifestyle modifications, the increased risk for CVD, and the strong association with metabolic syndrome (MS) in NAFLD patients, medications such as insulin sensitizers, lipid-lowering drugs, omega-3 polyunsaturated fatty acids, and vitamins have become important options for the treatment of NAFLD and may influence both NAFLD and its related cardiac and arrhythmic complications [5,6].

Resveratrol is a natural polyphenol found in grapes, peanuts, berries, and red wine. Currently, resveratrol is used as a dietary supplement. The acceptable daily intake is 450 mg/day. Trials have

* Corresponding authors at: Research Center of Nutrition and Food Safety, Chongqing Key Laboratory of Nutrition and Food Safety, The Third Military Medical University, No. 30 Gaotanyan Street, Shapingba District, Chongqing 400038, China. Tel.: +86 023 68752305; fax: +86 023 68752305.
E-mail addresses: zqianyong@sina.com (Q. Zhang), mimantian@hotmail.com (M. Mi).

http://dx.doi.org/10.1016/j.dld.2014.11.015
1590-8658/

shown that intake of 5000 mg/day did not result in obvious side effects [7].

In vitro, Renes et al. found that, when compared with calorie restriction, resveratrol treatment was more beneficial in reducing obesity-related metabolic complications and alleviating the inflammatory phenotype. Resveratrol can regulate liver lipid metabolism to prevent the development of NAFLD in animals [8]. Some studies conducted in rodent models have shown that resveratrol can inhibit the development of NAFLD [9,10] by decreasing the levels of AST, ALT and Apo-B, as well as by decreasing body weight, blood glucose, triglycerides, total cholesterol, and LDL-cholesterol [9,11,12]. Resveratrol can also improve glucose and lipid metabolism by attenuating oxidative stress *via* normalization of the Mn-SOD function in an AMPK/SIRT1-independent pathway in the kidneys of db/db mice [13]. In human studies, resveratrol induced SIRT1 expression and improved the human adipocyte secretome in a manner similar to that of low-glucose calorie restriction [14]. Timmers et al. observed modest improvements in insulin sensitivity, blood pressure, metabolic rate, hepatic steatosis, and pertinent biomarkers in healthy obese males after 150 mg/day resveratrol supplementation [15]. In type 2 diabetic patients, 10 mg/d resveratrol improved insulin sensitivity potentially due to a more efficient insulin signalling *via* the protein kinase B (Akt) pathway medicated by a resveratrol-induced decrease in oxidative stress [16]. A meta-analysis including 11 randomized controlled trials reported that resveratrol significantly improved glucose control and insulin sensitivity in persons with diabetes [17].

Clearly, resveratrol has a variety of biochemical and physiological effects including the following: anti-oxidative, decreased fatty acid availability, anti-inflammatory, anti-obesity, improved lipid metabolism, and improved insulin sensitivity [9,16,18]. But the true efficacy and mechanisms of action of resveratrol in NAFLD are not yet fully understood. Some studies found that resveratrol supplementation was not beneficial for metabolic functions or body composition in non-obese women with normal glucose tolerance or in obese men [19,20]. Currently, only one trial has been conducted to evaluate the effect of resveratrol on NAFLD; however, eight weeks of administration of resveratrol did not significantly improve any features of NAFLD [21]. Therefore, the aim of this study was to investigate whether resveratrol benefits patients with NAFLD.

2. Materials and methods

2.1. Study design

This study was a randomized, double blind, placebo-controlled trial. Community-dwelling subjects with a confirmed diagnosis of NAFLD by ultrasound were enrolled. This study took place at the health care centre of Southwest Hospital (Chongqing, China) from October 2012 to February 2013. Patients were randomly assigned to one of two parallel groups, initially at a 1:1 ratio, to receive either 2 placebo capsules (placebo group) or 2 150 mg resveratrol capsules (resveratrol group) twice daily. An independent investigator determined whether a patient would be treated with either placebo or resveratrol according to a computer-generated randomization list. The randomized sequence was created using Excel software. Both researchers and patients involved in the study were blinded to the randomization, which was revealed at the end of the study.

2.2. Participants

Eligible subjects were adult NAFLD patients aged 20–60 years and meeting the following inclusion criteria: presence of "bright liver" at ultrasonography, absence of known aetiologies of chronic liver disease, such as viral hepatitis, autoimmune hepatitis, drug-induced or other liver diseases, liver or kidney dysfunctions or malignant tumours, and with a body mass index (BMI) between 20 and 30, and fasting blood–glucose <7.8 mmol/L. Exclusion criteria were as follows: excessive alcohol consumption (more than 140 g/week for men and 70 g/week for women) or taking any medicine over the last 6 months that would influence glucose and lipid metabolism.

All participants provided written consent. The protocol was approved by the Medical Ethical Committee of The Third Military Medical University of Chongqing, China. This study complied with the standards of the Declaration of Helsinki and current ethical guidelines. The clinical trial number is ChiCTR-TRC-12002378.

2.3. Materials

Resveratrol powder, purified from natural products by high performance liquid chromatography (purity ≥98%), was purchased from Ciyuan Biotechnology Co. Ltd., Shanghai, China (CAS No. 501-36-0). Resveratrol and placebo capsules were identically packaged at the Pharmacy Department of the Southwest Hospital, in Chongqing, China. Each capsule of resveratrol contained 150 mg of resveratrol, as well as pullulan and maltodextrin, whereas the placebo capsule only contained pullulan and maltodextrin.

2.4. Dose calculation

According to the literature [22], the resveratrol dose was calculated by the body surface area normalization method. In this study, the resveratrol intervention dose was 600 mg/day.

