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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

# Efficacy of long-term ezetimibe therapy in patients with nonalcoholic fatty liver disease

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**Abstract**
*Background* Hyperlipidemia, insulin resistance, and oxidative stress can heavily contribute to the initiation and progression of nonalcoholic fatty liver disease (NAFLD). Currently, there is no established treatment for this disease. Recently, several studies have shown that ezetimibe (EZ), a lipid-lowering drug, attenuates liver steatosis in an experimental NAFLD model. This study was designed to assess the efficacy of long-term EZ monotherapy in patients with NAFLD.
*Methods* A total of 45 patients with newly diagnosed liver biopsy-proven NAFLD were treated with EZ (10 mg/day) for 24 months. NAFLD-related biochemical parameters, imaging by computerized tomography, and liver biopsy were studied before and after treatment.
*Results* Ezetimibe therapy significantly improved NAFLD-related metabolic parameters including visceral fat area, fasting insulin, homeostasis model assessment of insulin resistance (HOMA-R), triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-Ch), oxidative-LDL, the net electronegative charge modified-LDL, profiles of lipoprotein particle size and fatty acids component, and estimated desaturase activity. EZ therapy also significantly lowered serum alanine aminotransferase and high-sensitivity C-reactive protein levels, whereas no significant changes were found in serum type IV collagen 7S, adiponectin, leptin, and resistin levels. Histological features of steatosis grade ($P = 0.0003$), necroinflammatory grade ($P = 0.0456$), ballooning score ($P = 0.0253$), and NAFLD activity score (NAS) ($P = 0.0007$) were significantly improved from baseline. However, the fibrosis stage was not significantly ($P = 0.6547$) changed.
*Conclusion* The results in this study suggest that the long-term EZ therapy can lead to improvement in metabolic, biochemical, and histological abnormalities of NAFLD. Therefore, EZ may be a promising agent for treatment of NAFLD.

**Keywords** Ezetimibe · NAFLD · Insulin resistance · Lipid metabolism · Fatty acid metabolism

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## Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver injury in the world [1–3]. NAFLD is a metabolic condition which encompasses a wide spectrum of liver disease ranging from

simple steatosis to nonalcoholic steatohepatitis (NASH). Although the exact intricacies of the molecular and cellular mechanisms responsible for progression from simple steatosis to NASH have not been fully elucidated, hyperlipidemia, insulin resistance, and oxidative stress are major contributors to the initiation and progression of NAFLD [4–6]. A two-hit hypothesis has been proposed, whereby steatosis (first hit) sensitizes the liver to a variety of metabolic injuries (second hit) that lead to necrosis, inflammation, and fibrosis [6]. Several investigators have suggested that NASH is the hepatic manifestation of the metabolic syndrome [2–7]. While there are few proven beneficial therapies for NASH, its association with insulin resistance has provided the rationale for evaluation of medical therapies that increase insulin sensitivity. Indeed, several pilot studies have shown that treatment with the biguanides and the thiazolidinediones, two classes of insulin-sensitizing drugs, can lead to improvements in biochemical and histological features of NASH [8–16].

Ezetimibe (EZ) is a useful lipid-lowering agent that inhibits the absorption of dietary and biliary cholesterol by selectively binding to the intestinal cholesterol transporter Niemann–Pick C1-like 1 [17, 18]. Several recent studies in an experimental NAFLD model have shown that EZ monotherapy not only protects against diet-induced hyperlipidemia, but also attenuates liver steatosis in an experimental NAFLD model [19–21].

In the present study, we investigated the efficacy of long-term EZ monotherapy in patients with NAFLD.

## Patients and methods

### Patients

The study protocol was approved by the ethical committee of Saiseikai Suita Hospital and the Kyoto Prefectural University of Medicine, and informed consent was obtained from all subjects prior to enrollment in the study. A total of 45 patients who had been newly diagnosed histologically as having NAFLD at Saiseikai Suita Hospital and Kyoto Prefectural University Hospital between 2007 and 2009 were evaluated in this study.

