(7) 血清 BTR (BCAA/Tyr) 値

分岐鎖アミノ酸 (Leucine, Valine, Isoleucine) と芳香族アミノ酸 (Phenylalanine, Tyrosine) のモル比 (Fischer モル比) は肝の代謝, 予備能の評価, 肝障害の重症度判定に重要である[9]が, アミノ酸インバランスの結果として蛋白栄養障害が惹起される. したがって肝硬変患者の蛋白代謝状態を的確に評価するためには血清アルブミン値だけでなくアミノ酸代謝状態も検討することが不可欠である. BCAA/Tyr 比 (BTR) はアミノ酸代謝状態を評価できる簡便かつ有用な指標であるが臨床の現場ではあまり測定されていない. BTR を測定することにより血清アルブミン値の変化を予測することができる[10]ため分岐鎖アミノ酸の投与を予測することができる.

(8) 血清遊離脂肪酸

血清遊離脂肪酸は飢餓状態において高値を呈することが知られており, また肝硬変患者では早朝空腹時に飢餓状態にあるため肝硬変では血清遊離脂肪酸が上昇し[11], 就寝前夜食を行うことにより血清遊離脂肪酸は低下する[12]ことから早朝のエネルギー代謝状態の評価に有用であるが, 早朝飢餓状態にあることを血清遊離脂肪酸のみで判断するのは今のところは不可能である.

(9) その他

上記のように臨床の現場でも用いることができる栄養指標を用いることにより肝硬変患者の栄養評価を行うことが可能である.

3. おわりに

肝硬変患者にとって栄養治療は極めて重要である. 適切な栄養治療を必要な患者に提供するためには肝硬変患者にとって適切な栄養評価を行うことが重要である. 栄養指標の多くは肝疾患の病態の影響を受けやすいためその評価は慎重に行うべきである.

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Original Article

Effects of late evening snack on diurnal plasma glucose profile in patients with chronic viral liver disease

Kazutomo Suzuki,[1] Kagemasa Kagawa,[1] Kazuhito Koizumi,[1] Kazuyoshi Suzuki,[1] Hiromi Katayama[1] and Miwa Sugawara[2]

[1]*Department of Gastroeneterology & Hepatology and* [2]*Department of Nutrition, Koshigaya Hospital, Dokkyo Medical University, Saitama, Japan*

Aim: Glycemic control is important to improve the prognosis in cirrhotic patients with complications from diabetes. A late evening snack (LES) has been recommended for cirrhotic patients. We investigated the effects of LES on diurnal plasma glucose levels.

Methods: Subjects comprised 47 patients with chronic viral liver disease (chronic hepatitis, $n = 11$; cirrhosis, $n = 36$) treated in the Department of Gastroenterology & Hepatology, Dokkyo Medical University Koshigaya Hospital. Diurnal variations in plasma glucose were first investigated with three meals/day, in accordance with the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines. Starting the next day, patients were given four meals including a LES, without changing meal content. Diurnal variations in plasma glucose were examined on day 7, and urine C-peptide immunoreactivity (CPR), and homeostasis model assessment insulin resistance (HOMA-IR) were investigated.

Results: With a LES, plasma glucose levels in patients with chronic hepatitis were significantly lower 2 hours before and 2 hours after dinner. In cirrhotic patients, significant decreases in plasma glucose levels were seen 2 hours after breakfast, before lunch, and before dinner. Significant decreases were noted in average plasma glucose levels and highest plasma glucose levels with four meals including a LES in patients with liver cirrhosis. This decrease was greater when maximum plasma glucose levels were higher on the three-meal regimen.

Conclusions: Improvements in plasma glucose levels were seen with four meals per day, including a LES, in viral chronic liver disease, particularly cirrhosis.

Key words: chronic liver disease, diabetes, late evening snack, plasma glucose

INTRODUCTION

A HIGH RATE OF complications have long been known to occur with diabetes against a background of chronic liver disease, particularly hepatitis C virus (HCV)-related chronic liver disease. Patients with non-alcoholic chronic liver disease and diabetes are also known to show a high rate of liver cancer,[1,2] and cirrhotic patients with complications of diabetes show an increased frequency of liver disease-related death and worsened prognosis.[3] However, prognosis can be improved with strict glycemic control in HCV-related cirrhosis patients with diabetes.[4] Glycemic control in patients with chronic liver disease with diabetes is therefore crucial for preventing the onset of liver cancer and improving prognosis. Patients with liver cirrhosis are in a hypermetabolic state and can thus reach a state of extreme hunger during morning fasting, and the non-protein respiratory quotient (npRQ) decreases because of an increased fat-burning rate. A late evening snack (LES) to improve the morning starving state[5] is recommended for patients with liver cirrhosis.[6] However, dietary management of diabetes in patients with method of diet intake in patients with diabetes is not clear. We therefore investigated the effects of LES on diurnal fluctuations in plasma glucose in patients with chronic viral liver disease.

