Rapid alternative absorption of dietary long-chain fatty acids with upregulation of intestinal glycosylated CD36 in liver cirrhosis

Yasunori Yamamoto, Yoichi Hiasa, Hidehiro Murakami, Yoshio Ikeda, Hirofumi Yamanishi, Masanori Abe, Bunzo Matsuura, and Morikazu Onji

ABSTRACT
Background: Dietary long-chain fatty acid (LCFA) intake is an important risk factor for hepatic inflammation and hepatocarcinogenesis. An alternate route of dietary LCFA absorption has been suggested in patients with liver cirrhosis (LC).

Objective: We aimed to determine this alternate route and to identify its mechanism.

Design: Twenty healthy control subjects and 47 patients with LC—n = 23 with portal hypertension [PH(+)]LC] and 24 without portal hypertension [PH(-)]LC]—were enrolled. [1-13C]Palmitate (an LCFA) and octanoate (a medium-chain fatty acid [MCFA]) were administered by using gastrointestinal endoscopy. Breath 13CO2 was measured to quantify metabolized fatty acids. We also examined intestinal specimens of patients in these groups.

Results: A more rapid increase in metabolized palmitate, which showed a pattern similar to that of octanoate metabolism, was observed in patients with LC than in healthy control subjects. The increase in the PH(-)LC group was higher than that in the PH(+)LC group. However, the concentration of metabolized palmitate increased with treatment of the PH(+)LC group with a portal-systemic shunt. Morphologic changes such as expanded lymph and blood vessels were present, and glycosylated CD36 increased in the jejunum of the PH(+)LC group. This group had high serum concentrations of glucagon-like peptide-2. These data suggest that dietary LCFA, similar to MCFA, are absorbed via blood vessels in patients with LC.

Conclusions: Rapid absorption of LCFA by an alternative method occurred in patients with LC. This altered LCFA processing is likely related to upregulation of intestinal glycosylated CD36 and could contribute to pathogenesis in patients with LC. This trial was registered at http://www.umin.ac.jp/ctr/index.htm as UMIN000006637 and contribute to pathogenesis in patients with LC. This trial was registered as UMIN000006637.

INTRODUCTION
Dietary long-chain fatty acids (LCFAs)4 are an important risk factor for the development of hepatic inflammation (1, 2) and hepatocarcinogenesis (3). LCFAs are absorbed by small intestinal villi after their hydrolysis from triacylglycerols by pancreatic lipase. Subsequently, LCFAs are discharged as a component of chylomicrons into lymphatic vessels and then finally spread into the systemic circulation (4). Previous studies of patients with liver cirrhosis (LC) showed a blunted chylomicronic response after oral lipid loading (5). However, this response occurred in the absence of significant steatorrhea (5). Cabre et al (6) showed that incorporation of dietary LCFAs into plasma free fatty acids (FFAs) peaked earlier in cirrhotic patients with ascites than in healthy volunteers. Although these results suggest that an alteration in the absorption of LCFAs might occur in patients with LC, little is known about its mechanism.

To examine this mechanism, we developed a method to obtain precise data regarding FA absorption, in which [1-13C]-labeled FAs are directly administered into the duodenum by using gastrointestinal endoscopy. Using this method we evaluated 13CO2 breath excretion of [1-13C]sodium palmitate, which is one of the most common dietary LCFAs and is known to induce hepatic inflammation (7). As a control we similarly assessed [1-13C]sodium octanoate—a medium-chain fatty acid (MCFA). In addition, we evaluated the expression of molecules associated with the absorption of LCFAs in intestinal specimens of patients with LC, because we previously found a histologic change in the intestinal villi of patients with advanced LC (8). We focused on the multiligand scavenger receptor CD36, which is a leading candidate for promoting LCFA uptake across the microvillus membrane (9). We also analyzed glucagon-like peptide-2 (GLP-2), which increases the expression of intestinal glycosylated CD36 (10). Using these approaches, we aimed to identify alterations in the absorption of LCFAs in patients with LC and the underlying mechanism.

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4 Abbreviations used: BRTO, balloon-occluded retrograde transvenous obliteration; FA, fatty acid; FABPc, cytosolic fatty acid-binding protein; FATP4, fatty acid transporter protein 4; GLP-2, glucagon-like peptide-2; iAUC, incremental AUC; I-FABP, intestinal FABP; LC, liver cirrhosis; LCFA, long-chain fatty acid; L-FABP, liver FABP; MCFA, medium-chain fatty acid; MTTP, microsomal triglyceride transfer protein; PCR, polymerase chain reaction; PH(-)LC, patients with liver cirrhosis without portal hypertension; PH(+)LC, patients with liver cirrhosis with portal hypertension.