All liver biopsy specimens were examined by two experienced pathologists blinded to the patients' clinical or laboratory data or liver biopsy sequence. Histological features of samples were interpreted as outlined by Brunt et al. [22]. The stage of fibrosis was classified as follows: stage 0 = no fibrosis, stage 1 = zone 3 predominant pericellular fibrosis, stage 2 = zone 3 fibrosis plus periportal fibrosis, stage 3 = bridging fibrosis, stage 4 = cirrhosis. Necroinflammation was graded 0 (absent) to 3 (1, occasional ballooned hepatocytes and no or very mild inflammation; 2, ballooning of hepatocytes and mild-to-moderate portal inflammation; 3, intra-acinar inflammation and portal inflammation). The grade of steatosis was defined as mild (≤33%), moderate (34–65%), and advanced (≥66%). The NAFLD activity score (NAS) was calculated as the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3), and ballooning (0–2) as reported by Kleiner et al. [23], and as shown in Table 1 was used to classify NAFLD into "not NASH" (NAS ≤2), "borderline NASH" (NAS = 3–4), and "definite NASH" (NAS ≥5).

Prior to evaluation of liver histology we excluded patients with an alcohol intake exceeding 20 g/day and those who reported any sign, symptom, and/or history of known liver disease including viral, genetic, autoimmune, and drug-induced liver disease, previous use of anti-diabetic medication including insulin-sensitizing agents such as metformin and pioglitazone. All patients received EZ (10 mg/day) for 24 months.

### Estimation of energy and nutrient intake

All patients were asked to adhere to a dietary plan tailored to their energy requirements and metabolic control by a registered dietitian and/or physician, using the current Japan Diabetes Society recommendations. The patients

**Table 1** Baseline anthropometrics and demographics

| | |
|---|---|
| Male/female | 24/21 |
| Age (years) | 50.2 ± 9.4 |
| Body mass index (kg/m$^2$) | 26.9 ± 3.3 |
| Waist circumference (cm) | 92.3 ± 5.7 |
| Visceral fat area (cm$^2$) | 155.9 ± 38.9 |
| Subcutaneous fat area (cm$^2$) | 170.9 ± 51.3 |
| Obesity[a] (%) | 41 (91.1) |
| Hyperlipidemia[b] (%) | 45 (100) |
| Hypertension[c] (%) | 23 (48.9) |
| 75 g oral glucose tolerance test | |
| NGT (normal glucose tolerance; %) | 7 (15.6) |
| IGT (impaired glucose tolerance; %) | 28 (62.2) |
| Diabetes (%) | 10 (22.2) |
| NAFLD activity score (NAS) | |
| NAS ≤2 | 4 (11.1) |
| NAS 3–4 | 3 (8.9) |
| NAS ≥5 | 38 (80.0) |

*NAFLD* nonalcoholic fatty liver disease

[a] Obesity was defined as a body mass index of ≥25.1

[b] Hyperlipidemia diagnosed if serum total cholesterol level was ≥220 mg/dl and/or serum triglyceride level was ≥160 mg/dl on at least two occasions

[c] Hypertension was diagnosed if the patient was taking antihypertensive medication and/or had a resting recumbent blood pressure ≥140/90 mmHg on at least two occasions

recorded their daily dietary intake in a diary by using the calorie and lipid list in the Japan Diabetes Society recommendations guidebook. The dietary diary was collected every month, and the results were reported back to the subjects the following month. In addition, daily activity and physical condition were recorded every month using a checklist; and depending on the report, the physician checked the patient's condition and provided appropriate advice.

### Clinical and laboratory investigations

The intra-abdominal visceral (VSA) and subcutaneous fat areas (SFA) were determined at the umbilical level by a computed tomography (CT) scanning technique (TSX-012A, X-Vigor, Toshiba Co. Ltd, Tokyo, Japan) using a method described previously [24].