2.5. Interventions

Studies have found that weight loss, cardiorespiratory fitness improvement, and multiple conditions associated with metabolic syndrome reduction can be achieved by lifestyle changes and dietary restriction [23]. Therefore, to avoid any bias, during the trial all participants were asked to maintain their usual lifestyle and habitual dietary intake. Subjects were followed every month by a telephone call to obtain relevant information.

2.6. Data collection

All subjects filled out a questionnaire at baseline collecting their personal history including age, gender, race, alcohol consumption, medical history, health habits, and use of medications associated with NAFLD or that might affect glucose and lipid metabolism. At baseline and at the end of the trial, physical examination and blood sampling were also performed. The presence of other non-NAFLD aetiologies was ruled out by testing all participants for serum hepatitis B surface antigen, antibodies to hepatitis C, auto-antibodies, and ceruloplasmin.

2.7. Anthropometric parameters

Body weight, height, waist and hip circumference, and blood pressure were measured by a trained nurse at baseline and at the end of the study. The BMI was calculated as weight (kg)/height $(m)^2$. The waist/hip ratio was also calculated.

2.8. Liver ultrasound examination

Both before and at the end of the intervention, the same trained ultrasound reader performed a liver ultrasonography with a 3.5-MHz linear transducer (GE, Voluson 730, USA). A scoring system

Please cite this article in press as: Chen S, et al. Resveratrol improves insulin resistance, glucose and lipid metabolism in patients with non-alcoholic fatty liver disease: A randomized controlled trial. Dig Liver Dis (2015), http://dx.doi.org/10.1016/j.dld.2014.11.015

was adopted to obtain a semi-quantitative evaluation of the presence and severity of the fat deposits in the liver. Based on the appearance of the liver echo texture, the clarity of the hepatic blood vessel structures, as well as the echo penetration and visibility of the diaphragm, the degree of hepatic fatty infiltration was scored as described by Chiloiro et al. [24]. The fatty liver score ranged from 0 to 6. Patients were categorized as having mild, moderate, or severe steatosis if the overall score was 1–2, 3–4, or 5–6, respectively. A score of 0 indicated the absence of fatty liver. The intra-observer variability of the scores obtained for the assessment of liver fat was 6.8%.

2.9. Laboratory tests

Both at baseline and at the end of the trial, blood samples were obtained after an overnight fast. Laboratory evaluation comprised the following parameters: blood cell and haemoglobin concentration analysis; renal function including blood urea nitrogen (BUN) and creatinine; liver enzymes including alanine and aspartate transaminases (ALT, AST), and γ-glutamyl transpeptidase (GGT); parameters of glucose metabolism (fasting blood glucose, serum insulin, C-peptide); lipid metabolic profile including serum total cholesterol, triglycerides (TG), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and apolipoproteins A and B (Apo A and B). Moreover, the serum cytokines tumour necrosis factor alpha (TNF-α), adiponectin (APN), cytokeratin 18 (CK-18) fragment, and fibroblast growth factor 21 (FGF21) were also assessed.

The homeostasis model assessment insulin resistance index (HOMA-IR) was used to calculate insulin resistance according to the formula [fasting glucose (mmol/L) × fasting insulin (mIU/L)/22.5]. Higher values indicate greater insulin resistance.

The absorbance values of serum cytokines were measured with an ELISA Reader at 450 nm. Cytokines' concentrations in each sample were calculated from the calibration curve with Curve Expert 1.4. The intra and inter-assay coefficients of variation (CVs) for the measured biochemical parameters ranged from 5.9% to 7.9% and from 6.3% to 9.0%, respectively. The intra and inter-assay CVs of TNF-α, APN, CK-18 fragment, and FGF21 were 5.4% and 6.3%, 6.4% and 7.2%, 5.8% and 6.6%, and 5.9% and 7.0%, respectively.

2.10. Statistical analyses

All enrolled patients were included in the analysis according to the intention-to-treat principle. Statistical analyses were performed by SPSS software version 18.0 (SPSS, Inc., Chicago, USA). Data were presented as mean ± SD (for normally distributed data), median (and inter-quartile range, IQR, for skewed data), or frequency (*N*, for categorical factors). Skewed data were logarithmically transformed for statistical analyses. A $P < 0.05$ was considered significant in all analyses. Differences at baseline between the 2 groups were evaluated by using the Student's *t* test. At the end of the study, one-factor analysis of covariance (ANCOVA) of the 3-month values, or change values as dependent variables and baseline values as the covariates, were used to compare the changes in serum markers in patients taking resveratrol and placebo. The Pearson's chi-squared test was used for comparisons of categorical variables. Differences in the severity of fatty liver in the 2 groups at baseline and at the end of the study were assessed by Fisher's exact test.

Sample size and power calculations were based on changes in the mean value of blood glucose (where glucose was a measure of insulin resistance). According to Anty et al. [25] the mean blood glucose level in NAFLD was 5.1 mmol/L and the SD was 1.3; thus, to detect a 25% change in the primary outcome at a two-sided 0.05 significance level with a power of 0.90, twenty-two participants were required in each group. A 15% defaulter rate was expected during the trial period, thus at least twenty-six participants were enrolled in each group.

3. Results

The screening, enrolment, and retention of the treatment group are shown in Fig. 1. Fifty-seven subjects completed the study. Outcome analyses included all participants randomized by the intention-to-treat principle. At baseline, sixty participants were randomly assigned to the placebo group ($n = 30$) or resveratrol group ($n = 30$).