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Rapid Absorption of LCFA via CD36 in Cirrhosis

Subjects

Twenty healthy control subjects and 47 patients with LC—n = 23 with portal hypertension [PH(−)LC] and 24 without portal hypertension [PH(−+LC)]—were enrolled in this study. Portal hypertension was defined as esophageal or gastric varices and/or ascites. The clinical features of these patients are shown in Table 1, which indicates significant differences in the data of some of the liver-function tests between the 3 groups because of damage caused by LC. The control group did not have acute or chronic illness, had not undergone hepatobiliary surgery in the past, and had routine laboratory values within the normal range. None of the subjects were treated with β-blockers, glucocorticosteroids, or insulin-, glucose-, or lipid-lowering drugs or were administered ursodeoxycholic acid, cholestyramine, or other bile salt–chelating agents during the experimental period. Patients, especially those with LC, with specific disorders such as acute gastrointestinal bleeding, overt hepatic encephalopathy, portal thrombosis (assessed by enhanced computed tomography), surgical or intrahepatic portal-systemic anastomosis, acute or chronic pancreatic disease, extrahepatic cholestasis, gastrointestinal surgery, dyslipemia, or fasting hyperglycemia (glucose concentration ≥126 mg/dL) were excluded from the study.

All enrolled participants provided written informed consent to the research testing, which was performed by using protocols approved by the Institutional Review Boards of Ehime University Hospital (approval number: 0904007, University hospital Medical Information Network ID: UMIN000006637). The study protocol conformed with the ethical guidelines of the Declaration of Helsinki.

Breath $^{13}$CO$_2$ assay after loading of [1-$^{13}$C]-labeled fatty acids

The subjects underwent loading tests with the use of [1-$^{13}$C]-labeled fatty acids, and the amount of metabolized $^{13}$CO$_2$ in the breath was then assayed. The study design is depicted in Figure 1A. After an overnight fast of ≥12 h, all subjects received [1-$^{13}$C]-labeled fatty acids in the horizontal portion of the duodenum by using an upper gastrointestinal tract endoscope (GIF-Q260; Olympus Corporation), and breath samples were then taken over a period of 360 min (Figure 1A). Samples were collected before loading and at the following times after loading of the fatty acids: every 15 min over the first 30 min and then every 30 min over the following 5 h 30 min.

The LCFA that was loaded and then analyzed by using $^{13}$C breath tests was [1-$^{13}$C]sodium palmitate (200 mg; Cambridge Isotope Laboratories Inc), which was prepared as follows: [1-$^{13}$C]sodium palmitate powder (Figure 1B) was dissolved in 25 mL distilled water, heated at 70°C (Figure 1C, left), and then blended with 25 mL warmed (50°C) RACOL Liquid for Enteral Use (Ostuka Pharmaceutical Factory Inc), which was used to keep the fatty acid in a liquid state (Figure 1C, right). The solution was then cooled down to room temperature. The MCFA that was loaded was 200 mg [1-$^{13}$C]sodium octanoate (Cambridge Isotope Laboratories), which is easy to dissolve in water and which we dissolved in 25 mL distilled water at room temperature. These fatty acids were loaded into the horizontal portion of the duodenum by using a spraying tube and upper gastrointestinal endoscopy, which allowed standardization of the loading time of the fatty acids (Figure 1D).

Breath samples were analyzed by using nondispersive infrared spectrometry (POC one; Otsuka Electronics). Change in the $^{13}$CO$_2$/$^{12}$CO$_2$ ratio from the baseline is expressed as $\Delta$ (%) by comparison with a $^{13}$C Pee Dee Belemnite international standard. Time-dependent change in the $\Delta$ (%) was used as a surrogate marker of the metabolism of [1-$^{13}$C]-labeled fatty acids.