Blood samples were obtained in the morning after an overnight fast. Plasma glucose (PG) was measured by the glucose oxidase method and HbA1c was determined by high-performance liquid chromatography (HPLC: Arkray Inc., Kyoto, Japan). Plasma insulin immunoreactive insulin (IRI) concentrations were measured by an immunoradiometric assay (Insulin-RIAbead II, Abbott, Japan). The homeostasis model assessment of insulin resistance (HOMA-R) was calculated from fasting insulin and plasma glucose levels by the following equation: HOMA-R = fasting IRI (μU/ml) × fasting PG (mg/dl)/405. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (T-Ch), high-density lipoprotein cholesterol (HDL-Ch), low-density lipoprotein cholesterol (LDL-Ch), and triglyceride (TG) were measured by enzymatic methods using a chemical autoanalyzer (Hitachi Co., Tokyo, Japan). Serum type IV collagen 7S was measured by a radioimmunoassay kit (Mitsubishi Chemical Group, Tokyo, Japan). Serum high-sensitivity C-reactive protein (hs-CRP) was measured by nephelometry using a latex particle-enhanced immunoassay (Dade Behering, Tokyo, Japan). Serum oxidized LDL (oxLDL) was measured by an enzyme-linked immunoassay (ELISA) kit (Kyowa Medex Co., Ltd., Tokyo, Japan). The net electronegative charge modified-LDL (emLDL) was analyzed by using an agarose gel electrophoresis lipoprotein fraction system, according to the manufacturer's instructions (Chol/Trig Combo System™; Helena Labs, Saitama, Japan). The percentage frequency of emLDL was calculated on a computer from the migration distance ($b$) of the LDL fraction in the test samples and the migration distance ($a$) of normal control sera according to the following formula: emLDL density = $[b - a/a] \times 100\%$ .

Serum lipoproteins were also analyzed by an HPLC system according to the procedure described by Okazaki et al. [25], while lipoprotein particle size was determined based on individual elution times that corresponded to peaks on the chromatographic pattern of cholesterol fractions. In this study, we defined 3 VLDL, 4 LDL, and 5 HDL subclasses according to lipoprotein particle size, expressed as diameter.

Analysis of fatty acid composition in plasma cholesterol esters (CEs) was as follows: total lipid was extracted from plasma by using the method of Bligh and Dyer [26], followed by separation of the CEs by thin-layer chromatography using silica gel plates (Silica Gel 60, Merck, Darmstadt, Germany) and a solvent system of petroleum ether/ethyl ether/acetic acid (80:20:1, v/v/v). The spot corresponding to CEs was scraped from the plate and transmethylated with 2 ml of acetyl chloride/methanol (5:50, v/v) at 90°C for 2 h. Heptadecanoic acid (17:0) was used as an internal standard. Fatty acid methyl esters were quantified by using a model GC14A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a 25-m × 0.5-mm capillary column (HR-SS-10, Shinwa Chemical Industries, Ltd., Kyoto, Japan). Desaturase and elongase activities were estimated as the ratio product to the precursor of individual fatty acids in plasma CEs according to the following: D9-16D = 16:1n-7/16:1, D9-18D = 18:1n-9/18:1, D6D = 18:3n-6/18:2n-6, and D5D = 20:4n-6/20:3n-6.

### Statistical analysis

All statistical analyses were performed using Statview version 5.0 (Abacus Concepts, Berkeley, CA, USA). Data were summarized by frequencies and percentages for categorical variables, and means ± SD for continuous variables. Comparison of pre- and posttreatment of EZ data was carried out using nonparametric Wilcoxon signed rank test. A $P$ value less than 0.05 was considered statistically significant.

## Results

### Effect of ezetimibe on clinical and laboratory parameters

Compared to baseline, VFA level reduced significantly from 155.9 ± 38.9 to 146.5 ± 34.8 ($P < 0.05$) at the end of the study (Table 2). There were no significant changes in body mass index (BMI), waist circumference, and SFA at the end of the study.