Correspondence: Dr Kazutomo Suzuki, Department of Gastroeneterology & Hepatology, Koshigaya Hospital, Dokkyo Medical University, 2-1-50, Minami-Koshigaya, Koshigaya, Saitama 343-8555, Japan. Email: s-kazu@dokkyomed.ac.jp
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METHODS

Subjects

SUBJECTS COMPRISED 11 patients with chronic viral hepatitis (hepatitis B virus (HBV), $n = 2$; HCV, $n = 9$) and 36 patients with viral cirrhosis (HBV, $n = 8$;

Table 1 Data are expressed mean standard deviation. Turkey's method was used for statistical analysis

	n	Age (years) §	Male	Female	HBV	HCV	BMI (kg/m²)§
Chonic hepatitis	11	56 ± 14	8	3	2	9	23.5 ± 3.7
Liver cirrhosis	36	66 ± 8†	21	15	8	28	22.9 ± 3.7
Grade A‡	10	69 ± 4	6	4	1	9	23.5 ± 4.0
Grade B,C‡	26	65 ± 5	4	11	7	19	22.6 ± 3.6

†Chronic hepatitis vs liver cirrhosis, $P = 0.003$. ‡Child–Pugh classification. §Mean±standard deviation. BMI, body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus.

HCV, $n = 28$) hospitalized in the Department of Gastroenterology & Hepatology, Dokkyo Medical University Koshigaya Hospital between June 2002 and May 2004 (Table 1). Diagnoses were made comprehensively by liver specialists using blood biochemistry and abdominal ultrasonography. HCV-related hepatitis was diagnosed based on positive results for HCV-RNA from RT-PCR (real time polymerase chain reaction), and HBV-related hepatitis was diagnosed based on Hepatitis B surface antigen (HBsAg) positivity from enzyme immunoassay (EIA). This study was conducted in accordance with the Helsinki Declaration of the World Medical Association in 1975. Diurnal fluctuations in plasma glucose were monitored only in patients who had provided consent after receiving full explanations regarding chronic liver disease and glucose metabolism disorders. Patients complicated with liver cancer, patients with a history of taking branched-chain amino acid preparations and gastrointestinal tract surgery, and patients currently taking diabetes medications were excluded from this investigation.

Methods

In accordance with The European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines,[7] diet therapy was conducted with 25–35 kcal/day of non-protein and 1.0–1.2 g/day of protein per 1 kg of standard weight in three meals/day. Diurnal fluctuations in plasma glucose were measured on day 7 after starting diet treatment with three meals (early morning fasting (0600), 2 h after breakfast (0900), before lunch (1200), 2 h after lunch (1400), before dinner (1800), 2 h after dinner (2000), and 4 h after dinner (2200). Diet treatment with four meals including a late evening snack (LES) was started on the day after diurnal fluctuations in plasma glucose were measured in all subjects. We served LES at 2200. To maintain the same daily energy intake with four meals, 70 kcal was taken from each of the three meals and that reduction of approximately 200 kcal was provided in the LES.[5] Seven days after starting diet treatment with four meals including LES, diurnal fluctuations in plasma glucose were again measured, and the effects of four meals including a LES on these fluctuations were investigated. Average plasma glucose levels were taken as the mean levels of the seven measurements within a day, and the highest level within the daily fluctuations was used as maximum plasma glucose level. Changes in average and highest plasma glucose levels before and after starting LES were investigated. In addition, plasma insulin levels were measured by chemiluminescent enzyme immunoassay during the morning fast, and insulin resistance was investigated before and after LES. Insulin resistance was calculated as fasting glucose level (mg/dL) × fasting insulin level (μU/mL) using homeostasis model assessment insulin resistance (HOMA-IR).[8] To investigate effects on insulin secretion of four meals including LES, 24-h urine collection was conducted on the day diurnal fluctuations in plasma glucose were measured, and urinary C-peptide (CPR) was measured by EIA.

Statistical analysis

All statistical tests were performed using SPSS software (SPSS, Chicago, IL). Data are expressed as mean ± standard deviation. Analysis was performed using the paired *t*-test and Tukey's test. The level of statistical significance was taken as $P < 0.05$.