Changes in the serum concentration of apolipoprotein B-48 before and after oral loading after ingestion of a test meal

Ten of the healthy control subjects and 18 of the patients with LC [n = 10 PH(−)LC and 8 PH(+)LC] who had undergone the [1-$^{13}$C]sodium palmitate breath tests agreed to undergo the oral-meal-loading test. After a 12-h overnight fast, an intravenous catheter was inserted into a forearm vein for blood sampling. Each subject ingested a test meal containing 10.2–12.8 g fat/m$^2$, 60 g carbohydrates, and 16 g protein in 300 mL of water.
55.2–69.5 g carbohydrate/m², and 13.8–17.4 g protein/m², which had a total energy content of 650 kcal/m² of body surface area. The test meal consisted of a hospital diet that is strictly controlled by the Dietary Department of Ehime University, Japan. After the test meal, the subjects fasted for the next 4 h, except for water intake, and blood samples were collected 1, 2, and 4 h after the test meal. The serum concentrations of apolipoprotein B-48, which is a postprandial hyperlipidemia marker that reflects lipid transportation of chylomicron derived from the diet, were measured by using the Human Apo B-48-specific ELISA kit (AKHB48; Shibayagi) according to the manufacturer’s instructions (11). The lower limits of detection were 2.5 ng/mL, and all assays were performed in duplicate. To compare the net postprandial change in these variables, the AUC for apolipoprotein B-48 was calculated by using the trapezoidal method. Incremental AUC (iAUC) values were then identified by ignoring the area beneath the fasting concentration.

Analysis of breath 13CO2 and measurement of serum apolipoprotein B-48 before and after balloon-occluded retrograde transvenous obliteration treatment

Balloon-occluded retrograde transvenous obliteration (BRTO) is a treatment of gastric varices and was performed by using a standard technique (12). From December 2009 to June 2011, 9 PH(+)LC patients had gastric varices with danger of rupture that were subsequently treated with BRTO, and these patients agreed to enroll in the study of the analysis of breath 13CO2 and serum apolipoprotein B-48 assays. The clinical features of the enrolled PH(+)LC patients are indicated in Table 2. For the breath 13CO2 test, 200 mg [1-13C]sodium palmitate was loaded by using upper gastrointestinal endoscopy as described for Figure 1. Breath samples were collected over 120 min, and the ratio of 13CO2 divided by 12CO2 was determined by using nondispersive infrared spectrometry (POC one). This test was performed both before and after BRTO treatment. We also analyzed changes in the serum concentration of apolipoprotein B-48 over time after

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| Characteristics of PH(+)LC patients treated with BRTO

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<th>PH(+)LC with BRTO (n = 9)</th>
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<td>Age (y)</td>
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<td>Sex (male:female)</td>
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1 ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BRTO, balloon-occluded retrograde transvenous obliteration; Hb A1c, glycated hemoglobin; HBV, hepatitis B virus; HCV, hepatitis C virus; PH(+)LC, patients with liver cirrhosis with portal hypertension; γ-GTP, γ-glutamyltransferase.

2 Mean ± SD (all such values).
Endoscopic examination of the small intestine and collection of tissue samples from the small intestine

Twenty of the patients who underwent small intestinal endoscopy gave informed consent for tissue samples to be taken. Written agreements were obtained from all patients after the nature and purpose of the study were explained. Ten of these subjects were PH(+)LC patients and the other 10 subjects were patients used as control subjects, who had no particular abnormalities associated with liver disease but who needed to undergo close inspection of their small intestine by endoscopy for purposes associated with anemia or small intestine lesions. The backgrounds of these subjects are described in Table 3 and indicate significant differences in some of the liver-function data between the groups attributable to LC. The study for collection of tissue samples was approved by the Institutional Review Board of Ehime University Hospital, Ehime, Japan (approval number: 1011004, University hospital Medical Information Network ID: UMIN000006638). We used a double-balloon endoscope (EN-450P5/20 or EN-450T5/W; Fujifilm Medical Co) or a small intestine endoscope (SIF 10T; Olympus Corporation) for these endoscopic examinations. The endoscopy was inserted through the oral cavity in all patients, and detailed endoscopic views were recorded. Biopsy specimens were obtained from the jejum at a point ~15 cm along its length at the deep part beyond Treitz’ s ligament. Some biopsy specimens were fixed in 10% formalin. A few other samples were stored frozen at −80°C for subsequent protein assay or were incubated with RNA later (Ambion) overnight at 4°C and were then frozen at −80°C for subsequent RNA assay.