Mean ALT level decreased significantly by the end of the study from 62 ± 25 to 49 ± 23 ($P < 0.01$), whereas the AST level did not. The mean level of fasting insulin level and HOMA-R decreased significantly (both $P < 0.05$), although mean HbA1c and fasting glucose levels remained unchanged at the end of the study. Regarding lipid metabolism, the mean levels of TG, T-Ch, LDL-Ch, oxLDL, and

**Table 2** Clinical and laboratory parameters of baseline and after ezetimibe treatment

| | Baseline | At 12 months | At 24 months |
|---|---|---|---|
| Body mass index (kg/m$^2$) | 26.9 ± 3.3 | 26.0 ± 3.5 | 26.1 ± 3.2 |
| Waist circumference (cm) | 92.3 ± 5.7 | 90.5 ± 5.8 | 90.9 ± 6.0 |
| Visceral fat area (cm$^2$) | 155.9 ± 38.9 | 150.8 ± 33.6 | 146.5 ± 34.8* |
| Subcutaneous fat area (cm$^2$) | 170.9 ± 51.3 | 166.4 ± 41.5 | 167.1 ± 41.5 |
| HbA1c (%) | 6.3 ± 0.8 | 6.5 ± 0.7 | 6.4 ± 0.9 |
| Fasting glucose (mg/dl) | 113 ± 24 | 112 ± 27 | 112 ± 28 |
| Fasting insulin (μU/ml) | 10.9 ± 5.6 | 9.2 ± 5.8* | 9.4 ± 5.1* |
| HOMA-R | 3.04 ± 1.17 | 2.60 ± 1.33* | 2.62 ± 1.24* |
| Aspartate aminotransferase (IU/l) | 40 ± 22 | 36 ± 16 | 36 ± 16 |
| Alanine aminotransferase (IU/l) | 62 ± 25 | 48 ± 25** | 49 ± 23** |
| Triglycerides (mg/dl) | 168 ± 94 | 136 ± 90* | 138 ± 88* |
| Total cholesterol (mg/dl) | 228 ± 44 | 193 ± 36** | 194 ± 36** |
| HDL cholesterol (mg/dl) | 49 ± 13 | 53 ± 15 | 52 ± 14 |
| LDL cholesterol (mg/dl) | 136 ± 33 | 117 ± 34* | 114 ± 31* |
| Oxidative LDL (U/ml) | 14.1 ± 6.9 | 13.6 ± 7.1 | 11.8 ± 5.5* |
| Electronegative charge modified-LDL (ecd) | 6.4 ± 3.5 | 3.5 ± 3.6$^{\#}$ | 3.4 ± 3.2$^{\#}$ |
| Type IV collagen 7S (ng/dl) | 5.1 ± 2.9 | 4.7 ± 2.5 | 4.7 ± 2.5 |
| Adiponectin (μg/ml) | 5.8 ± 3.1 | 6.1 ± 3.4 | 6.1 ± 3.4 |
| Leptin (ng/l) | 4.0 ± 2.9 | 3.8 ± 3.1 | 3.8 ± 3.1 |
| Resistin (ng/ml) | 7.7 ± 3.1 | 7.4 ± 3.4 | 7.4 ± 3.4 |
| High-sensitivity C-reactive protein (ng/ml) | 883 ± 408 | 677 ± 392* | 685 ± 377* |

Data are the mean ± SD

*ecd* electronegative charge density

* $P < 0.05$, ** $P < 0.01$, and # $P < 0.005$ versus baseline

emLDL were decreased significantly at the end of study ($P < 0.05$, $P < 0.01$, $P < 0.05$, $P < 0.05$, and $P < 0.005$, respectively). However, there was no significant change in serum HDL-Ch levels during the study. Serum hs-CRP was decreased significantly ($P < 0.05$), whereas no significant changes were found in the levels of serum adiponectin, leptin, resistin, and type IV collagen 7S.

### Effect of ezetimibe on lipoprotein subclass according to particle size

As shown in Fig. 1, the levels of large VLDL (44.5–64.0 nm), corresponding to VLDL1 (Sf 60–400), decreased significantly compared with baseline from 6.6 ± 1.2 to 4.2 ± 1.4 mg/dl ($P < 0.005$). At the end of the study, the mean levels of small LDL (23 nm) and very small LDL (16.7–20.7 nm) were also significantly decreased compared with baseline (from 37.9 ± 5.4 to 33.2 ± 5.1 mg/dl, $P < 0.05$ and from 23.8 ± 4.8 to 18.6 ± 2.8 mg/dl, $P < 0.01$, respectively). No significant changes from baseline levels were observed in any of the HDL-Ch subclass at the end of the study.