At baseline, the distribution of age and gender was uniform ($P > 0.05$ for both, Table 1). There were no significant differences between the two groups in terms of anthropometric characteristics or degree of hepatic fatty infiltration ($P > 0.05$ for both, Table 1). Similarly, the levels of red and white blood cells, platelet and haemoglobin concentration, renal function test, ALT, AST, GGT, glucose and lipid metabolism determination, and serum cytokines' levels were also well matched ($P > 0.05$ for all, Table 2).

3.1. Results after 3 months of resveratrol consumption

Compliance assessment was based on pill counts. The compliance rates were 91.7% and 93.2% in the placebo and resveratrol

Table 1
Anthropometric characteristics and B ultrasound severity of fatty infiltration at baseline.[a]

	Total ($n = 60$)	Placebo ($n = 30$)	Resveratrol ($n = 30$)	P[b]
Anthropometric index				
Age at enrollment (year)	44.3 ± 10.5	43.5 ± 11.0	45.2 ± 10.0	0.526
Female sex *N* (%)	18 (30.0)	10 (33.3)	8 (26.7)	0.779
Height (cm)	165.6 ± 7.72	165.2 ± 7.95	166.1 ± 7.59	0.656
Weight (kg)	70.8 ± 9.65	71.6 ± 10.2	70.0 ± 9.15	0.513
BMI (kg/m^2)	25.7 ± 2.65	26.2 ± 3.08	25.3 ± 2.11	0.191
WC (cm)	88.3 ± 7.00	88.2 ± 7.10	88.4 ± 7.01	0.913
HC (cm)	96.8 ± 7.93	97.6 ± 6.02	95.9 ± 9.51	0.430
Waist:hip ratio	0.92 ± 0.10	0.90 ± 0.06	0.93 ± 0.13	0.313
SBP (mmHg)	127.9 ± 18.2	131.7 ± 21.7	124.1 ± 13.1	0.106
DBP (mmHg)	82.5 ± 12.0	84.5 ± 14.4	80.6 ± 8.76	0.207
Severity of fatty infiltration[c]				
Absent	0 (0)	0 (0)	0 (0)	1.000
Mild	7 (11.7)	3 (10.0)	4 (13.3)	
Moderate	48 (80.0)	24 (80.0)	24 (80.0)	
Severe	5 (8.3)	3 (10.0)	2 (6.7)	

BMI, body mass index; DBP, diastolic blood pressure; HC, hip circumference; SBP, systolic blood pressure; WC, waist circumference.
[a] Data are expressed as means ± SD or *N* (%).
[b] There were no significant differences for any variable by the independent-samples *t* test or Pearson's chi-square test between the 2 groups at baseline.
[c] There were no significant differences of the B ultrasound severity of fatty infiltration by Fisher's exact test between the 2 groups at baseline.

Please cite this article in press as: Chen S, et al. Resveratrol improves insulin resistance, glucose and lipid metabolism in patients with non-alcoholic fatty liver disease: A randomized controlled trial. Dig Liver Dis (2015), http://dx.doi.org/10.1016/j.dld.2014.11.015

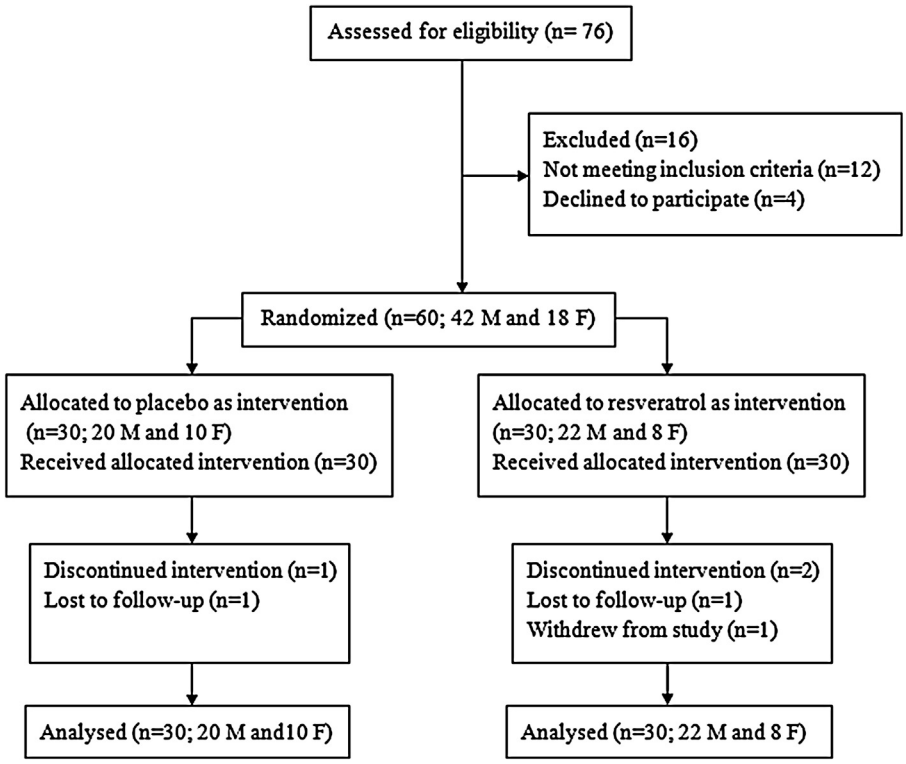

Fig. 1. Study flowchart: screening, enrolment and randomization of study subjects. F: female; M: male.

