Liver fat reduction with niacin is influenced by *DGAT-2* polymorphisms in hypertriglyceridemic patients

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Abstract Niacin reduces plasma triglycerides, but it may increase free fatty acids and insulin resistance during long-term treatment. We examined the effect of extended-release niacin on liver fat content in Chinese patients with dyslipidemia and whether the common diacylglycerol acyltransferase-2 (*DGAT2*) polymorphisms influenced this effect. The 39 patients (baseline liver fat content: 12.8 ± 7.6%, triglycerides: 3.30 ± 1.67 mmol/l) were treated with niacin, gradually increasing the dose to 2 g/day for a total of 23 weeks. The liver fat content and visceral/subcutaneous fat was measured before and after treatment. Subjects were genotyped for the *DGAT2* rs3060 and rs101899116 polymorphisms. There were significant ($P < 0.001$) reductions in plasma triglycerides (−34.9 ± 37.6%), liver fat content (−47.2 ± 32.8%), and visceral fat (−6.3 ± 15.8%, $P < 0.05$) after niacin treatment. Mean body weight decreased by 1.46 ± 2.7% (1.17 ± 2.44 kg, $P < 0.001$) during the study, but liver fat changes remained significant after adjustment for age, gender, and body weight changes [mean absolute change (95% CI): −6.1% (−8.0, −4.3), $P < 0.001$]. The *DGAT2* variant alleles were associated with a smaller reduction in liver fat content in response to niacin after adjustment for other covariates ($P < 0.01$). These findings suggest that niacin treatment may reduce liver fat content in Chinese patients with dyslipidemia and that the mechanism may involve inhibition of DGAT2. However, the findings might have been confounded by the small but significant reductions in body weight during the study. Future large randomized controlled trials are needed to verify these findings.—Hu, M., W. C. W. Chu, S. Yamashita, D. K. W. Yeung, L. Shi, D. Wang, D. Masuda, Y. Yang, and B. Tomlinson. **Liver fat reduction with niacin is influenced by *DGAT-2* polymorphisms in hypertriglyceridemic patients. *J. Lipid Res.* 2012.** 53: **802–809.**

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Nonalcoholic fatty liver disease (NAFLD) characterized by excessive accumulation of intrahepatic triglycerides is associated with multiple metabolic abnormalities that are important cardiovascular risk factors, including increased plasma triglycerides (TG), obesity, insulin resistance, diabetes, and the metabolic syndrome (1, 2). Hepatic steatosis, the earliest stage of the disease, arises from an imbalance between hepatic TG acquisition and removal (3). Increased supply to the liver of free fatty acids (FFA), the substrates for hepatic TG synthesis, from diet and adipose tissue and through increased de novo lipogenesis, promotes hepatic steatosis (3, 4).

Nicotinic acid or niacin, one of the naturally occurring B vitamins (vitamin B3), effectively reduces plasma TG levels. This effect was initially thought to be attributable to its antilipolytic effect in adipose tissue, thereby reducing FFA release from adipocytes and decreasing FFA flux to the liver (5, 6). However, although niacin initially reduces plasma FFA concentrations, this reduction is actually followed by a rebound within 1 to 9 h postdose, depending on the formulation used, and long-term treatment with niacin is associated with increases in plasma FFA, glucose, and insulin resistance (7, 8). These observations suggest that the reduction of the FFA delivery to the liver may not be the main mechanism explaining the consistent and maintained plasma TG-lowering effect of niacin.

Recent in vitro and animal studies suggested that the TG-lowering effect of niacin might be mediated by its direct and noncompetitive inhibitory effect on hepatic diacylglycerol acyltransferase-2 (DGAT2), a key enzyme that catalyzes the final step of TG synthesis (9, 10). Inhibition of mouse Dgat2 with antisense oligonucleotides in mice with obesity induced by high-fat diet or leptin-deficiency

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Abbreviations: hsCRP, high sensitivity C-reactive protein; DGAT2, diacylglycerol acyltransferase-2; ER, extended-release; ^{1}H-MRS, proton MR spectroscopy; MR, magnetic resonance; NAFLD, nonalcoholic fatty liver disease; SAT, subcutaneous adipose tissue; TG, triglyceride; VAT, visceral adipose tissue.

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for 7 weeks was associated with reduced hepatic TG synthesis and increased FFA oxidation in hepatocytes, which resulted in significantly improved hepatic steatosis and reduced plasma TG and FFA levels (11). Niacin has been widely used in patients with hypertriglyceridemia who have increased risk of metabolic syndrome and NAFLD, but whether it can improve liver fat content through its inhibitory effect on DGAT2 in humans is still unclear. The only study that tested the effect of niacin on liver fat content was reported by Fabbrini et al., who showed that 16 weeks of treatment with extended-released (ER) niacin (2 g per day) had no effect on liver fat content, which was measured with magnetic resonance (MR) imaging, or on plasma FFA levels in nine obese subjects with NAFLD; however, the sample size of the study might have been be too small to give a definitive answer, and the factors predisposing an individual to NAFLD may vary among individuals (12).