### Effect of ezetimibe on fatty acid composition in plasma CEs

The changes in plasma CEs fatty acid composition and estimated desaturase activities are shown in Table 3. Compared with baseline, myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (16:1n-7), oleic acid (C18:1n-9), and dihomo-γ-linoleic acid (C18:3n-6) were decreased significantly at the end of the study, while linoleic acid (C18:2n-6) was significantly increased. At the end of the study, activity of D9-16D, a major de no lipogenesis enzyme (known alternatively as stearoyl-CoA desaturase 1; SCD1), was increased significantly ($P < 0.005$). Activity of D5D was also increased significantly ($P < 0.01$) compared with baseline.

### Histological responses

Follow-up liver biopsies were performed on 33 patients at the end of the 24 months of EZ treatment. Table 4 shows the histologic changes before and after treatment. The mean level of steatosis grade (from 2.3 ± 0.7 to 1.9 ± 0.8, $P = 0.0003$), necroinflammatory grade (from 1.9 ± 0.7 to 1.8 ± 0.7, $P = 0.0456$), ballooning score (from 1.4 ± 0.5 to 1.3 ± 0.5, $P = 0.0253$), and NAS score (from 5.6 ± 1.6 to 5.1 ± 1.8, $P = 0.0007$) improved significantly during the study. Of 33 patients, 24 had a one or more point improvement in the NAS score, 8 had no change, and one had a one-point increase. In contrast, the mean level of fibrosis stage level did not change significantly (from 2.0 ± 0.8 to 2.1 ± 0.9, $P = 0.6547$). Overall, one patient had a one-point improvement, 29 had no change, and 3 had a one-point deterioration.

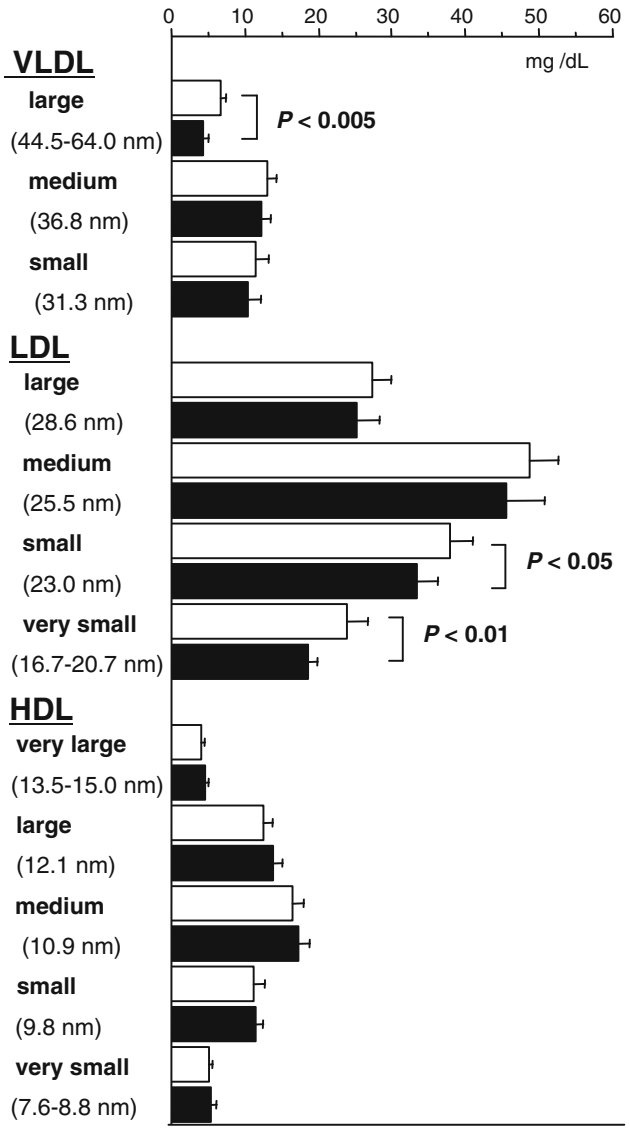


**Fig. 1** Changes in the cholesterol concentration of each lipoprotein subclass, grouped according to particle size, following ezetimibe administration. The values are shown as mean ± SD. The *open columns* represent baseline data and the *filled columns* represent data collected after 24 months of ezetimibe treatment