Table 2
Baseline characteristic of hematologic, renal function test, liver enzyme, glucose and lipid metabolism determination and serum cytokines.[a]

	Total ($n=60$)	Placebo ($n=30$)	Resveratrol ($n=30$)	P[b]
Hematologic measures				
RBC ($\times 10^{12}$/L)	4.92 ± 0.54	4.89 ± 0.48	4.95 ± 0.59	0.664
HGB (g/L)	147.4 ± 12.5	146.6 ± 11.5	148.2 ± 13.6	0.625
WBC ($\times 10^{9}$/L)	6.18 (5.36, 6.88)	6.25 (5.31, 6.89)	6.03 (5.36, 6.92)	0.662
Platelet ($\times 10^{9}$/L)	194.5 (154.0, 226.8)	204.0 (163.8, 223.5)	169.0 (151.0, 230.5)	0.355
Renal Function Tests				
BUN (mmol/L)	5.15 (4.60, 5.89)	5.25 (4.55, 5.80)	5.05 (4.55, 6.13)	0.725
Creatinine (μmol/L)	72.8 (64.6, 84.4)	72.7 (60.7, 85.4)	73.5 (65.0, 83.8)	0.883
Liver enzymes markers				
ALT (IU/L)	37.5 (31.3, 49.0)	34.5 (27.8, 47.0)	39.0 (33.8, 49.3)	0.899
AST (IU/L)	28.5 (25.0, 33.0)	27.5 (24.8, 33.0)	29.0 (26.0, 33.5)	0.613
GGT (IU/L)	40.5 (34.0, 56.8)	39.5 (26.5, 69.3)	41.0 (36.8, 54.3)	0.887
Glucose metabolic parameters				
Glucose (mmol/L)	5.52 ± 0.56	5.48 ± 0.59	5.55 ± 0.53	0.674
Insulin (mIU/L)	13.2 ± 5.17	13.1 ± 5.34	13.2 ± 5.09	0.922
HOMA-IR	3.21 ± 1.29	3.16 ± 1.22	3.26 ± 1.36	0.770
C-peptide (ng/mL)	4.35 (3.50, 5.01)	4.35 (3.49, 5.22)	4.27 (3.47, 4.85)	0.998
Lipid metabolic parameters				
Total cholesterol (mmol/L)	5.35 ± 0.76	5.39 ± 0.78	5.31 ± 0.75	0.696
Triacylglycerol (mmol/L)	2.65 ± 1.69	2.52 ± 1.41	2.78 ± 1.95	0.552
LDL cholesterol (mmol/L)	3.01 ± 0.59	2.95 ± 0.68	3.08 ± 0.48	0.412
HDL cholesterol (mmol/L)	1.26 ± 0.23	1.26 ± 0.25	1.26 ± 0.22	0.987
Apo B (g/L)	1.23 ± 0.24	1.21 ± 0.28	1.24 ± 0.19	0.633
Apo A-I (g/L)	1.47 ± 0.22	1.47 ± 0.23	1.46 ± 0.22	0.914
Serum cytokine tests				
TNF-α (pg/mL)	4.94 ± 0.96	4.97 ± 0.84	4.91 ± 1.07	0.800
APN (ng/mL)	4.93 (4.81, 5.31)	4.89 (4.72, 5.30)	4.95 (4.84, 5.35)	0.431
CK-18 (IU/L)	274.0 (251.2, 319.9)	284.8 (255.4, 310.1)	265.3 (248.3, 335.0)	0.733
FGF21 (pg/mL)	225.7 (211.3, 242.3)	226.9 (212.4, 244.7)	224.7 (208.9, 240.5)	0.970

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; HGB, haemoglobin; HOMA-IR, homeostasis model assessment insulin resistance index; LDL, low-density lipoprotein; RBC, red blood cells; WBC, white blood cells.

[a] Statistics presented are means ± SD or median (IQR).

[b] There were no significant differences for any variable by the independent-samples t test between the 2 groups at baseline.

Please cite this article in press as: Chen S, et al. Resveratrol improves insulin resistance, glucose and lipid metabolism in patients with non-alcoholic fatty liver disease: A randomized controlled trial. Dig Liver Dis (2015), http://dx.doi.org/10.1016/j.dld.2014.11.015

Table 3
Change from baseline to end of treatment in anthropometric characteristics and B ultrasound severity of fatty infiltration by treatment group.[a]

	Placebo ($n=30$)	Resveratrol ($n=30$)	P[b]
Anthropometric index			
Weight (kg)	0.42 ± 2.36	−0.02 ± 1.51	0.337
BMI (kg/m^2)	0.17 ± 0.86	−0.01 ± 0.54	0.262
WC (cm)	0.35 ± 4.99	1.18 ± 4.76	0.489
HC (cm)	1.23 ± 5.59	2.70 ± 9.38	0.807
Waist:hip ratio	−0.01 ± 0.05	−0.02 ± 0.14	0.589
SBP (mmHg)	1.73 ± 16.0	1.27 ± 14.4	0.446
DBP (mmHg)	0.63 ± 10.8	−1.20 ± 12.0	0.254
Severity of fatty infiltration[c]			
Absent	1 (3.3)	4 (13.3)	0.452
Mild	3 (10.0)	4 (13.3)	
Moderate	25 (83.3)	20 (66.7)	
Severe	1 (3.3)	2 (6.7)	

BMI, body mass index; DBP, diastolic blood pressure; HC, hip circumference; SBP, systolic blood pressure; WC, waist circumference.
[a] Data are expressed as means ± SD or *N* (%).
[b] *P*-value compares the change variables at the end of study between the 2 groups by one-factor ANCOVA test.
[c] There were no significant differences of the B ultrasound severity of fatty infiltration by Fisher's exact test between the 2 groups after treatment.

groups, respectively. No subjects reported adverse events during the trial period.