In the present study, we examined whether ER niacin treatment could influence the liver fat content in dyslipidemic Chinese patients with predominant hypertriglyceridemia. It has been suggested that *DGAT2* polymorphisms may influence the liver fat changes during lifestyle intervention in patients with fatty liver (13); therefore, this study also investigated whether the common *DGAT2* polymorphisms (rs3060, *19 T>C and rs10899116 C>T) influence the effect of niacin on liver fat content.

METHODS

Patients

Patients were Chinese men or women from 18 to 85 years of age with dyslipidemia (HDL-cholesterol $\leq$ 1.1 mmol/l for men, or HDL-cholesterol $\leq$ 1.3 mmol/l for women, or TG >1.7 mmol/l with or without statin therapy). Patients had to meet at least one of the following criteria: *i*) previously diagnosed with NAFLD; *ii*) central obesity according to Asian criteria with waist circumference > 90 cm for men or > 80 cm for women; *iii*) evidence of carotid atherosclerosis; or *iv*) increased risk of cardiovascular disease. Forty-six subjects who were in a clinically stable condition were recruited from the outpatient department in the Prince of Wales Hospital, a public hospital serving over one million residents in the New Territories of Hong Kong.

Subjects were excluded if they had a history of myocardial infarction, stroke, coronary artery bypass surgery, or other revascularization procedure; unstable angina or angioplasty within three months of screening; or impaired liver/renal functions or hyper- or hypothyroidism at the screening. Patients with poorly controlled (glycosylated hemoglobin > 8.5% at screening), unstable or new onset (within three months) diabetes were also excluded. Patients with diabetes or hypertension were required to be on a stable dose of antidiabetic or antihypertensive pharmacotherapy for at least six weeks prior to screening. Patients were not eligible if they consumed more than two alcoholic drinks per day, took anti-obesity therapy or cyclical hormonal contraceptives, or had intermittent use of hormone replacement therapies, systemic corticosteroids, or high-dose antioxidant vitamins. Patients on lipid-modifying therapy other than niacin and fibrates were eligible if they took the lipid-modifying drug for at least six weeks before enrollment and throughout the entire duration of the study. Patients were not recruited if they were engaged in high-intensity aerobic exercise and/or a special diet program for weight loss or if they had significant changes in lifestyle and/or body weight within six months of screening.

The study protocol was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong, Hong Kong, and written informed consent was obtained from all participants.

Study design

Intervention and procedures. Patients were treated with ER niacin (Niaspan®, Abbott Laboratories) once daily at bedtime in increasing doses of 375 mg, 500 mg, and 750 mg (each for 1 week); 1,000 mg and 1,500 mg (each for 4 weeks); and 2,000 mg (for 12 weeks). All participants were advised to continue with their usual diet and other aspects of lifestyle during the study. They were interviewed by research staff at weeks 1, 2, 3, 7, 11, 15, and 23 (the end of the study), and they were contacted by phone frequently during the study period to review any potential side effects and to enhance drug compliance, which was monitored by tablet count during each study visit. Anthropometric measurements, including body weight, waist circumference, hip circumference, and estimation of percentage body fat using an impedance device (TANITA Body Composition Analyzer BF-350) were performed at each study visit. Fasting blood samples were taken for lipid profile, fasting glucose, urate, and other laboratory safety tests at weeks 0, 7, 11, 15, and 23. Fasting FFA, insulin, glycated hemoglobin, high sensitivity C-reactive protein (hsCRP), and lipoprotein profiles were measured before and after niacin treatment.

Liver fact content, visceral adipose tissue, and subcutaneous adipose tissue measurements. The liver fat content, visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT) were measured before and after niacin treatment by using Proton MR spectroscopy (^{1}H-MRS) and MR imaging (Achieva 3.0T scanner, Philips Medical Systems, Best, The Netherlands), respectively. ^{1}H-MRS was performed using a single voxel point-resolved spectroscopy sequence; an echo time of 40 ms and repetition time of 5,000 ms were used. A survey scan was first performed in the abdominal region to help position a volume measuring 20 (AP) × 15 (RL) × 40 (FH) mm within the liver. The scanner's built-in body coil was used for both signal transmission and reception. No-water-suppressed spectra were acquired using 32 signal averages, and the data were exported for offline spectral analysis. Water (4.65 ppm) and lipid (1.3 ppm) peak amplitudes were measured to determine vertebral marrow fat content, which was defined as the relative fat signal amplitude in terms of a percentage of the total signal amplitude (water and fat) and calculated according to the following equation: fat content = $[I_{fat} / (I_{fat} + I_{water})] \times 100$, where I_{fat} and I_{water} are the peak amplitudes of fat and water, respectively. Correction for relaxation loss was not applied because of the relatively long repetition time and short echo time. A liver fat content of 5% was used to distinguish between patients with and without fatty liver (14).