## Discussion

This study involved a 24-month follow-up of histological and metabolic syndrome-related parameters. In this study, we demonstrated that a 24-month course of EZ in doses of 10 mg/day improved liver histology and several metabolic syndrome-related parameters, and serum ALT levels. While we were preparing this manuscript, Yoneda et al. [27] also reported that NAFLD-related clinical parameters and histological observations were significantly improved by the treatment with EZ for 6 months, although the number of patients was small and treatment period was short. Although we did not observe significant improvement in the serum type IV collagen 7S level, our findings are essentially consistent with their report.

A noteworthy finding of this study was that EZ decreased not only the levels of T-Ch and LDL-Ch but also the levels of oxLDL and emLDL, which are the most

**Table 3** Changes in plasma cholesterol esters fatty acid composition and estimated desaturase activities

| Fatty acids (% of total fatty acids) | Baseline | After treatment |
|---|---|---|
| C14:0 (myristic acid) | 0.56 ± 0.27 | 0.54 ± 0.31* |
| C16:0 (palmitic acid) | 12.58 ± 1.03 | 11.36 ± 1.09** |
| C16:1n-7 (palmitoleic acid) | 3.13 ± 1.02 | 2.81 ± 1.02** |
| C18:0 (stearic acid) | 1.09 ± 0.30 | 1.07 ± 0.31 |
| C18:1n-9 (oleic acid) | 19.30 ± 2.41 | 18.52 ± 2.41* |
| C18:2n-6 (linoleic acid) | 48.67 ± 4.32 | 50.83 ± 4.42** |
| C18:3n-6 ($\gamma$-linoleic acid) | 0.82 ± 0.28 | 0.82 ± 0.24 |
| C18:3n-3 ($\alpha$-linoleic acid) | 0.77 ± 0.35 | 0.74 ± 0.34 |
| C20:3n-6 (dihomo-$\gamma$-linoleic acid) | 0.74 ± 0.23 | 0.69 ± 0.28* |
| C20:4n-6 (arachidonic acid) | 5.63 ± 1.17 | 5.65 ± 1.23 |
| C20:5n-3 (eicosapentaenoic acid) | 2.13 ± 0.75 | 2.15 ± 0.70 |
| C22:6n-3 (docosahexaenoic acid) | 0.84 ± 0.31 | 0.85 ± 0.30 |
| Estimated desaturase index | | |
| D9–16D (16:1n-7/16:0) | 0.26 ± 0.08 | 0.22 ± 0.09# |
| D9–18D (18:1n-9/18:0) | 17.71 ± 4.05 | 17.31 ± 4.02 |
| D6D (18:3n-6/18:2n-6) | 0.016 ± 0.009 | 0.014 ± 0.010 |
| D5D (20:4n-6/20:3n-6) | 7.60 ± 2.38 | 8.18 ± 2.50** |

* $P < 0.05$, ** $P < 0.01$, and # $P < 0.005$ versus baseline

**Table 4** Histological changes in 33 patients with NAFLD

| | Baseline | After treatment | *P* value |
|---|---|---|---|
| Steatosis grade | 2.3 ± 0.7 | 1.9 ± 0.8 | 0.0003 |
| 0 | 0 (0) | 1 (3) | |
| 1 | 5 (16) | 9 (31) | |
| 2 | 14 (44) | 16 (44) | |
| 3 | 14 (41) | 7 (22) | |
| Necroinflammatory grade | 1.9 ± 0.7 | 1.8 ± 0.7 | 0.0456 |
| 1 | 10 (30) | 12 (36) | |
| 2 | 16 (48) | 16 (45) | |
| 3 | 7 (21) | 5 (18) | |
| Fibrosis stage | 2.0 ± 0.8 | 2.1 ± 0.9 | 0.6547 |
| 0 | 1 (3) | 1 (33) | |
| 1 | 6 (18) | 8 (24) | |
| 2 | 17 (52) | 12 (42) | |
| 3 | 9 (27) | 12 (30) | |
| 4 | 0 (0) | 0 (0) | |
| Ballooning score | 1.4 ± 0.5 | 1.3 ± 0.5 | 0.0253 |
| 0 | 0 (0) | 1 (3) | |
| 1 | 19 (64) | 22 (70) | |
| 2 | 14 (36) | 10 (27) | |
| NAS score | 5.5 ± 1.6 | 5.0 ± 1.8 | 0.0007 |