Histologic examination and immunohistochemistry

Hematoxylin and eosin staining was performed by using formalin-fixed intestinal tissue samples and standard procedures. Immunohistochemical staining of CD31 and D2-40 was performed on 5-μm sections of formalin-fixed, paraffin-embedded tissue samples. Glycosylated CD36 was similarly analyzed, except that frozen tissue samples were used. Paraffin-embedded samples were deparaffinized, and antigen retrieval was performed in a steamer by using citrate buffer (pH 6.0) for 30 min. After blocking with hydrogen peroxide and normal goat serum, D2-40, CD31, and glycosylated CD36 were detected by incubating the sections with mouse monoclonal anti-human D2-40 (1:50, no. IR072; DAKO JAPAN Inc), mouse monoclonal anti-human CD31 (1:100, no. JC70A; DAKO JAPAN Inc), and mouse monoclonal anti-human glycosylated CD36 (1:50, no. ab17044; Abcam) antibodies, respectively, overnight at 4°C. The sections were then incubated with the appropriate biotin-conjugated species-specific secondary antibodies for 60 min at room temperature (DAKO JAPAN Inc). Phosphate-buffered saline was used in place of primary antibody in negative control sections. Color was developed by incubation with 3,3′-diaminobenzidine tetrahydrochloride, and the slides were counterstained with hematoxylin.

Morphometric analysis of intestinal blood vessels and lymph vessels

Microscopic pictures were taken and saved as electronic files (ACT-1; Nikon). The files were converted for analysis by using the ImageJ program (NIH). Lymph vessels were identified as D2-40-positive vessels, and blood vessels were identified as CD31-positive vessels. The number of these vessels was counted by using the ImageJ program. Both vessels were manually traced on the monitor, and their luminal areas were calculated by using the ImageJ program.

RNA extraction and quantitative real-time reverse-transcription polymerase chain reaction

RNA was extracted from intestinal tissue samples that were treated with RNA later (Ambion) by using TRizol (Invitrogen) according to the manufacturer’s protocol. The RNA was reverse transcribed by using reverse-transcriptase polymerase chain reaction (RT-PCR) kits (Applied Biosystems) with an oligo d(T)16 primer (Invitrogen) under standard conditions. Real-time PCR amplification was performed by using LightCycler technology (Roche Diagnostics) and the SYBR green I dye. The PCR consisted of an initial denaturation step for 10 min at 95°C, followed by 40 cycles under the following conditions: 10 s at 95°C, 10 s at 58°C, and 15 s at 72°C. Primer sets for GAPDH were purchased from Search LC. We designed the primers for PCR analysis of the fatty acid transport protein 4 (FATP4), intestinal fatty acid–binding protein (I-FABP), liver fatty acid–binding protein (L-FABP), and liver fatty acid–binding protein (L-FABP).
binding protein (L-FABP), microsomal triglycerides transfer protein (MTTP), and apolipoprotein B, which are listed elsewhere (see Supplemental Table 1 under “Supplemental data” in the online issue). These genes were detected by using the appropriate primer set under the recommended conditions. GAPDH served as a housekeeping control, and the expression of other host genes is shown relative to that of the GAPDH gene. The relative mRNA expression levels of host genes divided by the amount of GAPDH mRNA were calculated and evaluated by statistical analysis.

Western blotting
Protein was extracted from intestinal specimens by lysing the specimens with 500 μL Nonidet P-40 buffer (0.5% Nonidet P-40, 10 mmol/L Tris, pH 7.4, 150 mmol/L NaCl, 1% sodium dodecyl sulphate) by using a mixer mill (MM400; Retsch). An aliquot of the protein lysate (50 μg) was loaded onto 4–12% Bis-Tris gradient gels (Invitrogen), and Western blotting was performed as previously described (13). A rabbit polyclonal anti-human CD36 antibody (1:100, no. 100011; Cayman Chemical), a rabbit polyclonal anti-human FATP4 antibody (no. ab72724; Abcam), a rabbit polyclonal anti-human MTTP antibody (no. ab63467; Abcam), and a mouse monoclonal anti-human β-actin antibody (Millipore) were used to detect the target proteins. Appropriate species-specific conjugated secondary antibody kits, which were commercially obtained (GE Health Care), were used for detection. Lysates of human platelets were used as a positive control for CD36, because glycosylated CD36 (molecular mass: 88 kDa) is strongly expressed in these cells. These platelet samples were further treated with PNGase F (P0704S; New England Biolabs Inc) to remove all N-linked oligosaccharides. This treatment converted CD36 to the unglycosylated form, which has a molecular mass of 54 kDa. The signal intensity of each band was analyzed by using the ImageJ program as described previously (13).