Fat-water separation images were acquired using a TFE multislice spoiled gradient echo sequence, similar to a dual-echo DIXON sequence (15). To cover the visceral fat of the entire abdomen, two separate breath-hold acquisitions were acquired, superiorly delineated by the dome of diaphragm and inferiorly by the pubic symphysis. The sequence parameters were TR/TE = 5.8/2.1 ms, NEX = 1, FOV = 450 mm, matrix = 128 × 128, slice thickness = 20 mm, slice gap = 0, slice number = 12, and scan duration = 18 s. The acquired axial fat-water separation MR images were transferred to an offline workstation for computational analysis. The automatic in-house analysis software was developed using Insight Segmentation and Registration Toolkit (ITK) (http://www.itk.org). The fat quantification using

the software and a semiautomatic segmentation performed by an expert radiologist on 16 subjects showed excellent correlation ($r^2 = 0.98$, $P < 0.01$) for VAT as well for SAT ($r^2 = 0.99$, $P < 0.01$).

Sample analysis. The lipid, glucose, glycated hemoglobin, and laboratory safety parameters were measured by routine methods. Fasting insulin level was measured using the human insulin ELISA kit (DAKO, Dako Denmark A/S, Glostrup, Denmark). The absorbance was read in an ELISA plate reader (Micro-Quant, Bio-Tek Instruments Inc., Winooski, VT). Apolipoprotein AI, AII, B, CII, CIII, and E were measured using the immunoturbidity methods. Concentrations of apoB-48 were measured using a chemiluminescence enzyme immunoassay (CLEIA) kit (Fujirebio Inc., Tokyo, Japan) carried out on the Lumipulse *f* fully automated immunoassay analyzer (Fujirebio Inc.). Fasting FFA and hsCRP levels were determined using the enzymatic method and the immunonephelometric assay (Sekisui Medical Co., Ltd., Tokyo, Japan), respectively. Free cholesterol and lipoprotein(a) were measured by using commercial kits (Sekisui Medical Co., Ltd., Tokyo, Japan).

Genotyping

A 10 ml EDTA blood sample was collected from all subjects for DNA extraction and genotyping for the *DGAT2* rs3060 and rs10899116 polymorphisms, which were selected from the literature due to their high frequency in Chinese populations (13, 16). The genotyping was performed using the Taqman SNP genotyping assays (C_8750930_10 and C_31731223_10) and the ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA). The *DGAT2* rs3060 and rs10899116 polymorphisms were in Hardy-Weinberg equilibrium (χ^2 test $P > 0.05$) and were nearly in complete linkage disequilibrium ($R^2 = 0.95$) with the number of variant alleles of these two SNPs being identical for individual patients, except for one subject having one copy of the rs3060 C variant allele but without having a variant for the rs10899116 polymorphism. Therefore, only the *DGAT2* rs3060 polymorphism was included in the subsequent analysis as the rs3060 is located in the 3′UTR and may influence mRNA stability, and the analysis for the rs10899116 polymorphism would produce an almost identical result (16).

Statistical analysis

Skewed data were logarithmically transformed before analysis. Changes in parameters examined during the study were evaluated by paired *t*-test. Relationships between baseline liver fat content and total abdominal fat, TG, and FFA were evaluated by Pearson's correlation test. The effect of treatment with niacin on liver fat content was determined by using repeated-measures ANOVA, with time as within-subject factor (before versus after treatment) and age, gender, and changes in body weight as covariates. Characteristics of subjects with body weight loss $\geq$ 1 kg and body weight loss < 1 kg were compared by Student *t*-test or Mann-Whitney U test where appropriate. Changes in parameters during the study among the *DGAT2* rs3060 genotype groups were assessed by ANCOVA (ANCOVA) followed by a posthoc Bonferroni test with age, gender, respective baseline values, and changes in body weight as covariates. A stepwise multiple linear regression analysis was performed to evaluate the determinants of liver fat changes during the intervention with age, gender, body weight changes, baseline liver fat content, and the *DGAT2* rs3060 polymorphism as dependent variables. Differences were considered to be statistically significant, if the 2-sided *P* value was < 0.05. Data were analyzed using SPSS version 17.0 (SPSS, Inc., Chicago, IL).

RESULTS

Of the 46 Chinese patients with dyslipidemia who participated in this study, 7 patients withdrew because of flushing or rash (n = 5) or other personal reasons (n = 2). Of the 39 patients completing the study [27 male, 8 with diabetes, 22 on statins, ages 55.1 ± 9.1 years, baseline liver fat content 12.8 ± 7.6% (range: 1.4–37.4%)], baseline liver fat content was significantly correlated to total abdominal fat ($r = 0.502$, $P = 0.001$) but not to the baseline plasma TG or FFA level. There were significant ($P < 0.001$) increases in HDL-cholesterol (29.7 ± 27.1%) and reductions in triglycerides (−34.9 ± 37.6%), liver fat content (−47.2 ± 32.8%), and visceral fat (−6.3 ± 15.8%) with niacin treatment (**Table 1**). Thirty-one subjects were found to have fatty liver (liver fat content > 5%) at baseline, whereas after 23 weeks of treatment, only 19 subjects had liver fat content > 5% (79.3% versus 48.7% of subjects, $P < 0.01$). The liver enzyme values decreased during the study (Table 1).