RESULTS

Intakes of energy and three main nutrients

IN PATIENTS WITH chronic hepatitis, non-nitrogen energy intake was 31.7 ± 3.6 kcal/kg/day per standard body weight, protein intake was 1.1 ± 0.1 g/kg/day, fat intake was 50.9 ± 5.4 g/day, and carbohydrate intake was 278.2 ± 16.6 g/day. In patients with liver cirrhosis, non-nitrogen energy intake was 32.3 ± 3.5 kcal/kg/day per standard body weight, protein intake was 1.1 ± 0.2 g/kg/day, fat intake was 49.2 ± 6.5 g/day, and carbohydrate intake was 276.9 ± 15.8 g/day. No

significant difference in intake of either energy or these three main nutrients was seen between groups.

Influence of four meals including LES on diurnal plasma glucose profile for patients with chronic liver disease

In patients with chronic hepatitis, significant decreases in plasma glucose levels were seen before the evening meal and 2 h after the evening meal ($P = 0.0281$, $P = 0.0406$) with four meals including LES. In patients with liver cirrhosis, plasma glucose levels were significantly decreased 2 h after breakfast, before lunch, and before dinner ($P = 0.0160$, $P = 0.0004$ and $P = 0.0440$, respectively) with four meals including LES. However, no significant differences in minimum plasma glucose levels during diurnal fluctuations were apparent between the 3- and 4-meal regimens. In patients with liver cirrhosis (Child–Pugh grade A), no significant decreases in plasma glucose levels were seen after the evening meal with four meals including LES. However, in patients with liver cirrhosis (Child–Pugh grades B and C) plasma glucose levels were significantly decreased before lunch and 2 h after lunch ($P = 0.0268$ and $P = 0.0006$, respectively) with four meals including LES (Table 2).

Changes of average plasma glucose levels and highest plasma glucose levels in patients with liver cirrhosis

In patients with chronic hepatitis, average plasma glucose levels were 204 ± 75 mg/dL in three meals/day and 167 ± 46 mg/dL after LES. Highest plasma glucose levels were 256 ± 100 mg/dL on three meals/day and 212 ± 56 mg/dL after LES. No significant differences were seen in patients with chronic hepatitis. In patients with liver cirrhosis, highest plasma glucose levels were 232 ± 97 mg/dL on three meals/day and 222 ± 98 mg/dL after LES. No significant differences were seen. However, in patients with liver cirrhosis, average plasma glucose levels were decreased significantly after LES (182 ± 82 vs. 168 ± 70) ($P = 0.0099$) (Fig. 1). Besides, in patients with chronic liver disease (HCV), average plasma glucose levels were 181 ± 80 mg/dL in three meals/day and 1163 ± 65 mg/dL after LES. Highest plasma glucose levels were 231 ± 99 mg/dL on three meals/day and 212 ± 90 mg/dL after LES ($P = 0.0215$). In patients with chronic liver disease (HBV), average plasma glucose levels were 209 ± 82 mg/dL for three meals/day and 186 ± 64 mg/dL after LES. Highest plasma glucose levels were 261 ± 93 mg/dL for three meals/day and 249 ± 84 mg/dL after LES. No significant

Table 2 Trends in diurnal changes of plasma glucose before and after LES in patients with chronic liver disease. Data are expressed mean standard deviation. A paired *t*-test was used. Before LES, 3 meals/day; After LES, 4 meals/day including LES