Measurement of plasma GLP-2 activities
After the subjects fasted for 12 h, blood samples were collected into blood collection tubes containing EDTA-2Na with aprotinin additive (SRL Inc). A DPP IV inhibitor (10 μg; LINCO Research Inc) was immediately added to the tube. The plasma was immediately separated by centrifugation and stored at −80°C until assayed. The concentration of plasma GLP-2 was determined by using a human GLP-2 EIA kit (Yanaihara Research Laboratory). The antibody (Millipore) was used to detect the target proteins. Apolipoprotein-B48, which is produced by the small intestine, is considered to be the main apolipoprotein component of the chylomicron (14). To evaluate the efficiency of LCFA transport to

RESULTS
Time course of breath 13CO2 excretion after loading of [1-13C]-labeled fatty acids
We performed loading tests using [1-13C]sodium palmitate (an LCFA) or [1-13C]sodium octanoate (an MCFA) in 3 groups of subjects: 23 patients with LC with portal hypertension [PH(+)LC], 24 patients with LC without portal hypertension [PH(−)LC], and 20 healthy control subjects. After loading, we analyzed the metabolism of these fatty acids by analysis of the time course and the concentration of 13CO2 excretion.

After loading [1-13C]sodium octanoate, there was a progressive increase in breath 13CO2 excretion in all groups early after loading, which peaked at 30 min and then gradually decreased over the following 330 min, although it did not decrease to baseline concentrations (Figure 2A). In comparison with the other groups, breath 13CO2 excretion was decreased in the PH(+)LC patients at some time points, especially in the early phase after loading. We calculated the AUCs for the different groups for early times (0–120 min) and for the total time (0–360 min) after loading (Figures 2B and 2C). These data indicated that, in comparison with the control group, breath 13CO2 excretion of [1-13C]sodium octanoate was significantly decreased in the PH(+)LC patients, especially during the early phase (0–120 min) after loading (P < 0.01 compared with the control) (Figure 2B). The AUC (0–120 min) was calculated as 396 ± 55.9, 364 ± 85.9, and 270.4 ± 53.5 min · %e (mean ± SD) for the control, PH(−)LC, and PH(+)LC groups, respectively (Figure 2B).

In the control group, the time course of 13CO2 excretion after loading [1-13C]sodium palmitate gradually increased, reaching a peak 240 min after loading, after which it then decreased (Figure 2D). However, the early phase of 13CO2 excretion was significantly higher in both the PH(−)LC and the PH(+)LC groups than in the control group. The AUC analysis indicated that LC patients showed the highest 13CO2 excretion in the early phase; mean (±SD) values for the PH(−)LC, PH(+)LC, and control groups were 541.8 ± 85.9, 471.5 ± 43.5, and 398.9 ± 55.9 min · %e, respectively, for the 0–120 min period (Figure 2E). However, the overall 13CO2 excretion was lower in the PH(+)LC group than in the control and PH(+)LC groups: mean (±SD) values for the control, PH(−)LC, and PH(+)LC groups were 1884.1 ± 273.5, 1741.3 ± 207.5, and 1506.2 ± 105.1 min · %e, respectively, for the 0–360-min period (Figure 2F).

Changes in the serum concentration of apolipoprotein B-48 over time after loading of an oral test meal
Apolipoprotein B-48, which is produced by the small intestine, is considered to be the main apolipoprotein component of the chylomicron (14). To evaluate the efficiency of LCFA transport to
lymph vessels via chylomicrons, we measured the serum concentration of apolipoprotein B-48 at various times after intake of a test meal, the details of which are described above in Subjects and Methods. Compared with the control group, the serum concentrations of apolipoprotein B-48 were low in LC patients, especially in PH(+)LC patients (Figure 3A). The iAUC analysis of apolipoprotein B-48 concentrations, which is thought to describe the integrated postprandial chylomicronic responses more accurately than other methods of analysis, showed concentrations lower in LC patients than in the control group (Figure 3B). On the basis of these results, we considered, at the least, that the transport of LCFAs to lymph vessels via chylomicron in the patients with LC was not upregulated.

