Improvement of Nonalcoholic Fatty Liver Disease With Carnitine-Orotate Complex in Type 2 Diabetes (CORONA): A Randomized Controlled Trial

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OBJECTIVE

We aimed to evaluate the effects of carnitine-orotate complex in patients with nonalcoholic fatty liver disease (NAFLD) and diabetes.

RESEARCH DESIGN AND METHODS

Eight hospitals in Korea participated in this randomized, controlled, double-blind trial of patients with diabetes and NAFLD. Seventy-eight patients were randomly assigned in a 1:1 ratio to receive carnitine-orotate complex (824 mg, three times daily) or matching placebo. The primary study outcome was decline in alanine aminotransferase (ALT) to the normal range. Secondary study outcomes were change in ALT, radiological hepatic steatosis, parameters for anthropometry, liver function, lipid profiles, and glycemic control. Hepatic steatosis was assessed using Hounsfield units on noncontrast computed tomography (CT) imaging with hepatic attenuation.

RESULTS

After 12 weeks of treatment, compared with placebo group, carnitine-orotate complex–treated participants had a significantly higher rate of normalization of serum ALT level (17.9% vs. 89.7%, $P < 0.001$). On hepatic CT analysis, participants treated with carnitine-orotate complex showed an increased liver attenuation index (0.74 ± 8.05 vs. 6.21 ± 8.96, $P < 0.008$). A significant decrease in HbA_{1c} was observed in the carnitine-orotate complex group (−0.33 ± 0.82% [−3.6 ± 9.0 mmol/mol], $P = 0.007$), but no significant change was seen in the placebo group.

CONCLUSIONS

Treatment with carnitine-orotate complex improves serum ALT and may improve hepatic steatosis as assessed by CT in patients with diabetes and NAFLD. Further studies using more advanced magnetic resonance imaging and liver histology as an end point are needed to assess its efficacy in NAFLD.

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Ectopic fat accumulation in a visceral organ is associated with insulin resistance (1). As an example of such ectopic fat accumulation, nonalcoholic fatty liver disease (NAFLD) is now recognized as the hepatic component of metabolic syndrome and is even reportedly associated with insulin resistance independent of obesity and other metabolic components. Therefore, NAFLD can be a major determinant of insulin

resistance (2,3). Recent study results have shown that NAFLD independently increases one's risk of diabetes and cardiovascular diseases (4–8), which suggests that treatment of fatty liver might ameliorate the risk of type 2 diabetes and cardiovascular diseases. Unfortunately, the only established treatment is weight loss by lifestyle intervention, including qualitative dietary changes and exercise, although recent randomized controlled trials have shown promising beneficial effects of pharmacological treatment with antioxidants, insulin sensitizers, pentoxifylline, ursodeoxycholic acid, and lipid-lowering drugs (9).

Reduced fatty acid oxidation in hepatocytes is related with hepatic steatosis. Mitochondrial dysfunction is reported to cause hepatic fat accumulation, and oxidative stress could explain the mitochondrial dysfunction found in NAFLD (10). Carnitine is a known modulator of mitochondrial free fatty acid transport and oxidation (11,12), and several studies have demonstrated the antioxidant activity of carnitine in hepatocytes (13–15). In a recent clinical trial, carnitine improved hepatic steatosis (16). Furthermore, in vivo, in vitro, and human studies have revealed a potential role of carnitine in the regulation of glucose and insulin sensitivity (16–18).

In this study, we aimed to evaluate the effects of carnitine-orotate complex (Godex, 412 mg/capsule; Celltrion Pharm, Seoul, Korea) in patients with NAFLD and diabetes.

RESEARCH DESIGN AND METHODS

Study Design

A multicenter, randomized, double-blind, placebo-controlled clinical trial of Carnitine-OROtate in NAFLD patients with diabetes (CORONA) was performed at eight hospitals in Korea between 7 September 2011 and 12 October 2012. Patients were randomly assigned to receive carnitine-orotate complex or placebo during the 12-week treatment period. One capsule (412 mg) of carnitine-orotate complex consists of carnitine-orotate (150 mg), biphenyl dimethyl dicarboxylate (25 mg), adenine (2.5 mg), cyanocobalamin (0.125 mg), pyridoxine (25 mg), and riboflavin (0.5 mg). Patients already taking stable doses of antidiabetic drugs continued their usual treatment throughout the study.

The primary study outcome was a decline in alanine aminotransferase (ALT) to the normal range (30 IU/L in men or <19 IU/L in women) at week 12 in subjects who entered the study with ALT 50–250 IU/L. The secondary study outcomes were change in ALT, radiological hepatic steatosis, other parameters for anthropometry, liver function, lipid profiles, and glycemic control from baseline. Hepatic steatosis was assessed using Hounsfield units (HU) on noncontrast computed tomography (CT) imaging with hepatic attenuation. The study was approved by the independent institutional review boards of each hospital before patient enrollment. Written informed consent was obtained from each participant. The trial was performed in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines.