The intervention had no significant effect on anthropometric characteristics ($P>0.05$, Table 3).

There were no significant differences between the 2 groups in the severity of hepatic fatty infiltration as assessed by ultrasound ($P=0.452$, Table 3).

Resveratrol consumption had no effect on red and white blood cell and platelet counts or on haemoglobin concentration ($P>0.05$, Table 4).

There were no significant differences in BUN ($P=0.384$) or creatinine levels ($P=0.904$) between the two groups (Table 4).

The median serum ALT levels were significantly lower at the end of the study in the resveratrol group [−7.00 (−11.0, −2.50) IU/L *vs.* −1.00 (−8.00, 4.50) IU/L, $P=0.002$, Table 4].

Similarly, a significant reduction in AST [−6.00 (−9.00, −3.00) IU/L *vs.* −0.50 (−4.50, 2.25) IU/L, $P<0.001$] was observed in the resveratrol group (Table 4).

GGT levels did not differ significantly between the 2 groups [−5.00 (−9.50, 0.00) IU/L *vs.* −2.00 (−10.8, 3.00) IU/L, $P=0.196$, Table 4].

A significantly greater decrease in the mean serum glucose level (−0.64 ± 0.31 mmol/L) was found in the resveratrol group compared with the placebo group (−0.10 ± 0.82 mmol/L, $P=0.001$, Table 4).

Similarly, the changes in HOMA-IR were significantly greater in the resveratrol group than in the placebo group (−0.60 ± 1.15 *vs.* 0.09 ± 1.37, $P=0.016$; Table 4).

By contrast, changes in serum insulin (−1.09 ± 4.25 *vs.* 0.39 ± 6.30 mIU/L, $P=0.162$), and C-peptide levels were not significantly different between the 2 groups [−0.13 (−0.28, 0.02) *vs.* −0.11 (−0.33, 0.04) ng/mL, $P=0.382$, Table 4].

3.2. Lipid metabolism markers

Total cholesterol was significantly reduced in the resveratrol group compared with the placebo group (−0.67 ± 0.50 *vs.* −0.15 ± 0.77 mmol/L, $P=0.002$; Table 4).

A significant change in LDL cholesterol was also observed in the resveratrol group (−0.41 ± 0.35 *vs.* 0.08 ± 0.71 mmol/L, $P=0.001$; Table 4).

Table 4
Changes from baseline to the end of treatment in variables by treatment group.[a]

	Placebo ($n=30$)	Resveratrol ($n=30$)	P[b]
Hematologic measures			
RBC ($\times10^{12}$/L)	−0.07 ± 0.41	−0.12 ± 0.41	0.851
HGB (g/L)	−2.20 ± 10.7	−0.90 ± 11.5	0.224
WBC ($\times10^9$/L)	−0.23 (−0.99, 0.26)	−0.49 (−1.30, 0.11)	0.741
Platelet ($\times10^9$/L)	−3.50 (−14.3, 22.0)	−0.00 (−20.8, 26.0)	0.816
Renal Function Tests			
BUN (mmol/L)	0.15 (−1.23, 1.00)	0.10 (−0.33, 1.00)	0.384
Creatinine (μmol/L)	1.90 (−4.40, 10.6)	4.10 (−11.9, 14.0)	0.904
Liver enzymes markers			
ALT (IU/L)	−1.00 (−8.00, 4.50)	−7.00 (−11.0, −2.50)	0.002
AST (IU/L)	−0.50 (−4.50, 2.25)	−6.00 (−9.00, −3.00)	<0.001
GGT (IU/L)	−2.00 (−10.8, 3.00)	−5.00 (−9.50, 0.00)	0.196
Glucose metabolic parameters			
Glucose (mmol/L)	−0.10 ± 0.82	−0.64 ± 0.31	0.001
Insulin (mIU/L)	0.39 ± 6.30	−1.09 ± 4.25	0.162
HOMA-IR	0.09 ± 1.37	−0.60 ± 1.15	0.016
C-peptide (ng/mL)	−0.11 (−0.33, 0.04)	−0.13 (−0.28, 0.02)	0.382
Lipid metabolic parameters			
Total cholesterol (mmol/L)	−0.15 ± 0.77	−0.67 ± 0.50	0.002
Triacylglycerol (mmol/L)	−0.22 ± 0.62	−0.38 ± 0.49	0.324
LDL cholesterol (mmol/L)	0.08 ± 0.71	−0.41 ± 0.35	0.001
HDL cholesterol (mmol/L)	−0.02 ± 0.41	0.04 ± 0.36	0.382
Apo B (g/L)	−0.09 ± 0.35	−0.02 ± 0.28	0.074
Apo A-I (g/L)	0.07 ± 0.23	0.15 ± 0.39	0.273
Serum cytokine tests			
TNF-α (pg/mL)	−0.16 ± 1.00	−0.53 ± 1.30	0.030
APN (ng/mL)	0.06 (−0.21, 0.44)	1.22 (−0.37, 1.60)	0.025
CK-18 (IU/L)	−0.95 (−49.5, 29.0)	−26.9 (−70.3, 5.12)	0.030
FGF21 (pg/mL)	−2.95 (−27.4, 12.1)	−23.3 (−43.0, 0.31)	<0.001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment insulin resistance index; LDL, low-density lipoprotein.
[a] Statistics presented are means ± SD or median (IQR). There were no significant differences for any variable by the independent-samples *t* test between the 2 groups at baseline.
[b] *P*-value compares the change variables at the end of study between the 2 groups by the one-factor ANCOVA test.