	Chronic hepatitis			Liver cirrhosis			Child–Pugh grade A			Child–Pugh grade B,C		
	Before LES	After LES	*P*-value	Before LES	After LES	*P*-value	Before LES	After LES	*P*-value	Before LES	After LES	*P*-value
Cases (*n*)	11	11		36	36		10	10		26	36	
Fasting glucose (mg/dL)	142 ± 37	138 ± 51	0.7681	123 ± 47	120 ± 45	0.4766	117 ± 37	106 ± 21	0.2589	125 ± 50	125 ± 50	0.0875
2 h after breakfast (mg/dL)	239 ± 93	201 ± 64	0.1985	221 ± 102	193 ± 98	0.0160	205 ± 112	176 ± 119	0.3029	227 ± 100	200 ± 91	0.9935
Before lunch (mg/dL)	197 ± 101	163 ± 86	0.3838	181 ± 94	158 ± 81	0.0004	163 ± 107	149 ± 84	0.2781	187 ± 91	161 ± 82	0.0268
2 h after lunch (mg/dL)	228 ± 98	183 ± 52	0.1147	199 ± 101	187 ± 93	0.0928	190 ± 116	179 ± 97	0.2565	203 ± 96	190 ± 93	0.0006
Before dinner (mg/dL)	180 ± 65	136 ± 35	0.0281	158 ± 81	139 ± 68	0.0440	144 ± 81	129 ± 47	0.3602	163 ± 82	143 ± 74	0.1824
2 h after dinner (mg/dL)	230 ± 78	177 ± 58	0.0406	203 ± 93	194 ± 78	0.3522	203 ± 99	178 ± 66	0.1487	202 ± 92	201 ± 83	0.0800
4 h after dinner (mg/dL)	216 ± 78	173 ± 49	0.1682	190 ± 95	184 ± 81	0.5280	182 ± 103	173 ± 70	0.5198	193 ± 94	189 ± 86	0.8622
Minimum plasma glucose (mg/dL)	132 ± 32	113 ± 23	0.1055	117 ± 49	108 ± 41	0.0661	108 ± 40	101 ± 24	0.4849	120 ± 52	110 ± 46	0.6963

LES, late evening snack.

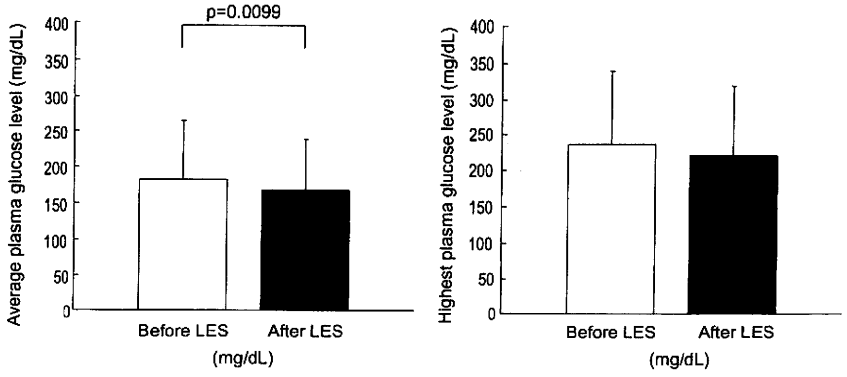

Figure 1 Changes of average plasma glucose levels and highest plasma glucose levels before and after LES in patients with liver cirrhosis. A paired *t*-test was used. Before LES, 3 meals/day; After LES, 4 meals/day including LES. LES, late evening snack.

differences were seen in patients with chronic liver disease (HBV).

Relationship between fasting plasma glucose levels before LES and changes of average plasma glucose levels after LES in patients with liver cirrhosis

Relationship between fasting plasma glucose levels before LES and changes of average plasma glucose levels after LES were investigated in patients with liver cirrhosis. Average plasma glucose levels were significantly decreased ($P = 0.0034$) after starting LES, when fasting plasma glucose level was higher before LES (Fig. 2), but no significant change was seen in patients with chronic hepatitis.

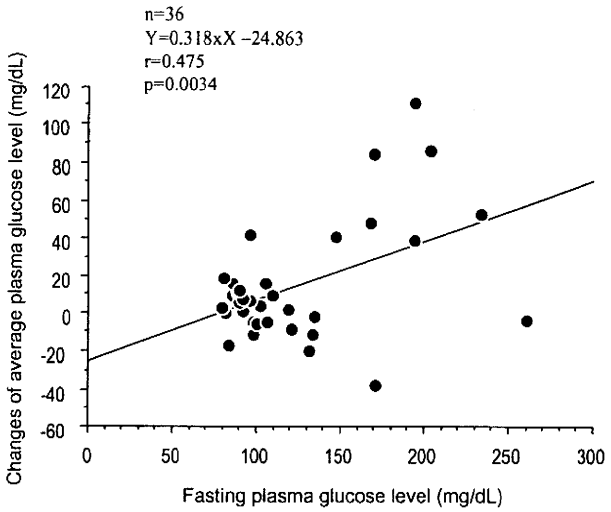

Figure 2 Relationship between fasting plasma glucose levels in 3 meals/day and changes of average plasma glucose levels after LES in patients with liver cirrhosis. The investigation was performed using Pearson's correlation coefficient.