The efficiency of [1-13C]sodium palmitate metabolism was altered by treatment of the collateral vessels in patients with portal hypertension

We further analyzed 9 patients with PH(+)LC, who needed BRTO. We performed the [1-13C]sodium palmitate loading test both before and after the treatment of these patients with BRTO (Figure 3C). In the early phase (15–60 min) after loading, 13CO2 excretion increased after BRTO treatment. The AUC analysis of the 0–120-min period indicated that 13CO2 excretion increased in all of the patients after BRTO treatment (546.1 ± 685.9), and this increase was statistically significant compared with that before BRTO treatment (373.7 ± 55.9) (P < 0.001 for both comparisons; Figure 3D). Thus, portal-systemic shunting affected...
the dynamics of absorption and metabolism of sodium palmitate in the early phase after loading. In contrast, the serum concentration of apolipoprotein B-48 in these patients did not change before and after BRTO treatment (Figure 3, E and F). These results indicated that transport of LCFAs to lymph vessels via chylomicrons was not altered by BRTO treatment.

Morphologic examination of the jejunum in patients with LC with portal hypertension

LCFAs are absorbed mainly in the jejunum. We therefore examined morphologic changes in the jejunum of the PH(+)/LC and control groups using double-balloon endoscopy. An endoscopic view of the jejunum in a representative healthy control subject and in a PH(+)/LC patient is shown in Figure 4A. In the PH(+)/LC group, the mucosa of the jejunum was edematous with swollen and rounded villi. This feature was typically observed in 4 of the 10 PH(+)/LC patients examined (40%). Histologic examination of biopsy specimens from small intestinal tissues also showed that the villi of the PH(+)/LC patients were more round and edematous than those of the control group (Figure 4B). Lymph vessels were clearly distinguished in these specimens by staining with an anti D2-40 antibody (Figure 4C), and blood vessels were distinguished by staining with an anti CD31 antibody (Figure 4D). The number and the luminal areas of the lymph vessels (Figure 4E), and the number of blood vessels (Figure 4F), were analyzed. This analysis indicated that the mean luminal area of lymph vessels was significantly higher in the PH(+)/LC group than in the control group \(P < 0.05\). Moreover, the number and the luminal areas of blood vessels were significantly higher in the PH(+)/LC patients than in the control group \(P < 0.05\).
CD36 was upregulated and activated in the jejunum of patients with LC with portal hypertension.

CD36 is a key molecule for the uptake of LCFAs in the jejunum. We therefore analyzed the expression of glycosylated CD36, which is an activated form of CD36, in the jejunum of PH(+)LC patients. Immunohistochemical analysis showed that glycosylated CD36 is located in the brush border membrane of enterocytes and blood vessels and that the concentration of glycosylated CD36 was greater in the PH(+)LC patients than in the control group (Figure 5, A and B). Western blotting did show higher protein concentrations of glycosylated CD36 in the PH(+)LC patients than in the control group (Figure 5, C and D).

The concentrations of plasma GLP-2 were higher in patients with LC and correlated with the expression of glycosylated CD36, especially in patients with portal hypertension.

GLP-2 is a gastrointestinal-derived intestinotropic hormone that links nutrient absorption to intestinal structure and function. GLP-2 is also known to increase the expression of glycosylated CD36 (10). We evaluated the concentrations of GLP-2 in plasma samples from all groups under fasted conditions using an ELISA (Figure 5E). In patients with LC, with or without portal hypertension, the plasma concentrations of GLP-2 were significantly higher than those in the control group: 25.5 ± 14.4, 18.4 ± 10.2, and 6.9 ± 1.5 ng/dL in the PH(+)LC, PH(−)LC, and control groups (P < 0.01 for both comparisons). The plasma concentrations of GLP-2 were higher in the PH(+)LC group than in the PH(−)LC group (P < 0.05). Moreover, we found a positive correlation between the expression of glycosylated CD36 and the concentrations of plasma GLP-2 in these patients (r = 0.769, P < 0.0001; Figure 5F).

Analysis of the concentrations of molecules that contribute to the transport of LCFAs in patients with LC.