By contrast, the changes in TG, HDL cholesterol, Apo B, and Apo A-I concentrations were not significant when comparing the 2 groups (Table 4).

3.3. Serum cytokines

A significant decrease in serum TNF-α (−0.53 ± 1.30 *vs.* −0.16 ± 1.00 pg/mL, $P=0.030$) was observed in the resveratrol group compared with the placebo group.

The change in APN [1.22 (−0.37, 1.60) *vs.* 0.06 (−0.21, 0.44) ng/mL, $P=0.025$] was greater in the resveratrol group than in the placebo group (Table 4).

Also the level of CK-18 fragment [−26.9 (−70.3, 5.12) *vs.* −0.95 (−49.5, 29.0) IU/L, $P=0.030$] declined significantly in the resveratrol group compared with the placebo group.

Finally, in the resveratrol group, the reduction of the FGF21 concentration was significantly different than that in the placebo group [−23.3 (−43.0, 0.31) *vs.* −2.95 (−27.4, 12.1) pg/mL, $P<0.001$].

4. Discussion

To date, there is only one study focusing on the effects of resveratrol in NAFLD patients; thus, our knowledge on this subject is still incomplete. We here evaluated the efficacy of resveratrol supplementation for three months in patients with NAFLD, and resveratrol showed beneficial effects in NAFLD patients. NAFLD has now become the most common cause of liver disease worldwide and is usually associated with overnutrition and underactivity,

Please cite this article in press as: Chen S, et al. Resveratrol improves insulin resistance, glucose and lipid metabolism in patients with non-alcoholic fatty liver disease: A randomized controlled trial. Dig Liver Dis (2015), http://dx.doi.org/10.1016/j.dld.2014.11.015

insulin resistance, visceral obesity, dysglycemia, dyslipidemia, and hypertension. Liver inflammation, cytokines, and genetic factors may all play a role in the origin and progression of NAFLD [26]. Despite the increased understanding of the pathogenic mechanisms of NAFLD, only few effective therapies are now available.

In this study, treatment of NAFLD patients with resveratrol significantly reduced liver enzymes, glucose, HOMA-IR, total cholesterol, and LDL cholesterol. These results are in conflict with those of Chachay et al. [21], whose study did not find significant improvements of any NAFLD feature. The participants in the abovementioned study were 10 overweight or obese men diagnosed with NAFLD, while in our study the subjects were 20 male and 8 female NAFLD patients. In the Chachay study, the patients used a resveratrol dose of 3000 mg/d and the intervention duration was of 8 weeks, while in our study we used a dose of 600 mg/d for 3 months. Therefore, the different findings in these two studies may also be related to the differences in sample size, intervention duration, and baseline characteristics of the participants. Furthermore, other studies using 10 mg/d, 150 mg/d, 250 mg/d, or 1 g/d resveratrol had positive effects on glucose control and insulin sensitivity [15,17].

Even though we did find some positive effects of resveratrol on serum liver function and metabolic parameters, we found no benefit in terms of fatty liver reduction based on the ultrasonographic images of our patients. These results are in contrast with those of Scaglioni et al. [27]. These authors found that a short-term change in lifestyle, diet, and physical activity could improve not only the metabolic features and liver enzymes' levels, but also the liver fat content measured by semi-quantitative ultrasound scores. These contrasting results may be due to the different interventions, number of participants, and scoring systems. Although the result was not statistically significant, we also found that some participants exhibited an improvement in their liver fatty infiltration. We thus speculate that a much longer intervention time may correlate with the positive results in the liver ultrasound performance of the treatment group. Therefore, larger-scale, well-controlled, long-term studies as well as dose–effect relationship studies are needed to evaluate the definite therapeutic effects and effective doses of resveratrol for NAFLD.

Recently, inflammatory mediators have been postulated to play an important role in the pathogenesis of NAFLD. Studies have shown that a significantly increased level of TNF-α is an independent predictor of histological fibrosis in NASH patients, and that the level of TNF-α is higher in patients with NAFLD [28]. Therefore, TNF-α is considered an important inflammatory cytokine in the pathogenesis of NAFLD. TNF-α induces hepatic steatosis, which may enhance the gene expression of sterol regulatory element binding protein-1c (SREBP-1c) [29]. In contrast to TNF-α, blood APN levels were higher in patients with low-grade liver steatosis [30]. A reduced adiponectin concentration was associated with an increased risk of insulin resistance [31]. Our data showed that supplementation with 600 mg resveratrol for 3 months decreased the TNF-α concentration and increased the level of APN. These findings are consistent with the results of Palsamy et al. [32], who showed that resveratrol significantly lowered the TNF-α concentration and increased the APN level in renal tissues of diabetic rats by attenuating hyperglycaemia-mediated oxidative stress and renal inflammatory cytokines *via* the Nrf2–Keap1 pathway. Therefore, our results indicate that resveratrol could normalize the levels of TNF-α and APN to protect against NAFLD by inhibiting hepatic inflammation and exerting antioxidant activities.

Hepatocyte apoptosis plays an important role during liver injury and NASH development [33]. During the process of apoptosis, effector caspases (mainly caspase 3) are activated and cleave a number of different substrates inside the cell, including CK-18 fragment, the major intermediate filament protein in the liver, resulting in the characteristic morphological changes of apoptosis, which are enhanced in various liver diseases [34]. Studies have shown that the level of CK-18 fragment is markedly increased in patients with NASH, which highlights the potential usefulness of this marker as a noninvasive diagnostic means of predicting the severity of NASH [33,35]. FGF21, a member of the FGF family, is primarily produced in liver tissue [36] and has been shown to have lipid-lowering effects. It also exerts potent beneficial effects on glucose and insulin sensitivity in animal models [37]. In human studies, patients with obesity, metabolic syndrome, type 2 diabetes and NALFD had elevated serum FGF21 levels, suggesting that the level of serum FGF21 has the potential to be an important biomarker for the early diagnosis of NASH [38,39].