Relationship between highest plasma glucose levels in three meals/day and changes of average plasma glucose levels and highest plasma glucose levels after LES

The highest plasma glucose levels in diurnal fluctuations before LES and changes in average plasma glucose levels and highest plasma glucose levels before and after LES were investigated (Fig. 3). In patients with chronic hepatitis, there were no significant changes in average plasma glucose levels and highest plasma glucose levels before and after LES in patients with chronic hepatitis. However, decreasing degrees of average plasma glucose levels and highest plasma glucose levels were significantly greater ($r = 0.830$, $P = 0.0016$, $r = 0.826$, $P = 0.0017$) in proportion to the degree of highest plasma glucose levels.

The highest plasma glucose levels in diurnal fluctuations before LES and changes in average plasma glucose levels and highest plasma glucose levels before and after LES were investigated in patients with liver cirrhosis (Fig. 4). Decreasing degrees of average plasma glucose levels and highest plasma glucose levels were significantly greater ($r = 0.558$, $P = 0.0004$, $r = 0.373$, $P = 0.0250$) in proportion to the degree of highest plasma glucose levels.

Effects of four meals with LES on insulin resistance

In patients with chronic hepatitis patients, insulin resistance (HOMA-IR) was 3.28 ± 1.37 before LES and 4.37 ± 1.55 with four meals including LES, showing no significant change. HOMA-IR in patients with liver cirrhosis was 5.75 ± 8.69 before LES and 3.77 ± 2.41 with four meals including LES, again showing no significant change.

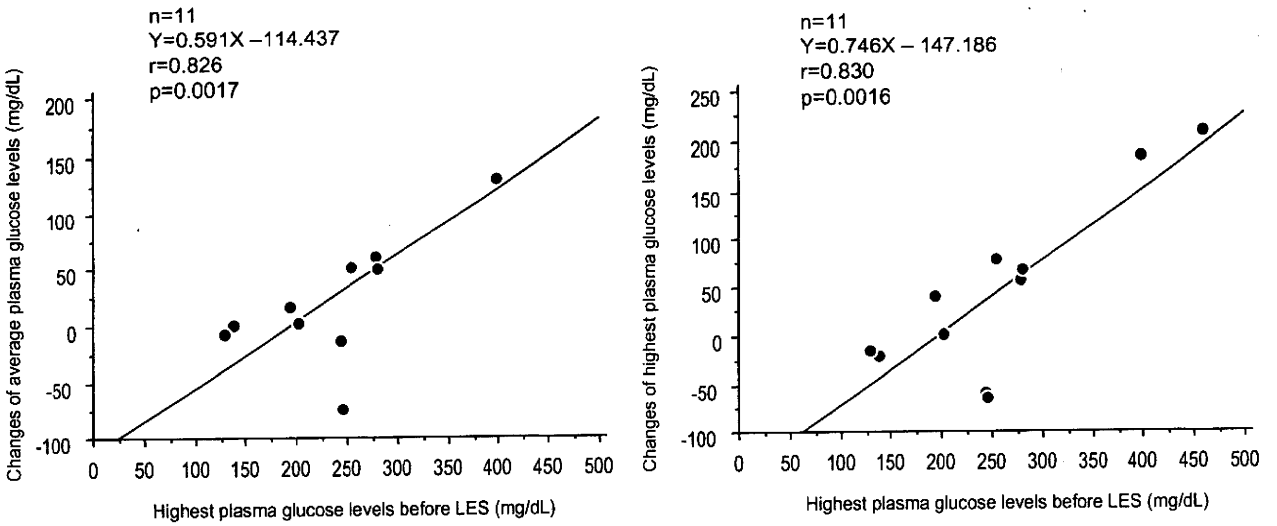

Figure 3 Relationship between highest plasma glucose levels in 3 meals/day and changes of average plasma glucose levels and highest plasma glucose levels after LES in patients with chronic hepatitis. The investigation was performed using Pearson's correlation coefficient.

Effects on insulin secretion of four meals including LES

In patients with chronic hepatitis urine C-peptide was 96.6 ± 17.2 μg/day before LES and 92.3 ± 34.1 μg/day with four meals including LES. Similarly, in patients with liver cirrhosis urine C-peptide was 97.6 ± 104.1 μg/day before LES and 90.4 ± 93.5 μg/day with four meals including LES. Neither change was significant. In addition, no significant differences were seen after stratifying the patients according to average plasma glucose levels and highest plasma glucose levels.