Subjects

Individuals aged 20–70 years with known diabetes who met all of the following inclusion criteria were eligible to participate in the study: 1) a previous diagnosis of type 2 diabetes at least 3 months before screening, 2) glycated hemoglobin (HbA$_{1c}$) greater than 6.4% (46 mmol/mol) or fasting plasma glucose 130–300 mg/dL (inclusive) at screening, and 3) ALT 50–250 IU/L at screening. We excluded subjects with alcohol intake >30 g/day in men or >20 g/day in women, those with positive serologic markers for hepatitis A, B, or C virus at screening, those with known history or clinical evidence of liver cirrhosis (serum total bilirubin ≥1.8 mg/dL, albumin <3.5 g/dL, international normalized ratio >1.3, or platelet count <150,000/mm^3), and those with other liver disease such as acute hepatitis or drug-induced hepatitis (19). We also excluded subjects taking thiazolidinediones for treatment of diabetes, those who received an antiobesity drug within 1 month before screening, those with a history of malignancy, those with a history of severe heart disease (angioplasty, stent placement, bypass surgery, myocardial infarction, unstable angina pectoris, congestive heart failure, or ventricular arrhythmia within 6 months before screening), and women who were pregnant or lactating.

Individuals who met inclusion and exclusion criteria (n = 88) underwent hepatic CT at each study site. Subjects

without fatty liver on hepatic CT (n = 10) were then excluded. Of 123 patients who were screened, 78 were eligible for the study. Among the 88 patients who underwent hepatic CT before randomization, contrast-enhanced CT was performed in 6 (CT protocol deviation). All 6 of these patients with protocol deviation showed fatty liver on the contrast CT image, did not undergo follow-up CT, and were excluded from the hepatic CT attenuation analysis (Supplementary Fig. 1).

Randomization

Investigators enrolled patients, and eligible participants were randomly assigned in a 1:1 ratio to receive the carnitine-orotate complex capsule or matching placebo. The randomization sequence was produced by an independent clinical research organization (Medical Excellence, Seoul, Korea) and was computer-generated and stratified by sites with block sizes of four. Allocation concealment was implemented by use of sequentially numbered, opaque, and sealed envelopes. All patients and investigators were masked to the treatment assignment. The matching placebo capsule was identical in appearance, taste, and smell to the carnitine-orotate complex capsule.

Procedure

After baseline vital signs and anthropometric measurements were collected, participants were randomly assigned to treatment groups and instructed to take the first dose of the study drug at the study site. Randomization was performed within 28 days after the blood sample was collected at the screening visit. Throughout the 12-week treatment period, all trial medications were provided by Celltrion Pharm. Patients were instructed to take the medication as two capsules orally, three times daily after a meal. In the treatment group, a daily dose of carnitine-orotate complex totaled 2,472 mg. Participating patients were instructed to maintain their usual diet and exercise patterns throughout the study period. Participating investigators were instructed to maintain the patient's usual medication regimen, including antidiabetic, antihypertensive, antiplatelet, and lipid-lowering drugs without changes during the 12-week treatment period. The concurrently used medications were reviewed at every visit.

At screening, participants were surveyed for demographic information,

medical histories, and information on alcohol consumption. Vital sign and anthropometric measurements, except for height, were collected at the screening, on day 1 of treatment (baseline), and at weeks 6 and 12. Height was measured only at screening. Blood samples for hematologic and biochemical assays were obtained from the antecubital vein after an overnight fast ($\geq$12 h) at screening and at weeks 6 and 12. Blood chemistry testing included liver function tests, lipid profile, and glycemic parameters. Serological testing for hepatitis A, B and C was performed at the screening. Changes in insulin resistance were assessed with the HOMA of insulin resistance (HOMA-IR): HOMA-IR = fasting insulin (μIU/mL) $\times$ fasting plasma glucose (mmol/L)/22.5. β-Cell insulin secretion was assessed with the HOMA of β-cell function (HOMA-B).

Safety variables were adverse events and abnormal findings related to physical examination, vital signs, and laboratory testing. Treatment-emergency adverse events were analyzed from the time the patient was first given the study drug to 28 days after the end of treatment. All clinical and safety laboratory samples were analyzed by a central laboratory (Neodin Medical Institute, Seoul, Korea; Clinical Laboratory Improvement Amendments certified) using standard validated methods. Plasma ALT was measured by the enzymatic method using a commercial kit (Sekisui Medical, Tokyo, Japan) on a Hitachi 7180 biochemical analyzer (Hitachi Ltd., Tokyo, Japan). Measurement of HbA$_{1c}$ was performed using high-performance liquid chromatography with a Tosoh HLC-723 G8 automatic analyzer (Tosoh Corp., Tokyo, Japan).

CT Imaging

Noncontrast CT scans of the liver were performed before randomization at 120 kVp, 50–75 mAs, and 5-mm slice thickness. Liver CT examinations for follow-up were performed at the end of the study visit (week 12) in the same way. Contiguous transverse images were obtained from the dome of the diaphragm to the bottom of the liver during a single breath hold. All scans were performed in each hospital, and five different multidetector CT scanners were used, including a 16-detector LightSpeed Ultra 16 (GE Healthcare Milwaukee, WI)

($n = 12$), a 16-detector Somatom Sensation 16 (Siemens Healthcare, Forchheim, Germany) ($n = 20$), a 64-detector LightSpeed VCT (GE Healthcare) ($n = 18$), a 64-detector Brilliance 64 (Philips Healthcare, Best, the Netherlands) ($n = 12$), and a 128-detector Somatom Definition AS+ (Siemens Healthcare) ($n = 16$).