In this study, we also found that the concentrations of CK-18 fragment and FGF21 were significantly elevated in patients with NAFLD. These findings were consistent with a previous investigation of these two molecules in NAFLD. The levels of CK-18 fragment and FGF21 were significantly decreased after 3 months of treatment with resveratrol, indicating that resveratrol may ameliorate liver injury by modulating the process of hepatocellular apoptosis, slowing the progress of liver steatosis, and regulating glucose and lipid metabolism in patients with NAFLD.

While our study provided some interesting outcomes that may help us gain a better understanding of the treatment of NAFLD, it has a main limitation. As a pilot study, patients with NAFLD were diagnosed only by ultrasound, thus making diagnosis somewhat subjective and operator-dependent. In clinical practice, and especially in the general population, ultrasonography is the preferred imaging method used to assess the significance of abnormal liver function tests. Furthermore, this method has a sensitivity of 91.3%, a specificity of 83.8%, and an accuracy of 86.7% in the diagnosis of fatty liver [40]. A recent meta-analysis showed that liver ultrasonography had a sensitivity of 84.8% and a specificity of 93.6% in detecting moderate to severe fatty liver in forty-nine studies (overall including 4720 participants) [41]. We did not perform biopsies since they are invasive and our participants declined them. This limitation was partly compensated by the strict adherence to the inclusion and exclusion criteria and the very tight control of the intervention and experimental procedures, which allowed the comprehensive assessment of the factors involved in NAFLD.

In conclusion, the results of this clinical trial suggest that resveratrol supplementation in patients with NAFLD has a beneficial effect on liver enzymes, insulin resistance, as well as glucose and lipid metabolism. These beneficial effects may be due to the ability of resveratrol to improve insulin sensitivity, modulate the process of hepatocellular apoptosis, and slow down the progress of liver steatosis in addition to its anti-inflammatory properties. Therefore, resveratrol offers a novel approach in the reduction of the risks correlated with NAFLD.

Conflict of interest

None declared.

Funding

This study was supported by the National Natural Science Foundation of China (No. 30972469; No. 81273059), the Science and Technology Key Project Foundation of Chongqing (No. CSTC, 2011AB5040), and the National Science-technology Support Plan Projects Foundation of China (No. 2012BAI35B02).

References

[1] Matteoni CA, Younossi ZM, Gramilich T, et al. Non-alcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413–9.