Significant reductions in plasma free cholesterol, phospholipids, apolipoproteins AII, B, CII, CIII, and E, and hsCRP levels were observed in the study (Table 1). However, the effects of niacin on LDL-cholesterol and lipoprotein(a) levels were not significant. There were significant increases in fasting plasma FFA (48.2 ± 73.3%, $P < 0.005$), insulin (31.8 ± 55.0%, $P < 0.05$), glucose, and glycated hemoglobin levels during the study, and two patients needed increases in oral hypoglycemic therapy. The uric acid level was not changed in this group of patients.

Although the average body weight decreased by 1.5 ± 2.7% (1.17 ± 2.44 kg, $P < 0.001$) during the study, the liver fat changes with niacin treatment remained significant after adjustment for age, gender, and body weight changes, with a mean absolute change (95% CI) of −6.1% (−8.0, −4.3) ($P < 0.001$). There were no significant changes in total body fat and abdominal fat (Table 1). Greater reductions in liver fat content and VAT were observed in 20 subjects with weight loss $\geq$ 1 kg than in subjects with weight loss < 1 kg (n = 8) or weight gain (n = 11) during the study (**Table 2**). In 11 subjects with weight gain (+1.51 ± 1.03 kg), the liver fat content was reduced from 11.0 ± 5.0% at baseline to 8.5 ± 5.8% after treatment ($P < 0.05$).

The variant alleles of the *DGAT2* rs3060 or rs10899116 polymorphism were significantly associated with liver fat content changes in response to niacin treatment after adjustment for other covariates (**Table 3**, **Fig. 1**). Subjects with two copies of the variant allele had a smaller decrease in liver fat content compared with those with the homozygous wild-type (Table 3, Fig. 1). No relationship was found between the *DGAT2* rs3060 polymorphism and baseline liver fat content or other parameters at baseline or changes in body weight, VAT, plasma TG, FFA, insulin, or liver enzyme level (Table 3).

The scatter plots showed that the greater reductions in liver fat content in the *DGAT2* rs3060 TT genotype group may be largely driven by the three subjects with absolute reductions in liver fat content of > 20% (Fig. 1A). These three subjects were all females who had severe fatty liver at baseline with liver fat content of 23.8–37.4%, and their

TABLE 1. Subject characteristics before and after treatment with niacin

Parameter	Pre	Post	*P*
Body composition			
Body weight (kg)	80.9 ± 16.8	79.7 ± 16.7	0.005
Body mass index (kg/m^2)	29.9 ± 5.0	29.4 ± 5.0	0.003
Body fat (%)	31.8 ± 8.2	31.7 ± 8.7	0.950
Waist circumference (cm)	99.0 ± 10.8	97.7 ± 11.2	0.016
Hip circumference (cm)	103.2 ± 9.2	102.7 ± 9.5	0.425
Total abdomen fat (cm^3)	11947 ± 3897	11557 ± 4011	0.063
Visceral adipose tissue (cm^3)	3751 ± 1489	3530 ± 1579	0.031
Subcutaneous fat (cm^3)	8196 ± 3369	8027 ± 3334	0.226
Liver fat (%)	12.8 ± 7.6	6.7 ± 6.1	<0.001
Fatty liver (liver fat content > 5%)	31 (79.5%)	19 (48.7%)	<0.01
Metabolic characteristics			
Total cholesterol (mmol/l)	4.82 ± 0.72	4.53 ± 0.95	0.018
HDL-cholesterol (mmol/l)	1.03 ± 0.21	1.32 ± 0.27	<0.001
LDL-cholesterol (mmol/l)	2.40 ± 0.82	2.35 ± 0.87	0.623
Triglycerides (mmol/l)	3.30 ± 1.67	1.88 ± 1.54	<0.001
Free fatty acids (μEq/l)	552.4 ± 186.1	768.2 ± 332.6	0.004
Free cholesterol (mg/dl)	54.4 ± 8.1	48.3 ± 10.5	<0.001
Phospholipids (mg/dl)	214.2 ± 26.5	192.0 ± 35.0	<0.001
apoAI (mg/dl)	125.4 ± 15.3	130.2 ± 19.0	0.017
apoAII (mg/dl)	29.9 ± 4.8	27.4 ± 6.2	0.002
apoB (mg/dl)	101.5 ± 19.2	86.0 ± 23.3	<0.001
apoB-48 (μg/ml)	7.92 ± 5.84	6.45 ± 5.98	0.270
apoCII (mg/dl)	7.38 ± 3.13	5.19 ± 3.94	<0.001
apoCIII (mg/dl)	16.1 ± 5.29	11.5 ± 5.58	<0.001
apoE (mg/dl)	5.41 ± 2.03	3.53 ± 2.01	<0.001
Lp(a) (mg/dl)	14.62 ± 21.73	14.42 ± 18.74	0.838
hsCRP (mg/l)	0.325 ± 0.320	0.254 ± 0.278	0.043
Fasting insulin (μIU/ml)	17.05 ± 10.32	21.17 ± 14.06	0.023
Fasting glucose (mmol/l)	5.66 ± 0.79	6.46 ± 1.79	0.001
Glycated hemoglobin (%)	5.85 ± 0.80	6.37 ± 1.15	<0.001
Uric acid (mmol/l)	0.43 ± 0.08	0.43 ± 0.09	0.703
Bilirubin (μmol/l)	11.9 ± 5.7	12.5 ± 4.8	0.299
Gamma-glutamyltransferase (U/l)	41.6 ± 18.2	37.1 ± 26.6	0.019
Alkaline phosphatase (U/l)	33.0 ± 12.5	28.5 ± 11.6	0.003
Alanine aminotransferase (IU/l)	67.2 ± 15.7	63.0 ± 16.0	0.026