Images were reviewed on a picture archiving and communication system (Centricity 1.0; Integrated Imaging Solutions, GE Medical Systems, Mt. Prospect, IL) monitor by one abdominal radiologist blinded to patient data and randomization status. When interpreting follow-up CT images, the radiologist was also blinded to initial CT image results. Hepatic steatosis was assessed using HU on CT with hepatic attenuation. For each case, the hepatic attenuation was measured by means of 12 circular regions of interest (ROIs) on three transverse sections at different hepatic levels containing the confluence of the right hepatic vein, the umbilical portion of left portal vein, and the posterior branch of the right portal vein. At each representative level, the liver was divided into four sectors (right posterior, right anterior, left medial, and left lateral). One ROI was randomly drawn inside each sector, avoiding the large vessels and any focal lesions. Mean splenic attenuation was also calculated by three random area ROI values of attenuation measurement on three transverse sections at different splenic levels. The size of each ROI was defined as 1.0–1.1 cm^2 (20). With splenic attenuation acting as a control or reference value, the liver attenuation index (LAI), defined as the difference between mean hepatic attenuation and mean splenic attenuation, was used as an indicator of the degree of hepatic steatosis. Fatty liver disease was defined as a LAI below 5 HU on the unenhanced hepatic CT image at screening (21) and as an LAI below -18.5 HU on the contrast-enhanced CT image (22). LAI in all subjects who underwent contrast-enhanced CT ($n = 6$) was evaluated between 80 and 120 s after injection of a contrast bolus.

Statistical Analyses

Except for hepatic fat content analysis, all randomly assigned patients (intention to treat) were included in the analysis of results (Supplementary Fig. 1). The independent t test and paired t test were used for analyzing normally

distributed continuous variables. The Wilcoxon rank sum test, Wilcoxon signed rank test, and Kruskal-Wallis test were used for analyzing nonnormally distributed continuous variables. Differences in baseline characteristics between carnitine-orotate complex and placebo groups were analyzed using the independent t test or the Wilcoxon rank sum test. Intrasubject differences in variables between baseline and week 12 were analyzed using the paired t test or the Wilcoxon signed rank test with last observation carried forward imputation. The Wilcoxon rank sum test or the independent t test was used for comparing the changes in all baseline variables between treatment groups except ALT, aspartate aminotransferase (AST), and BMI. Because the baseline BMI, AST, and ALT levels were higher in the carnitine-orotate complex group, ANCOVA with baseline values as covariates was used for analyzing the changes in these variables between treatment groups. Simple correlation analysis was performed between the change in ALT levels and the change in the LAI. When subjects who underwent follow-up CT scan were classified into tertiles by LAI changes from baseline at 12 weeks, the Kruskal-Wallis test was used to compare the changes in variables between tertile groups. Statistical data analysis was performed using SAS 9.3.1 software (SAS Institute, Inc., Cary, NC). All the reported P values are two-tailed, and statistical significance was set at $P < 0.005$.

The sample size was calculated assuming that the proportion of patients with a decline in ALT to the normal range at week 12 would be 65% in the treatment group and 30% in the placebo group. A sample size of 64 patients would provide statistical power of 82%, with two-sided α level of 0.05. A final sample size of 78 was selected assuming 20% lost to follow-up.

RESULTS

The participants were obese, with a mean BMI of 27.4 kg/m^2 and waist circumference of 93.7 cm. Participants were a mean age of 51 years, and 69% were men. Baseline clinical characteristics of the two randomized treatment groups were similar, except for the BMI, AST, and ALT levels, which were higher in the carnitine-orotate complex group than in the placebo group (Table 1). In particular,

Table 1—Baseline characteristics of participants

	Carnitine complex ($n = 39$)	Placebo ($n = 39$)	P value
Age (years)	50.6 ± 9.3	52 ± 9.4	0.522*
Male	25 (64.1)	29 (74.4)	0.326†
Weight (kg)	76.3 ± 11.5	74.8 ± 12.0	0.567*
BMI (kg/m^2)	28.2 ± 2.6	26.7 ± 3.7	0.007‡
Waist circumference (cm)	93.6 ± 6.5	93.8 ± 9.0	0.641*
Fasting glucose (mg/dL)	143.6 ± 32.2	153.4 ± 64.4	0.317‡
HbA$_{1c}$ (%)	7.64 ± 0.94	7.64 ± 0.95	0.988‡
HbA$_{1c}$ (mmol/mol)	60.0 ± 10.3	60.0 ± 10.4	0.988‡
HOMA-IR	5.69 ± 4.41	6.29 ± 6.99	0.320‡
HOMA-B	80.7 ± 63.1	86.0 ± 104.3	0.579‡
AST (IU/L)	61.8 ± 25.5	51.7 ± 22.1	0.034‡
ALT (IU/L)	94.9 ± 36.4	79.2 ± 27.2	0.008‡
ALP (IU/L)	151.8 ± 103.2	169.6 ± 122.7	0.441‡
γ-GT (IU/L)	94.6 ± 78.2	73.5 ± 51.3	0.242‡
Systolic BP (mmHg)	129.6 ± 13.5	126.1 ± 12.1	0.226*
Triglyceride (mg/dL)	227.6 ± 273.9	188.1 ± 164.3	0.310‡
HDL-cholesterol (mg/dL)	44.3 ± 7.1	44.1 ± 7.9	0.893*
LDL-cholesterol (mg/dL)	97.8 ± 36.9	96.4 ± 28.0	0.844*
CT attenuation (HU)	$n = 36$	$n = 36$	
Whole liver	39.6 ± 10.8	42.8 ± 9.3	0.213‡
Spleen	50.7 ± 3.4	51.9 ± 4.3	0.225‡
Whole liver-spleen (LAI)	−11.1 ± 10.3	−8.8 ± 9.3	0.308‡
Concomitant medication			
Metformin	34 (87.2)	32 (82.1)	0.530†
Sulfonylurea	15 (38.5)	23 (59.0)	0.070†
DPP-4 inhibitor	18 (46.2)	14 (35.9)	0.357†
Statin	26 (66.7)	23 (59.0)	0.482†
Antiplatelet	14 (35.9)	23 (59.0)	0.041†
ACE inhibitor/AT-1 antagonist	15 (38.5)	14 (35.9)	0.815†