atherogenic forms of lipoproteins. Analysis of lipoprotein subclass by HPLC also revealed that EZ treatment caused significant decrease in large VLDL (VLDL1), small LDL, and very small LDL. An increased serum level of large VLDL (VLDL1) is a common characteristic of the dyslipidemia associated with insulin resistance and type 2 diabetes mellitus [28–30]. Griffin and Packard [31] demonstrated that large VLDL1 is a precursor of small dense LDL, and that it is produced preferentially in the liver during development of insulin resistance. Adiels et al. reported that overproduction of large VLDL particles is driven by increased liver fat content [32] and that acute suppression of VLDL1 secretion by insulin is associated with hepatic fat content and insulin resistance [33]. In the present study, the change in large VLDL levels associated with EZ therapy correlated positively with changes in very small LDL ($r = 0.706$, $P < 0.001$), emLDL ($r = 0.412$, $P < 0.01$), and HOMA-R ($r = 0.565$, $P < 0.01$). Taken together these previous data and the findings of the present study suggest strongly that EZ ameliorates both hepatic and systemic vascular insulin resistance.

Another noteworthy finding of this study was that the long-term EZ therapy was associated with significant decrease in the levels of myristic acid, palmitic acid, palmitoleic acid, oleic acid, dihomo-$\gamma$-linoleic acid, and D9-16D activity, and significant increase in linoleic acid and D5D activity. It has been reported that EZ causes significant reduction in the absorption of several saturated fatty acids in diet-induced obese and diabetic mice [34]. Joshi-Barve et al. [35] reported that palmitic acid induces interleukin-8 from hepatocytes and consequent liver injury. Several recent studies have demonstrated that palmitic acid induces apoptosis in liver cells [36–38]. In the present study, palmitic acid levels were significantly and positively correlated with ALT levels ($P < 0.05$). Therefore, the reducing of palmitic acid levels may inhibit liver injury. Stearoyl-CoA desaturase 1(SCD1; known alternatively as D9D) is the final step in de novo lipogenesis and converts saturated fatty acid to monounsaturated fatty acid, whereas $\Delta$5- and $\Delta$6-desaturases participate in the metabolism of polyunsaturated fatty acids. Miyazaki et al. [39] have shown that the biosynthesis of hepatic CEs and triglycerides is highly dependent on the expression of the SCD1 gene. On the other hand, it has been reported that the activities of both D5D and D6D are linked with insulin sensitivity [40–43]. These previous data and the findings of the present study, including histological examinations, imply that EZ inhibits the development and progression of liver steatosis and insulin resistance.

Previous studies of the natural history of serial liver biopsies revealed that fibrosis stage progressed in 30–40%, remained stable in 30–40%, and regressed in 20–30% of cases, and that severity of steatosis, inflammation, hepatocytes ballooning, and Mallory's hyaline usually improved [44, 45]. In the present study, fibrosis stage progressed in 9%, remained in 88%, and regressed in 3% of cases over the 2-year period. Liver histologic findings were also improved in steatosis grade, necroinflammatory grade, ballooning score, and NAS score in this study. However, 3 of 33 patients had progression of fibrosis stage over the 2-year period. It is unclear whether this divergent response represents sampling error or heterogeneity in the population. Further studies are needed in order to clarify this point.

In conclusion, the results of this study suggest that the long-term EZ therapy can lead to improvement in metabolic, biochemical, and histological abnormalities of NAFLD through both improvement of insulin resistance and reduction in absorption of monosaturated fatty acids, especially palmitic acid. Therefore, EZ may be a promising agent for treatment of NAFLD. To confirm our finding, an appropriately designed, large-scale, controlled trial is need.

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