Apo, apolipoprotein; hsCRP, high sensitivity C-reactive protein; Lp(a), lipoprotein(a).

body weights were reduced by 0.7 kg, 3.4 kg, and 4.6 kg, respectively, during the study. The association tended to become less evident after removing these three subjects from the analysis, but the difference between the two homozygote groups remained statistically significant (−6.4 versus −5.7 versus −1.5, $P = 0.013$ by ANCOVA).

Multivariable regression analysis revealed that baseline liver fat content ($r = -0.640$, $P < 0.001$), body weight changes ($r = 0.392$, $P < 0.001$), the *DGAT2* rs3060 polymorphism ($r = 0.331$, $P = 0.002$), and age ($r = -0.321$, $P = 0.004$) were determinants of the absolute liver fat change during the study, and these factors explained 67% of the variance in liver fat changes in response to niacin treatment.

DISCUSSION

It has been well known that long-term treatment with niacin is associated with a rebound in plasma FFA levels and insulin resistance, but its effect on liver fat content has not been established. As increased circulating FFA flux to the liver is associated with excessive accumulation of TG within hepatocytes, such a rebound occurring during chronic niacin treatment might worsen hepatic steatosis. However, the present study, for the first time demonstrated that ER niacin (2 g/day) treatment significantly reduced liver fat content in Chinese patients with dyslipidemia and that this effect might be mediated by inhibition of DGAT2 in the liver.

Liver fat content is closely related to obesity, and body weight reduction is usually associated with significant improvement in hepatic steatosis (17). An early lifestyle intervention program reported that a 3.1% body weight reduction with diet modification and increases in physical activity was associated with a 29.1% decrease in liver fat content in Caucasian subjects (13). Niacin did not seem to affect body weight in previous randomized controlled trials (12, 18), but in the present study, there was a small but significant reduction (1.5%) in mean body weight, which is likely to be due to changes in lifestyle in study subjects participating in the clinical trial. The liver fat content was reduced by 47.2% during the study, which is much greater than that reported in the lifestyle intervention study, and this change remained significant after adjustment for body weight changes. There were significant increases in plasma FFA and insulin levels, suggesting a significant drug effect as weight reduction is usually associated with decreases in FFA and insulin levels. The change in FFA levels during niacin treatment was correlated to the change in insulin ($r = 0.332$, $P < 0.05$).

The effect of niacin on liver fat content, which was previously examined in a small randomized controlled trial in obese subjects with NAFLD, showed that ER niacin treatment (16 weeks, 2 g/day) had no effect on liver fat content in nine subjects compared with those on placebo (n = 9, 8 weeks) (12). The discrepancy between the two studies may be due to the different study design. The previous study was conducted in obese subjects (mean body mass index: 36 kg/m^2) with NAFLD (mean liver fat content: 23%) who had plasma TG level of < 3.4 mmol/l (mean TG level: 1.7 mmol/l), whereas our study was performed in Chinese dyslipidemic patients who were less obese (mean body mass index: 29.9 kg/m^2) but who had higher plasma TG levels (mean value: 3.3 mmol/l). The different causes of fat accumulation in the liver between the two studies probably contribute to the variable outcomes. These results suggest that niacin might reduce liver fat content in subjects with elevated plasma TG levels but perhaps not in subjects with other causes for NAFLD, such as obesity or diabetes. Although the small reduction in body weight may contribute to liver fat reduction in our study, the liver fat content was still reduced by 24.3% [95% CI (4.5, 44.2), $P < 0.05$] in the 11 subjects showing an increase in body weight.