Data are mean ± SD or n (%). ALP, alkaline phosphatase; AT-1, angiotensin II type 1 receptor; BP, blood pressure; DPP-4, dipeptidyl peptidase 4; γ-GT, γ-glutamyl-transpeptidase. *By independent t test. †By Pearson χ^2 test. ‡By Wilcoxon rank sum test.

no differences were seen in mean liver attenuation level, LAI, glycemic level, or diabetes medications between the two randomized treatment groups at baseline.

After 12 weeks of treatment, 89.7% (35 of 39) of participants treated with carnitine-orotate complex showed normalization of serum ALT levels (<30 IU/L in men or <19 IU/L in women), whereas only 17.9% showed normalization in the placebo group ($P < 0.001$ between groups) (Fig. 1A). At weeks 6 and 12, a significant reduction in ALT levels from baseline was noted in the carnitine-orotate complex group ($P < 0.001$ vs. placebo) (Supplementary Fig. 2). The mean change in the ALT level from baseline at week 12 was −73.7 ± 38.7 IU/L in the carnitine-orotate complex group ($P < 0.001$) compared with −5.4 ± 37.1 IU/L in the placebo group ($P = 0.369$) (Fig. 1B and Table 2). On the hepatic CT analysis, participants treated with carnitine-orotate complex showed an increase in mean liver attenuation values ($P = 0.002$ vs. placebo) and the LAI ($P = 0.008$ vs. placebo) after 12 weeks of treatment. Mean changes in the LAI level from baseline at week 12 were 6.21 ± 8.96 in the carnitine-orotate complex group ($P < 0.001$) compared with 0.74 ± 8.05 in the placebo group ($P = 0.582$) (Fig. 1C and Table 2). After 12 weeks of treatment, the changes in the ALT level were inversely correlated with the changes in the LAI ($r = −0.41$, $P < 0.001$; Supplementary Fig. 3).

At week 12, a significant decrease from baseline in HbA$_{1c}$ was observed in the carnitine-orotate complex group ($P = 0.007$), but no significant change was seen in the placebo group. Mean change in HbA$_{1c}$ from baseline was −0.33 ± 0.82% (3.6 ± 9.0 mmol/mol) in the carnitine-orotate complex group. However, no significant changes were seen in fasting glucose, HOMA-IR, HOMA-B, lipid profile, and blood pressure in either treatment group.

When subjects were classified into tertiles by LAI changes from baseline at 12 weeks, participants in the highest tertile of LAI changes showed a significant decrease in fasting glucose, HbA$_{1c}$, and HOMA-IR from baseline. However, there was no significant change from baseline in HOMA-β and lipid profile in the highest tertile of LAI changes. The reduction in AST and ALT levels from baseline at week 12 increased with increasing tertiles of LAI changes ($P < 0.001$ between LAI change tertiles) (Table 3).

Compliance and Adverse Events

At the study end point, drug compliance (defined as taking more than 70% of the given trial medicine) was 89.5% in the carnitine-orotate complex group and 86.5% in the placebo group ($P = 0.736$, not significant). During 12 weeks of treatment, 17 adverse events were reported by 13 patients (33.3%) in the carnitine-orotate complex group, and 21 adverse events were reported by 20 patients (51.3%) in the placebo group ($P = 0.109$, not significant). One serious adverse event (rib fracture) was reported by one patient in the placebo group (Supplementary Table 1).

CONCLUSIONS

In this CORONA trial, carnitine-orotate complex significantly improved hepatic steatosis in patients with diabetes and NAFLD. Carnitine-orotate complex also improved the HbA$_{1c}$ level in relation to improvement of hepatic steatosis.