[2] Lonardo A, Sookoian S, Chonchol M, et al. Cardiovascular and systemic risk in non-alcoholic fatty liver disease – atherosclerosis as a major player in the natural course of NAFLD. Current Pharmaceutical Design 2013;19:5177–92.
[3] Musso G, Gambino R, Cassader M, et al. A meta-analysis of randomized trials for the treatment of non-alcoholic fatty liver disease. Hepatology 2010;52:79–104.
[4] Nascimbeni F, Pais R, Bellentani S, et al. From NAFLD in clinical practice to answers from guidelines. Journal of Hepatology 2013;59:859–71.
[5] Ballestri S, Lonardo A, Bonapace S, et al. Risk of cardiovascular, cardiac and arrhythmic complications in patients with non-alcoholic fatty liver disease. World Journal of Gastroenterology: WJG 2014;20:1724–45.
[6] Mazzella N, Ricciardi LM, Mazzotti A, et al. The role of medications for the management of patients with NAFLD. Clinics in Liver Disease 2014;18:73–89.
[7] Brown VA, Patel KR, Viskaduraki M, et al. Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis. Cancer Research 2010;70:9003–11.
[8] Mukherjee S, Dudley JI, Das DK. Dose-dependency of resveratrol in providing health benefits. Dose–Response 2010;8:478–500.
[9] Gomez-Zorita S, Fernandez-Quintela A, Macarulla MT, et al. Resveratrol attenuates steatosis in obese Zucker rats by decreasing fatty acid availability and reducing oxidative stress. British Journal of Nutrition 2012;107:202–10.
[10] Shang J, Chen LL, Xiao FX, et al. Resveratrol improves non-alcoholic fatty liver disease by activating AMP-activated protein kinase. Acta Pharmacologica Sinica 2008;29:698–706.
[11] Baek SH, Shin WC, Ryu HS, et al. Creation of resveratrol-enriched rice for the treatment of metabolic syndrome and related diseases. PLOS ONE 2013;8:1–10.
[12] Cho IJ, Ahn JY, Kim S, et al. Resveratrol attenuates the expression of HMG-CoA reductase mRNA in hamsters. Biochemical and Biophysical Research Communications 2008;367:190–4.
[13] Kitada M, Kume S, Imaizumi N, et al. Resveratrol improves oxidative stress and protects against diabetic nephropathy through normalization of Mn-SOD dysfunction in AMPK/SIRT1-independent pathway. Diabetes 2011;60:634–43.
[14] Renes J, Rosenow A, Roumans N, et al. Calorie restriction-induced changes in the secretome of human adipocytes, comparison with resveratrol-induced secretome effects. Biochimica et Biophysica Acta 2014;1844:1511–22.
[15] Timmers S, Konings E, Bilet L, et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metabolism 2011;14:612–22.
[16] Brasnyo P, Molnar GA, Mohas M, et al. Resveratrol improves insulin sensitivity, reduces oxidative stress and activates the Akt pathway in type 2 diabetic patients. British Journal of Nutrition 2011;106:383–9.
[17] Liu K, Zhou R, Wang B, et al. Effect of resveratrol on glucose control and insulin sensitivity: a meta-analysis of 11 randomized controlled trials. American Journal of Clinical Nutrition 2014.
[18] Kim S, Jin Y, Choi Y, et al. Resveratrol exerts anti-obesity effects via mechanisms involving down-regulation of adipogenic and inflammatory processes in mice. Biochemical Pharmacology 2011;81:1343–51.
[19] Yoshino J, Conte C, Fontana L, et al. Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance. Cell Metabolism 2012;16:658–64.
[20] Poulsen MM, Vestergaard PF, Clasen BF, et al. High-dose resveratrol supplementation in obese men: an investigator-initiated, randomized, placebo-controlled clinical trial of substrate metabolism, insulin sensitivity, and body composition. Diabetes 2013;62:1186–95.
[21] Chachay VS, Macdonald GA, Martin JH, et al. Resveratrol does not benefit patients with non-alcoholic fatty liver disease. Clinical Gastroenterology and Hepatology 2014.
[22] Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB Journal 2008;22:659–61.
[23] Centis E, Marzocchi R, Suppini A, et al. The role of lifestyle change in the prevention and treatment of NAFLD. Current Pharmaceutical Design 2013;19:5270–9.
[24] Chiloiro M, Caruso MG, Cisternino AM, et al. Ultrasound evaluation and correlates of fatty liver disease: a population study in a Mediterranean area. Metabolic Syndrome and Related Disorders 2013;11:349–58.
[25] Anty R, Marjoux S, Iannelli A, et al. Regular coffee but not espresso drinking is protective against fibrosis in a cohort mainly composed of morbidly obese European women with NAFLD undergoing bariatric surgery. Journal of Hepatology 2012;57:1090–6.
[26] Farrell GC, Larter CZ. Non-alcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology 2006;43:S99–112.
[27] Scaglioni F, Marino M, Ciccia S, et al. Short-term multidisciplinary non-pharmacological intervention is effective in reducing liver fat content assessed non-invasively in patients with non-alcoholic fatty liver disease (NAFLD). Clinics and Research in Hepatology and Gastroenterology 2013;37:353–8.
[28] Jarrar MH, Baranova A, Collantes R, et al. Adipokines and cytokines in non-alcoholic fatty liver disease. Alimentary Pharmacology & Therapeutics 2008;27:412–21.
[29] Endo M, Masaki T, Seike M, et al. TNF-alpha induces hepatic steatosis in mice by enhancing gene expression of sterol regulatory element binding protein-1c (SREBP-1c). Experimental Biology and Medicine 2007;232:614–21.
[30] Aller R, Luis DA, Fernandez L, et al. Influence of insulin resistance and adipokines in the grade of steatosis of non-alcoholic fatty liver disease. Digestive Diseases and Sciences 2008;53:1088–92.
[31] Yamamoto Y, Hirose H, Saito I, et al. Adiponectin, an adipocyte-derived protein, predicts future insulin resistance: two-year follow-up study in Japanese population. Journal of Clinical Endocrinology and Metabolism 2004;89:87–90.
[32] Palsamy P, Subramanian S. Resveratrol protects diabetic kidney by attenuating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines via Nrf2-Keap1 signaling. Biochimica et Biophysica Acta – Molecular Basis of Disease 2011;1812:719–31.
[33] Wieckowska A, Zein NN, Yerian LM, et al. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in non-alcoholic fatty liver disease. Hepatology 2006;44:27–33.
[34] Malhi H, Gores GJ. Cellular and molecular mechanisms of liver injury. Gastroenterology 2008;134:1641–54.
[35] Feldstein AE, Wieckowska A, Lopez AR, et al. Cytokeratin-18 fragment levels as noninvasive biomarkers for non-alcoholic steatohepatitis: a multicenter validation study. Hepatology 2009;50:1072–8.
[36] Nishimura T, Nakatake Y, Konishi M, et al. Identification of a novel FGF,FGF-21, preferentially expressed in the liver. Biochimica et Biophysica Acta 2000;1492:203–6.
[37] Dostálová I, Haluzíková D, Haluzík M. Fibroblast growth factor 21: a novel metabolic regulator with potential therapeutic properties in obesity/type 2 diabetes mellitus. Physiological Research 2009;58:1–7.
[38] Chen C, Cheung BMY, Tso AWK, et al. High plasma level of fibroblast growth factor 21 is an independent predictor of type 2 diabetes a 5.4-year population-based prospective study in Chinese subjects. Diabetes Care 2011;34:2113–5.
[39] Dushay J, Chui PC, Gopalakrishnan GS, et al. Increased fibroblast growth factor 21 in obesity and non-alcoholic fatty liver disease. Gastroenterology 2010;139:456–63.
[40] Osawa H, Mori Y. Sonographic diagnosis of fatty liver using a histogram technique that compares liver and renal cortical echo amplitudes. Journal of Clinical Ultrasound: JCU 1996;24:25–9.
[41] Hernaez R, Lazo M, Bonekamp S, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 2011;54:1082–90.

Please cite this article in press as: Chen S, et al. Resveratrol improves insulin resistance, glucose and lipid metabolism in patients with non-alcoholic fatty liver disease: A randomized controlled trial. Dig Liver Dis (2015), http://dx.doi.org/10.1016/j.dld.2014.11.015