The DGAT2 enzyme catalyzes the final step of triglyceride biosynthesis, in which fatty acyl-CoA and diacylglycerol molecules covalently join to form TG. Overexpression of hepatic DGAT2 causes significant hepatic steatosis as evidenced by increased hepatic TG levels but not insulin resistance in mice (19), whereas inhibition of Dgat2 with an optimized antisense oligonucleotide in obese mice

TABLE 2. Characteristics of subjects with body weight loss ⩾ 1 kg or body weight loss < 1 kg

Parameter	Weight Loss		P
	⩾1 kg (n = 20)	<1 kg (n = 19)	
Age	54.2 ± 8.0	56.1 ± 10.4	0.728
Male, n (%)	12 (60.0)	15 (78.9)	0.2
Diabetes, n (%)	6 (30.0)	2 (10.5)	0.235
Body weight at baseline (kg)	81.7 ± 18.3	80.1 ± 15.6	0.768
VAT at baseline (cm^3)	3857 ± 1705	3530 ± 1293	0.505
SAT at baseline (cm^3)	8575 ± 2995	7717 ± 3686	0.429
Liver fat content at baseline (%)	14.6 ± 8.0	11.0 ± 6.9	0.206
HDL-cholesterol at baseline (mmol/l)	1.01 ± 0.16	1.06 ± 0.25	0.396
LDL-cholesterol at baseline (mmol/l)	2.18 ± 0.79	2.63 ± 0.80	0.091
Triglycerides at baseline (mmol/l)	3.39 ± 1.89	3.20 ± 1.46	0.735
Absolute change in body weight (kg)	−2.9 ± 2.0	0.7 ± 1.3	<0.001
% Change in body weight	−3.5 ± 1.8	0.7 ± 1.6	<0.001
% Change in VAT	−11.0 ± 15.2	−0.9 ± 15.0	0.047
% Change in SAT	−1.7 ± 12.4	−1.9 ± 10.6	0.675
% Absolute change in liver fat content	−8.3 ± 6.3	−3.9 ± 6.2	0.006
% Change in liver fat content	−58.6 ± 25.7	−35.3 ± 35.9	0.026
% Change in fasting free fatty acids	53.2 ± 93.5	46.2 ± 44.8	0.627
% Change in fasting insulin	20.5 ± 49.2	43.7 ± 59.5	0.19
% Change in HDL-cholesterol	34.0 ± 28.0	25.2 ± 26.1	0.109
% Change in triglycerides	−44.1 ± 37.0	−25.2 ± 36.8	0.119
% Change in LDL-cholesterol	3.5 ± 33.3	−1.2 ± 24.5	0.631

SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

resulted in marked reduction in hepatic TG as well as plasma TG, diacylglycerol, and FFA levels (11). In vitro and animal studies also suggested that upregulation of DGAT2 may be involved in the hepatic fat accumulation induced by chronic alcohol consumption (20). Niacin was demonstrated to directly and noncompetitively inhibit the activity of DGAT2 in cell lines (9), and our study is the first to show a significant association between the *DGAT2* polymorphism and the liver fat changes in response to niacin treatment in patients with dyslipidemia. The result of the study suggests that DGAT2 may play a role in the pharmacological effect of niacin. There was no statistically significant difference in plasma TG responses among the *DGAT2* genotype groups, although subjects with variant alleles tended to have a smaller reduction in plasma TG levels.

In humans, the *DGAT2* gene is located at chromosome 11q13.3 comprising eight exons (21). Seventeen mutations have been identified in the coding region, the predicted promoter region, and in the 5′ noncoding exon in human *DGAT2*, but the functionality of these polymorphisms in the human *DGAT2* gene have not been evaluated or reported (16). Most of these reported mutations are rare in Chinese populations. This study evaluated the two common polymorphisms in *DGAT2*, rs3060 and rs101988116, which are located at the 3′ UTR and 5′ UTR in *DGAT2*, respectively. Although rs10899116 did not significantly affect liver fat changes during lifestyle intervention in a previous study in patients with fatty liver (13), the present study showed that the variant alleles of the *DGAT2* rs3060 or rs101988116 polymorphism were associated with smaller liver fat changes in response to niacin treatment, suggesting these noncoding polymorphisms might be related to functional effects and could affect the pharmacodynamics of niacin. A recent study in pigs, in which the *DGAT2* gene is 90% identical to the homologous gene of humans, showed that an A/G variation in the 3′ UTR of *DGAT2* was

TABLE 3. Study parameters stratified by the *DGAT2* rs3060 (T>C) genotype

Parameters	TT (n = 17)	TC (n = 16)	CC (n = 6)	P
Baseline body weight (kg)	76.5 (68.1, 85.0)	81.6 (74.3, 88.9)	91.2 (67.2, 115.3)	0.181
Absolute change in weight (kg)	−1.09 (−2.39, 0.20)	−0.81 (−2.09, 0.48)	−2.34 (−4.54, −0.14)	0.470
% Change in body weight	−1.47 (−2.91, −0.03)	−1.02 (−2.45, 0.42)	−2.59 (−5.04, −0.14)	0.524
Baseline VAT (cm^3)	3381 (2826, 3936)	4036 (3118, 4954)	3692 (1611, 5773)	0.471
Absolute change in VAT (cm^3)	−337 (−668, −6)	−161 (−510, 188)	−43 (−596, 510)	0.612
% Change in VAT	−10.4 (−18.8, −2.0)	−3.6 (−12.5, 5.2)	−1.0 (−15.5, 13.0)	0.409
% Baseline liver fat	15.0 (10.4, 19.6)	10.4 (7.1, 13.7)	13.3 (7.4, 19.3)	0.218
% Absolute change in liver fat	−8.2 (−10.1, −6.2)	−5.7 (−7.7, −3.7)	−1.5 (−4.8, 1.9)[a]	0.005
% Change in liver fat	−60.9 (−73.7, −48.2)	−41.0 (−54.1, −27.9)	−25.2 (−46.8, −3.5)[b]	0.015
% Change in triglycerides	−41.9 (−57.2, −26.6)	−33.1 (−48.5, −17.7)	−20.0 (−46.2, 6.3)	0.361
% Change in free fatty acids	47.6 (10.9, 84.3)	48.9 (11.5, 86.3)	48.2 (−13.9, 110.3)	0.999
% Change in insulin	32.6 (3.8, 61.4)	21.9 (−7.0, 50.8)	55.8 (6.3, 105.2)	0.485