In our study, no weight change occurred in the carnitine-orotate complex group during the 12-week treatment period. Despite no changes in weight or usual medications, serum ALT levels normalized in 89.7% of the participants treated with carnitine-orotate complex. Furthermore, hepatic CT analysis showed reduction in hepatic fat content in participants treated with carnitine-orotate complex. Carnitine acts as a carrier for fatty acids across the mitochondrial membrane for subsequent β-oxidation, which is essential for the body to convert fat into energy (18). Reduced oxidation of fatty acids in hepatocytes increases the amount of hepatic free fatty acids, causing increased triglyceride accumulation within the cytoplasm of hepatocytes.
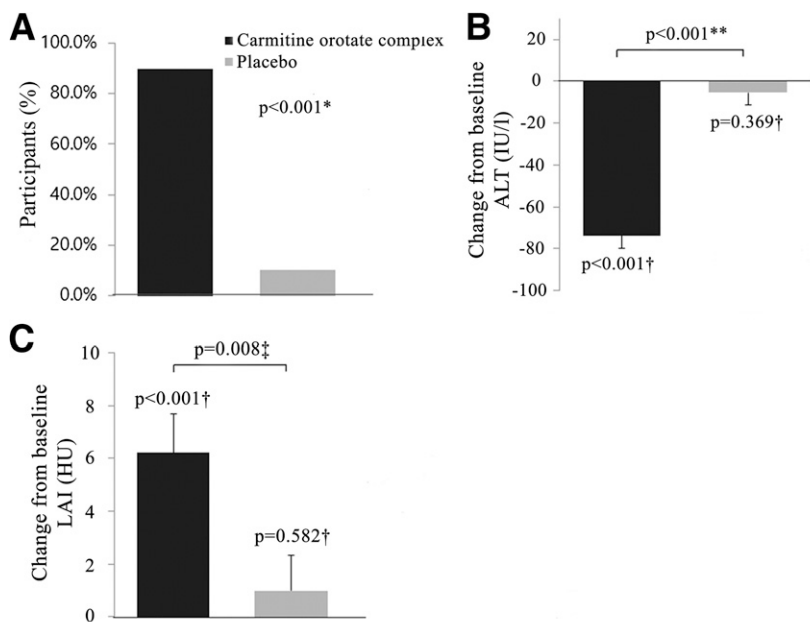
Figure 1—Biochemical and radiological improvement with carnitine-orotate complex. A: Proportion of participants who returned to normal ALT level (<30 IU/L in men or <19 IU/L in women) at week 12. Change from baseline in ALT (B) and LAI (C) at 12 weeks. *By Pearson χ^2 test. **By ANCOVA with baseline values as covariate. †By paired t test. ‡By independent t test. The error bars show the SEM.

Mitochondria are the primary sites for fatty acid oxidation, and hepatocytes are rich in mitochondria, which occupy 18% of the entire liver cell volume (10). Meanwhile, accumulating evidence suggests that hepatic mitochondrial dysfunction is crucial to the pathogenesis of NAFLD (23–26), and excessive reactive oxygen species production is considered one of the possible mechanisms of the mitochondrial dysfunction found in NAFLD patients and animal models (10,27). During NAFLD, fatty acid oxidation increases in mitochondria as a metabolic adaptation to fat accumulation, but this adaptation secondarily induces oxidative stress resulting in mitochondrial dysfunction (26,27). Carnitine has shown antioxidant activity protecting hepatocytes or hepatocyte cell lines against intracellular oxidative stress in several studies (13–15). These roles of carnitine in oxidation of mitochondrial free fatty acids and reducing oxidative stress in hepatocytes might explain the improvement of hepatic steatosis and liver function in our participants treated with carnitine-orotate complex.

In addition to improvement of hepatic steatosis, improved glycemic control was also shown in our participants treated with carnitine-orotate complex. Besides its role in hepatocytes, the beneficial effect of carnitine supplementation on glucose tolerance and insulin sensitivity has been reported in several human studies and different animal models (18). It is well known that carnitine enhances mitochondrial oxidation of long-chain fatty acids. Accumulation of long-chain fatty acids and other fatty acid metabolites impair insulin signaling and contribute to the development of

Table 2—Changes from baseline in metabolic parameters at week 12

	Carnitine complex (n = 39)		Placebo (n = 39)		
	Change from baseline	P value*	Change from baseline	P value*	P value†
Weight (kg)	−0.4 ± 1.6	0.078	−0.7 ± 1.4	0.002	0.512
BMI (kg/m^2)	−0.14 ± 0.58	0.142	−0.25 ± 0.48	0.002	0.471**
Waist circumference (cm)	−0.8 ± 3.1	0.115	−1.5 ± 2.9	0.002	0.388
Fasting glucose (mg/dL)	−2.2 ± 34.5	0.976	−16.9 ± 65.2	0.519	0.642
HbA$_{1c}$ (%)	−0.33 ± 0.82	0.007	−0.09 ± 0.96	0.574	0.085
HbA$_{1c}$ (mmol/mol)	−3.6 ± 9.0	0.007	−1.0 ± 10.5	0.574	0.085
HOMA-IR	−0.83 ± 4.10	0.293	−0.80 ± 7.41	0.935	0.494
HOMA-B	−10.4 ± 49.6	0.357	−4.6 ± 84.8	0.795	0.475
AST (IU/L)	−26.1 ± 22.9	<0.001	−0.72 ± 30.1	0.814	<0.001**
ALT (IU/L)	−73.7 ± 38.7	<0.001	−5.38 ± 37.1	0.369	<0.001**
γ-GT (IU/L)	−14.1 ± 47.5	0.01	2.5 ± 42.3	0.555	0.016
Systolic blood pressure (mmHg)	−2.1 ± 13.5	0.343	−6.4 ± 9.8	<0.001	0.109
Triglyceride (mg/dL)	4.2 ± 220.3	0.023	6.9 ± 84.3	0.614	0.241
HDL-cholesterol (mg/dL)	0.6 ± 5.6	0.535	0.4 ± 5.3	0.634	0.902
LDL-cholesterol (mg/dL)	3.5 ± 23.2	0.393	0.4 ± 13.9	0.873	0.415
CT attenuation (HU)	(n = 36)		(n = 36)		
Whole liver	6.10 ± 8.51	<0.001	1.04 ± 6.95	0.377	0.002
Whole liver-spleen (LAI)	6.21 ± 8.96	<0.001	0.74 ± 8.05	0.582	0.008

Data are mean ± SD. γ-GT, γ-glutamyl-transpeptidase. *By paired t test or Wilcoxon signed rank test. †By Wilcoxon rank sum test or independent t test. **By ANCOVA with baseline values as covariate.