We additionally evaluated the expression of molecules that could contribute to the transport and chylomicron synthesis of LCFAs. We evaluated the mRNA concentrations of these molecules in biopsy samples from the jejunum using real-time RT-PCR (Figure 6). One of these molecules, FATP4, is expressed in the endoplasmic reticulum and is known to function as an intracellular enzyme that indirectly facilitates fatty acid uptake because of its acyl-CoA synthetase activity (15). The concentration of FATP4 mRNA was significantly lower in PH(+)LC patients than in the control group (Figure 6A). We also evaluated the mRNA concentration of cytosolic fatty acid–binding protein (FABPc), which has a high affinity for LCFAs and which facilitates fatty acid transport through the cytosol of enterocytes (16). Two different types of FABPc are expressed along the small intestine: I-FABP (17) and L-FABP (18). The mRNA concentrations of I-FABP and L-FABP did not differ between PH(+)LC patients and the control group (Figure 6, B and C). MTTP stabilizes apolipoprotein B-48 and contributes to chylomicron synthesis from triglycerides, during which apolipoprotein B-48 is incorporated (19). Although the mRNA concentrations of apolipoprotein B mRNA were not significantly different between PH(+)LC patients and the control group (Figure 6D), the MTTP mRNA concentration was significantly lower in PH(+)LC patients than in the control group (P < 0.05; Figure 6E). The lower expression of FATP4 and MTTP mRNA in the PH(+)LC patients was confirmed at the protein level by Western blotting of several patients in both groups (Figure 6F).

DISCUSSION

The current study was designed to assess the absorption and metabolic pathway of dietary LCFAs in patients with LC than in healthy control subjects after loading of [1-13C]sodium palmitate, which is one of the most abundant dietary FAs. A rapid
increase in metabolized \( [1-^{13}C] \) sodium palmitate over the first 120 min after administration was observed in patients with LC that was greater than that seen in healthy control subjects. This early pattern of LCFA metabolism in patients with LC was similar to that of the metabolism of \( [1-^{13}C] \) sodium octanoate, which is an MCFA that is absorbed via the portal vein. We performed an additional assay of patients with PH(+)LC, who were treated with BRTO for portal-systemic shunt. In these patients, the AUC of \( ^{13}CO_2 \) over the first 120 min after loading was increased after BRTO treatment. However, serum apolipoprotein B-48 concentration, which is a chylomicron marker, was not altered by BRTO. We considered that, in patients with LC, dietary LCFAs are absorbed via the portal vessels in a similar manner to MCFAs.

We administered test substrates directly into the duodenum by using gastrointestinal endoscopy to avoid the influence of gastric emptying. Although palmitic acid is a saturated fatty acid that is a solid at room temperature and is insoluble in water, \( [1-^{13}C] \) sodium palmitate was maintained in a liquid state during administration in this study because of the emulsifying effect of enteral nutrients. The use of this approach for FA administration resulted in a more precise evaluation of the absorption of dietary FAs from the small intestine than that obtained by using the traditional approach of oral FA administration (6).

We further examined the mechanism by which alteration of the absorption of LC FAs could occur in LC patients with portal hypertension. We identified morphologic changes, including expanded lymph and blood vessels, in the jejunum of PH(+)LC patients. The back pressure of lymph vessels is known to be elevated in patients with LC (20), which results in impairment of the absorption of LC FAs by lymph ducts. However, we also observed increased expression of glycosylated CD36 in the blood vessels of these patients. CD36 is a key molecule in the facilitative
uptake of LCFAs (21–23). We therefore hypothesized that this increase in glycosylated CD36 would ensure the absorption of LCFAs into the blood vessels in the jejunum. Moreover, the concentration of plasma GLP-2 was higher in patients with LC than in the control group. A positive correlation was observed between the expression of glycosylated CD36 and plasma GLP-2 concentrations. GLP-2 is a 33-residue peptide, formed from the cleavage of proglucagon peptide (24). Hsieh et al (10) reported that GLP-2 significantly increases the expression of glycosylated CD36. Therefore, the increased expression of glycosylated CD36 in patients with LC could be due to the upregulation of serum GLP-2. A recent study reported that a change in gut microbiota increases endogenous GLP-2 production (25). These effects were related with the production of short-chain fatty acids, which stimulate the release of GLP-2 (26), during bacterial fermentation (27). It is known that there is bacterial overgrowth in the small intestine of patients with LC (28, 29). Therefore, these bacteria might contribute to the upregulation of GLP-2 in patients with LC (26).

In our study, the concentrations of FATP4 and MTTP in the jejunum were significantly decreased in patients with PH(+)LC. It is known that oxidative stresses occur more often in the enterocytes of patients with LC (30, 31). Indeed, imbalances in protein oxidation and in the glutathione redox state have been observed in the intestinal mucosa of a rat model of LC (30–32). Increased oxidative stress in LC could inhibit the function of the endoplasmic reticulum, including the function of FATP4 and MTTP, which are located in the endoplasmic reticulum in patients with LC, and the latter would result in a decrease in the chylomicronemic response. On the basis of the combined data, we considered that both blunted chylomicron formation and the blunting of its transport could contribute to the absorption of higher amounts of LCFAs via the blood vessels in the jejunum.