VAT, visceral adipose tissue. Data are mean (95% CI). Changes in body weight were adjusted for body weight at baseline, age, and gender. Changes in liver fat and other parameters were adjusted for the respective parameters at baseline, age, gender, and body weight changes during the study. Baseline values were compared by ANOVA.

[a] $P = 0.004$ for TT versus CC.

[b] $P = 0.023$ for TT versus CC.

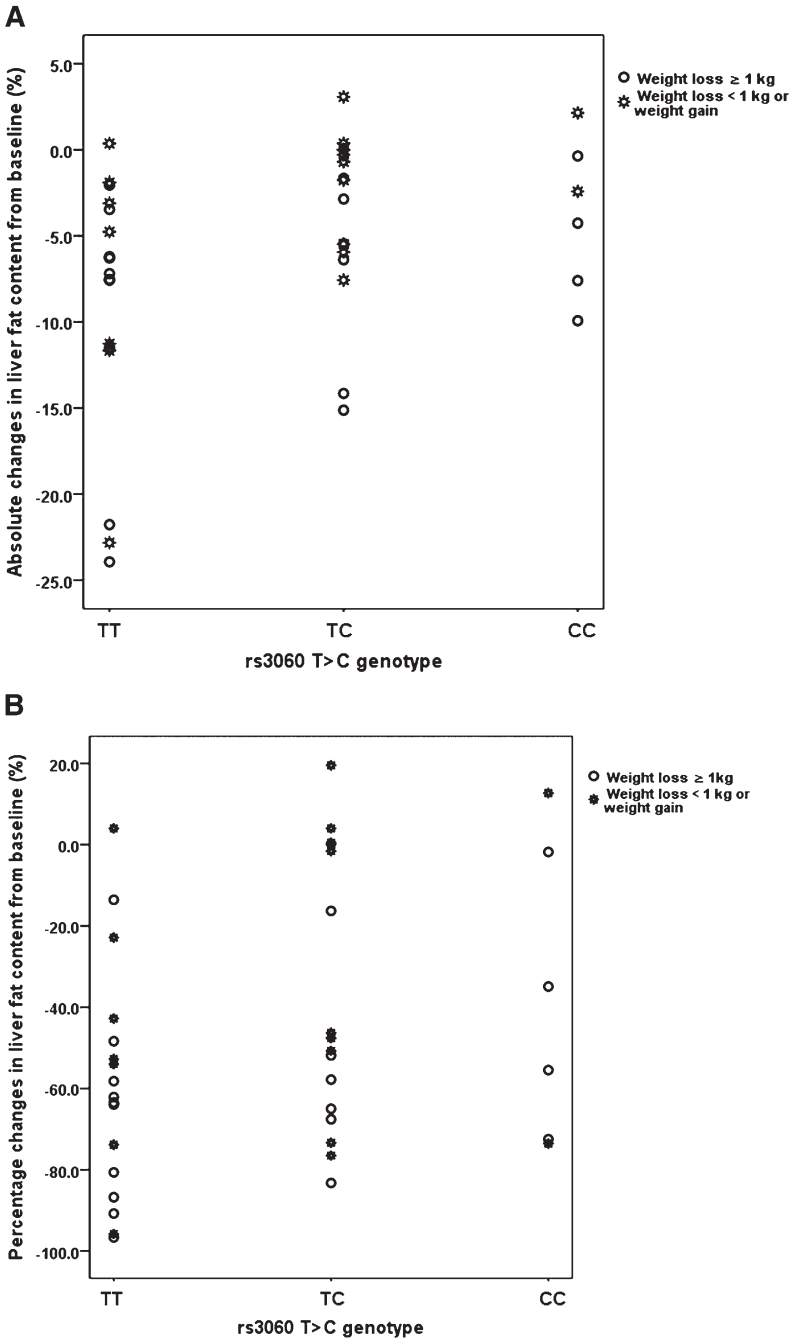

Fig. 1. Effect of the *DGAT2* rs3060 polymorphism on the absolute changes (A) and percentage changes (B) in liver fat content during the study.

significantly associated with back-fat thickness, and this may indicate that polymorphisms in the 3′ UTR of *DGAT2* influence the function of the gene (22). The *DGAT2* polymorphisms were not associated with obesity or body weight changes during the study, as shown in previous studies (13, 16),