Table 3—Changes from baseline in metabolic parameters according to tertile by LAI changes from baseline at 12 weeks

	≤0 (HU) (n = 22)	P*	0–8 (HU) (n = 22)	P*	>8 (HU) (n = 24)	P*	P†
			LAI change tertile				
AST	2.0 ± 20.7	0.685	−15.6 ± 34.6	0.035	−26.3 ± 17.8	<0.001	<0.001
ALT	−15.1 ± 38.1	0.068	−40.2 ± 53.2	<0.001	−67.9 ± 35.3	<0.001	<0.001
Fasting glucose (mg/dL)	−15.9 ± 69.7	0.974	0.3 ± 37.5	0.277	−24.4 ± 47.2	0.019	0.035
HbA_{1c} (%)	−0.09 ± 0.92	0.676	−0.19 ± 1.05	0.375	−0.34 ± 0.85	0.039	0.274
HbA_{1c} (mmol/mol)	−1.0 ± 10.1	0.676	−2.1 ± 11.5	0.375	−3.7 ± 9.3	0.039	0.274
HOMA-IR	−1.82 ± 6.12	0.486	−1.27 ± 4.79	0.887	−1.97 ± 4.35	0.022	0.309
HOMA-B	−17.03 ± 91.6	0.987	−12.89 ± 66.28	0.661	5.47 ± 31.01	0.648	0.932
Triglyceride (mg/dL)	19.2 ± 112.6	0.661	11.8 ± 92.7	0.205	−36.3 ± 259.8	0.424	0.909
LDL-cholesterol (mg/dL)	−1.3 ± 15.6	0.733	1.5 ± 15.7	0.426	2.0 ± 24.3	0.955	0.824
HDL-cholesterol (mg/dL)	−0.14 ± 4.87	0.68	2.09 ± 6.38	0.177	0.67 ± 4.53	0.393	0.464
Weight (kg)	0.47 ± 1.97	0.386	−0.22 ± 1.38	0.519	−1.00 ± 1.17	0.001	0.025
Carnitine-orotate group (n)	7	—	10	—	17	—	—

Data are mean ± SD, except as indicated. *By Wilcoxon signed rank test. †P for between LAI change tertiles are by the Kruskal-Wallis test.

insulin resistance in skeletal muscle and heart (18). Some studies reported that subjects with diabetes have reduced plasma free carnitine concentrations compared with healthy subjects, indicating an association between impaired carnitine status and glucose intolerance (18,28,29). All participants in our study had diabetes and experienced significant decreases in HbA_{1c} after 12 weeks of carnitine-orotate complex treatment compared with no significant change in the placebo group. Evidence suggests that oxidative stress damages mitochondrial DNA, resulting in mitochondrial respiratory dysfunction, which aggravates the insulin signaling pathway (30–32). A recent clinical study showed that treatment with carnitine-orotate complex (Godex, Celltrion Pharm) reduced systemic oxidative stress and increased mitochondrial DNA copy number in patients with impaired glucose metabolism (33). Several other mechanisms of action of carnitine on glucose tolerance have also been documented (18).

Although the serum ALT value is used as a surrogate marker of liver injury, ALT values do not correlate well with the severity of NAFLD because the entire histologic spectrum of NAFLD can be seen in individuals with normal ALT values (34). Instead of comparing ALT activity itself with severity of NAFLD, we focused on changes in ALT level and hepatic fat content on CT. In our study, subjects with NAFLD were identified by elevated ALT, and the reduction in the ALT level correlated well with the increments in the LAI. This result suggests

that improvement of ALT activity represents improvement of hepatic steatosis in a concentration-dependent manner in individuals with fatty liver identified by presence of elevated ALT.

When subjects were classified into tertiles by LAI changes from baseline at 12 weeks, participants in the highest tertile of LAI changes showed significant decreases in fasting glucose, HbA_{1c}, and HOMA-IR from baseline. These results indicate that improvement of hepatic steatosis is associated with improvement of glycemic control in relation to insulin resistance. Hepatic steatosis is well known to be closely associated with insulin resistance, which plays an integral role in the pathogenesis of type 2 diabetes (2,3,35). Despite this close association of hepatic steatosis with insulin resistance, a causal relationship between NAFLD and glucose metabolism has not been clearly established. Only a few studies evaluating NAFLD as a risk factor for the development of diabetes have been reported (4,5,7). In the context of a suspected causal relationship, the results obtained at week 6 appear to provide further information. Reductions in the HbA_{1c} level and hepatic fat content were both seen in patients with diabetes and NAFLD after 12 weeks of treatment with carnitine-orotate complex. At week 6, however, there was no significant change in glycemic control despite a significant reduction in ALT levels from baseline in the carnitine-orotate complex group (Supplementary Table 2). Taken together with the above results, this study demonstrates that an

improvement of hepatic steatosis might have an effect on the improvement of glycemic control in relation to insulin resistance. Also, our results would support a previous finding that the presence of NAFLD can aggravate insulin resistance independent of other metabolic components (3).