We summarized our results in a schematic illustration of the proposed alteration of LCFA absorption in the small intestine of patients with PH(+)/LC (Figure 7). We propose that the absorption of dietary LCFAs through the portal capillary vessels occurs through the following 4 alternative processes: 1) morphologic changes in the jejunum, including the expansion of lymph and blood vessels that occur as a result of portal hypertension, which results in the occurrence of alternative absorption of LCFAs through the blood vessels; 2) promotion of intestinal absorption of LCFAs by glycosylated CD36 that is upregulated in the enterocyte apical membrane; 3) a decrease in the chylomicronemic response attributable to a decrease in FATP4 and MTTP; and 4) an increase in the number and the luminal area of blood vessels attributable to portal hypertension. Although the back pressure of blood capillary vessels would be increased by

![Figure 7. Model of the alternative absorption of dietary LCFAs in patients with liver cirrhosis. This alternative absorption is likely to be associated with 4 alternative processes: 1) morphologic change in the jejunum, including an increase in the luminal area of lymph and blood vessels attributable to portal hypertension; 2) an increase in glycosylated CD36 in the brush border membrane of enterocytes; 3) a blunted chylomicronemic response attributable to the impairment of lymphatic chylomicron transport and the downregulation of molecules (FATP4 and MTTP) that contribute to the transport of LCFA; 4) increased LCFA transport into the blood vessels that express glycosylated CD36, rather than into lymphatic vessels because of the back pressure of these vessels as a result of portal hypertension. ACBP, acyl-CoA-binding protein; ACS, acyl-CoA synthetase; Apo, apolipoprotein; ER, endoplasmic reticulum; FACoA, fatty acyl-CoA; FATP4, fatty acid transporter protein 4; I-FABP, intestinal fatty acid–binding protein; LCFA, long-chain fatty acid; L-FABP, liver fatty acid–binding protein; MTTP, microsomal triglycerides transfer protein.](image)
portalmembrane, the increased expression of glycosylated CD36 in these vesicles may be sufficient to maintain LCFA transport into blood capillary vessels. The portal absorption of dietary LCFAs in patients with LC could be related to the rapid increased absorption of LCFAs, and this rapid increase might relate to the pathogenesis of LC attributable to the LCFA metabolism for patients with LC.

An alteration in the metabolism of [1-13C]sodium octanoate was also observed in the PH(+)LC patients. The oxidation of octanoate was lower in those patients than in the other groups (Figure 2A and 2B). Metabolism of octanoate is considered to be preserved, even in patients with LC (33). Indeed, the lower octanoate oxidation was seen only in the early phase (0–150 min) after administration. This oxidation was influenced by the first blood pass of octanoate from the intestine, which would decrease because of collaterals in the patients with PH(+)LC. The combined data suggest that the low level of octanoate oxidation in PH(+)LC patients was associated with a change in blood flow rather than with a change in the oxidizing ability of the cirrhotic liver. In a comparison of the amount of oxidation of palmitate and octanoate in the early phase after administration (compare Figure 2A and 2D), it looks blunted in palmitate rather than in octanoate. We considered that this difference in oxidation would be affected by differences in the mechanisms of both absorption and of oxidation of palmitate and octanoate. Thus, it is known that palmitate needs to be incorporated into micelles by intraluminal bile acids in the intestine, and that this micelle formation is usually decreased in patients with LC (34). Moreover, palmitate needs a special element, such as carnitine, for oxidation. The carnitines are known to be decreased in patients with LC (33). Indeed, the lower octanoate oxidation was seen only in the early phase (0–150 min) after administration. This oxidation was influenced by the first blood pass of octanoate from the intestine, which would decrease because of collaterals in the patients with PH(+)LC.

In conclusion, a rapid alternative absorption of LCFA after dietary intake occurred in patients with LC. This alternative pathway occurs via portal capillary vessels in the jejunum and is accompanied by upregulation of glycosylated CD36 and an increase in plasma GLP-2 concentrations. This alteration in the absorption of LCFA might influence the pathogenesis of patients with LC, and its clinical effect should be evaluated in future studies.

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