Animal studies suggested that inhibition of Dgat2 with antisense oligonucleotide caused a reduction in liver fat and plasma TG and FFA levels (11, 23). In the present study, the plasma FFA levels taken at 12 h after dosing were increased, although the plasma FFA levels within 4 h after dosing might still be reduced as observed in an early metabolic study (8). The mechanism of FFA rebound with chronic niacin treatment remains unknown, but a recent microarray analysis revealed that the FFA rebound after 24 h niacin infusion in rats was associated with many changes in gene expression in adipocytes, including a significant reduction in expression of phosphodiesterase 3B, which would increase cAMP levels and thus lipolysis, and reductions in the expression of key enzymes of TG synthesis, such as 1-acylglycerol-3-phosphate *O*-acyltransferase and DGAT (24). These changes in gene expression in adipocytes may contribute to the increase in basal lipolysis in adipocytes and FFA rebound, which may be responsible for the development of insulin resistance during long-term treatment with niacin (24, 25).

This study showed that long-term niacin treatment improved hepatic steatosis, but whether this effect is beneficial is unknown. Emerging data provide convincing evidence that hepatic TG accumulation does not cause insulin resistance or

cellular injury in the liver, although it may not be totally benign, whereas the non-TG fatty acid metabolites may play a central role in hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis (26). Studies in animal models of progressive NAFLD demonstrated that inhibition of DGAT2 with antisense oligonucleotides improved liver fat content in obese diabetic mice but worsened liver injury and fibrosis (23), which is probably due to increased hepatic FFA levels leading to induction of fatty acid oxidizing systems that increased hepatic oxidative stress and liver damage (23, 27). Niacin, in particular in its sustained-release form, has been shown to be associated with hepatotoxicity (28). In the present study, niacin reduced liver fat content and increased plasma FFA levels (at 12 h after dosing), which might be associated with increased hepatic FFA levels and increased hepatic oxidative stress. However, improvements in liver enzymes, including ALT, ALP, and GGT, were observed during the study, and these may be associated with liver fat reduction in the study.

This study demonstrated that treatment with ER niacin significantly improved multiple atherogenic lipid profiles in Chinese patients with dyslipidemia as expected (5). However, the LDL-cholesterol and lipoprotein(a) levels were unchanged during the study. Further analysis revealed that the LDL-cholesterol and lipoprotein(a) responses appeared to be inversely related to baseline TG levels in this group of patients with hypertriglyceridemia ($r = -0.439$, $P < 0.01$ and $r = -0.35$, $P < 0.05$, respectively), with subjects with higher baseline TG levels having a smaller reduction or even an increase in LDL-cholesterol in response to niacin. Increased LDL-cholesterol accompanied by reductions in TG has been reported with treatment of high doses of fish oil or certain fibrates in patients with hypertriglyceridemia (29, 30). These observations may be due to increased lipolysis of very low-density lipoprotein (VLDL) particles promoting formation of larger and less dense LDL particles, which were thought to be less atherogenic than small, dense LDL particles, or down-regulation of the LDL receptors and increased oxidation of LDL particles (31, 32). Although the underlying mechanism for the association between LDL-cholesterol response to niacin and TG levels is unclear, it may be related to the complex interaction between TG, VLDL-cholesterol, and LDL-cholesterol synthesis and degradation pathways.

To our knowledge, this study is the first to report that ER niacin may reduce plasma and hepatic TG levels in Chinese patients with dyslipidemia who have an increased risk of NAFLD. The study was strengthened by employing the most accurate and reliable technique and validation methods to quantify liver fat content and by closely monitoring drug compliance during the study. This study has several limitations. One potential limitation is its uncontrolled design. However, a double-blind randomized controlled design may not be feasible with niacin treatment due to the common side effect of flushing induced by the drug. The small but significant reduction in the body weight during the study may confound the outcome of the study. This and the uncontrolled design make it difficult to assess the exact effect of niacin on the liver fat changes, as liver fat content is very sensitive to small changes in energy balance. Future large randomized controlled trials are needed to verify the true effect of niacin on liver fat change. In addition, whether the rs3060 polymorphism in *DGAT2* examined in the study could influence the function of the gene remains unknown and needs to be evaluated. Furthermore, this study only measured the plasma FFA at approximately 12 h after dosing but did not assess the acute effect of ER niacin on FFA levels, although previous metabolic data with ER niacin (2 g for 12 weeks) in subjects with metabolic syndrome may provide a clue to changes in FFA over time after the dose (8). The histological response assessment may help to provide a better understanding of the effect of niacin on NAFLD, but this was not evaluated in the study.

In conclusion, this pilot study suggested that ER niacin therapy reduced liver fat content in Chinese patients with dyslipidemia and that the common polymorphisms in *DGAT2* might influence the hepatic TG response to niacin; however, this result may be significantly confounded by the body weight changes during the study. Future large randomized studies are warranted to confirm or refute this finding and to investigate the significance of this hepatic TG reduction on clinical outcomes in patients with dyslipidemia. The increases in FFA and insulin levels during long-term treatment of niacin may offset some of the beneficial effects on cardiovascular risk.

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