The trial medication, the carnitine-orotate complex capsule, consists of various components besides carnitine. Although the effect of carnitine on NAFLD or glucose metabolism has been documented in several human and animal studies (11–16,18), those of other components have not been revealed. These other components might have an effect on NAFLD or glucose metabolism. Indeed, orotate, the other main component of the trial medication, has been reported to have a favorable effect on energy metabolism by promoting fatty acid oxidation in the heart (36).

Limitations
Despite the reduction in HbA_{1c}, there was no change in markers related to insulin sensitivity after treatment. The short trial duration of 12 weeks, small sample size, and the use of low-dose carnitine (carnitine-orotate, 900 mg/day) may limit conclusions about metabolic efficacy. Indeed, several clinical trials with longer treatment duration, a larger sample size, and the use of higher dose carnitine (2–4 g/day) have shown the benefits of carnitine treatment on metabolic morbidity improving anthropometric parameters, lipid profiles, and insulin

resistance (18). There was no liver biopsy, and subjects with simple steatosis cannot be differentiated from those with nonalcoholic steatohepatitis (NASH). Thus, we do not have an idea whether patients who were included in the trial were candidates for treatment, because NAFLD without NASH is not an indication for treatment. Also, we could not evaluate whether there was improvement in inflammatory changes after treatment.

The use of CT to diagnose fatty liver was also another limitation. CT is reasonably accurate but cannot identify mild fatty infiltration of the liver below the threshold of 30% (37) and might have resulted in the exclusion of subjects with NAFLD. Magnetic resonance (MR)–based assessment is better than CT in quantifying liver fat content, and based on the results from recent clinical trials, MR-based imaging was even better than histology in assessing quantitative changes in liver fat (37–40). In our further study, we plan to use advanced MR imaging and liver histology as an end point to assess the effects of carnitine-orotate complex in patients with NASH.

Summary

Participants treated with carnitine-orotate complex showed biochemical and radiological improvement in NAFLD as well as improved glycemic control. An improvement of ALT activity correlated well with the improvement of hepatic steatosis in a concentration-dependent manner in individuals with fatty liver. Improvement of ALT activity preceded the improvement of glycemic control, suggesting that improvement of hepatic steatosis may have an effect on the improvement of glycemic control.

The results obtained from the CORONA trial suggest that treatment with carnitine-orotate complex improves hepatic steatosis in patients with diabetes and NAFLD and has a beneficial effect on glucose metabolism, particularly in relation to improvement of hepatic steatosis.

References

1. Lim S. Ectopic fat assessment focusing on cardiometabolic and renal risk. Endocrinol Metab (Seoul) 2014;29:1–4
2. Rector RS, Thyfault JP, Wei Y, Ibdah JA. Nonalcoholic fatty liver disease and the metabolic syndrome: an update. World J Gastroenterol 2008;14:185–192
3. Bae JC, Cho YK, Lee WY, et al. Impact of nonalcoholic fatty liver disease on insulin resistance in relation to HbA1c levels in nondiabetic subjects. Am J Gastroenterol 2010;105:2389–2395
4. Bae JC, Rhee EJ, Lee WY, et al. Combined effect of nonalcoholic fatty liver disease and impaired fasting glucose on the development of type 2 diabetes: a 4-year retrospective longitudinal study. Diabetes Care 2011;34:727–729
5. Chang Y, Jung HS, Yun KE, Cho J, Cho YK, Ryu S. Cohort study of non-alcoholic fatty liver disease, NAFLD fibrosis score, and the risk of incident diabetes in a Korean population. Am J Gastroenterol 2013;108:1861–1868
6. Kim D, Choi SY, Park EH, et al. Nonalcoholic fatty liver disease is associated with coronary artery calcification. Hepatology 2012;56:605–613
7. Shibata M, Kihara Y, Taguchi M, Tashiro M, Otsuki M. Nonalcoholic fatty liver disease is a risk factor for type 2 diabetes in middle-aged Japanese men. Diabetes Care 2007;30:2940–2944
8. Targher G, Bertolini L, Padovani R, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care 2007;30:1212–1218
9. Musso G, Gambino R, Cassader M, Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. Hepatology 2010;52:79–104
10. Wei Y, Rector RS, Thyfault JP, Ibdah JA. Nonalcoholic fatty liver disease and mitochondrial

dysfunction. World J Gastroenterol 2008;14:193–199
11. Murosaki S, Lee TR, Muroyama K, et al. A combination of caffeine, arginine, soy isoflavones, and L-carnitine enhances both lipolysis and fatty acid oxidation in 3T3-L1 and HepG2 cells in vitro and in KK mice in vivo. J Nutr 2007;137:2252–2257
12. Noland RC, Koves TR, Seiler SE, et al. Carnitine insufficiency caused by aging and overnutrition compromises mitochondrial performance and metabolic control. J Biol Chem 2009;284:22840–22852
13. Li JL, Wang QY, Luan HY, Kang ZC, Wang CB. Effects of L-carnitine against oxidative stress in human hepatocytes: involvement of peroxisome proliferator-activated receptor alpha. J Biomed Sci 2012;19:32
14. Jun DW, Cho WK, Jun JH, et al. Prevention of free fatty acid-induced hepatic lipotoxicity by carnitine via reversal of mitochondrial dysfunction. Liver Int 2011;31:1315–1324
15. Dobrzyńska I, Szachowicz-Petelska B, Skrzydlewska E, Figaszewski Z. Effect of L-carnitine on liver cell membranes in ethanol-intoxicated rats. Chem Biol Interact 2010;188:44–51
16. Malaguarnera M, Gargante MP, Russo C, et al. L-carnitine supplementation to diet: a new tool in treatment of nonalcoholic steatohepatitis—a randomized and controlled clinical trial. Am J Gastroenterol 2010;105:1338–1345
17. Power RA, Hulver MW, Zhang JY, et al. Carnitine revisited: potential use as adjunctive treatment in diabetes. Diabetologia 2007;50:824–832
18. Ringseis R, Keller J, Eder K. Role of carnitine in the regulation of glucose homeostasis and insulin sensitivity: evidence from in vivo and in vitro studies with carnitine supplementation and carnitine deficiency. Eur J Nutr 2012;51:1–18
19. Wong VW, Vergniol J, Wong GL, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology 2010;51:454–462
20. Kodama Y, Ng CS, Wu TT, et al. Comparison of CT methods for determining the fat content of the liver. AJR Am J Roentgenol 2007;188:1307–1312
21. Limanond P, Raman SS, Lassman C, et al. Macrovesicular hepatic steatosis in living related liver donors: correlation between CT and histologic findings. Radiology 2004;230:276–280
22. Jacobs JE, Birnbaum BA, Shapiro MA, et al. Diagnostic criteria for fatty infiltration of the liver on contrast-enhanced helical CT. AJR Am J Roentgenol 1998;171:659–664
23. Caldwell SH, Swerdlow RH, Khan EM, et al. Mitochondrial abnormalities in non-alcoholic steatohepatitis. J Hepatol 1999;31:430–434
24. Sobaniec-Lotowska ME, Lebensztejn DM. Ultrastructure of hepatocyte mitochondria in nonalcoholic steatohepatitis in pediatric patients: usefulness of electron microscopy in the diagnosis of the disease. Am J Gastroenterol 2003;98:1664–1665
25. Ibdah JA, Perlegas P, Zhao Y, et al. Mice heterozygous for a defect in mitochondrial trifunctional protein develop hepatic steatosis and insulin resistance. Gastroenterology 2005;128:1381–1390
26. Begriche K, Igoudjil A, Pessayre D, Fromenty B. Mitochondrial dysfunction in NASH: causes,

consequences and possible means to prevent it. Mitochondrion 2006;6:1–28

27. Begriche K, Massart J, Robin MA, Bonnet F, Fromenty B. Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease. Hepatology 2013;58:1497–1507

28. Winter SC, Simon M, Zorn EM, et al. Relative carnitine insufficiency in children with type I diabetes mellitus. Am J Dis Child 1989;143:1337–1339

29. Poorabbas A, Fallah F, Bagdadchi J, et al. Determination of free L-carnitine levels in type II diabetic women with and without complications. Eur J Clin Nutr 2007;61:892–895

30. Victor VM, Apostolova N, Herance R, Hernandez-Mijares A, Rocha M. Oxidative stress and mitochondrial dysfunction in atherosclerosis: mitochondria-targeted antioxidants as potential therapy. Curr Med Chem 2009;16:4654–4667

31. Victor VM, Rocha M, Herance R, Hernandez-Mijares A. Oxidative stress and mitochondrial dysfunction in type 2 diabetes. Curr Pharm Des 2011;17:3947–3958

32. Petersen KF, Befroy D, Dufour S, et al. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 2003;300:1140–1142

33. Hong ES, Kim EK, Kang SM, et al. Effect of carnitine-orotate complex on glucose metabolism and fatty liver: a double-blind, placebo-controlled study. J Gastroenterol Hepatol 2014;29:1449–1457

34. Mofrad P, Contos MJ, Haque M, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology 2003;37:1286–1292

35. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia 2003;46:3–19

36. Pôrto LC, de Castro CH, Savergnini SS, et al. Improvement of the energy supply and contractile function in normal and ischemic rat hearts by dietary orotic acid. Life Sci 2012;90:476–483

37. Roldan-Valadez E, Favila R, Martínez-López M, Uribe M, Méndez-Sánchez N. Imaging techniques for assessing hepatic fat content in nonalcoholic fatty liver disease. Ann Hepatol 2008;7:212–220

38. Loomba R, Sirlin CB, Ang B, et al. Ezetimibe for the treatment of nonalcoholic steatohepatitis: Assessment by novel magnetic resonance imaging and magnetic resonance elastography in a randomized trial (MOZART trial). Hepatology 2015;61:1239–1250

39. Noureddin M, Lam J, Peterson MR, et al. Utility of magnetic resonance imaging versus histology for quantifying changes in liver fat in nonalcoholic fatty liver disease trials. Hepatology 2013;58:1930–1940

40. Le TA, Chen J, Changchien C, et al.; San Diego Integrated NAFLD Research Consortium (SINC). Effect of colesevelam on liver fat quantified by magnetic resonance in nonalcoholic steatohepatitis: a randomized controlled trial. Hepatology 2012;56:922–932