DISCUSSION

A HIGH RATE OF complications is seen with diabetes in patients with liver cirrhosis, and diabetes in patients with liver cirrhosis reduces long-term survival

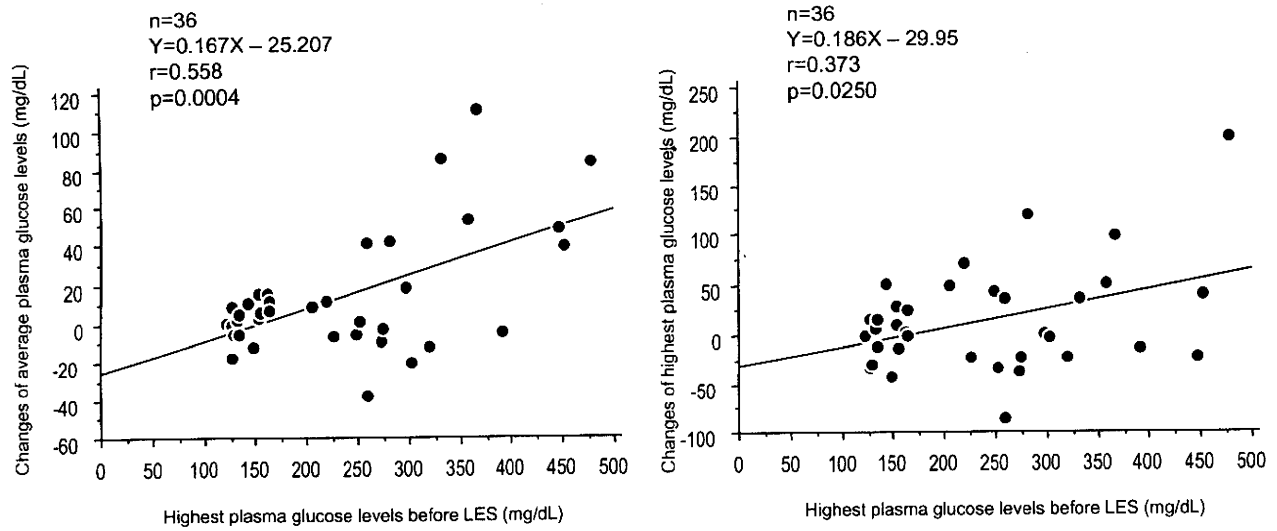

Figure 4 Relationship between highest plasma glucose levels in 3 meals/day and changes of average plasma glucose levels and highest plasma glucose levels after LES in patients with liver cirrhosis. The investigation was performed using Pearson's correlation coefficient.

in liver disease patients. Cause of death in these patients is often hepatic failure.[3] Variables that were found to have a significantly increased hazard ratio of liver cancer without the interaction term were sex, baseline serum albumin level, concurrent diabetes, baseline BMI, and baseline AFP level.[9] In hepatitis C, the virus itself is related to insulin resistance, and this insulin resistance has also been indicated to influence the progression of liver fibrosis.[10] In addition, HCV-related chronic hepatitis shows a high rate of liver cancer from complications with diabetes,[2,11] and hyperinsulinemia in liver cancer patients accelerates the rate of tumor growth for the liver cancer. If insulin secretion is suppressed by administration of octreotide, the rate of tumor growth is slowed,[12] indicating a relationship between insulin secretion and the rate of liver cancer growth. A relationship has also been indicated between insulin therapy and postoperative recurrence of liver cancer complicated with cirrhosis when the patient has diabetes.[13] Therefore, controlling plasma glucose to treat diabetes in patients with liver cirrhosis may reduce mortality rates related to liver disease. Moreover, suppressing insulin secretion through dietary measures may delay liver cancer recurrences and tumor growth rate.

Dietary measures for diabetes in patients with liver cirrhosis are important in consideration of the hepatic usage of glucose. In an investigation of glucose usage using an indirect calorimeter in liver cirrhosis, glucose usage was found to be slowed in liver cirrhosis, but increased in peripheral skeletal muscle and increased overall.[14] Compared with three meals/day, four meals/day including LES decreases the glucose intake per meal, so glucose can be supplied gradually with four meals including LES. Considering the state of glucose metabolism in cirrhotic patients, frequent meals may be effective in controlling plasma glucose in patients with liver cirrhosis.

We therefore investigated the effect of four meals including LES in diurnal fluctuations of plasma glucose in hospitalized patients. In patients with chronic hepatitis, the glucose contained in a single meal can be adequately processed in the liver, even with three meals/day, so we assumed that no major changes would be seen in the diurnal profile of plasma glucose with four meals including LES. In cirrhosis, however, uptake of glucose in the liver is slower than that in patients with chronic hepatitis, so with three meals the glucose contained in a single meal cannot be completely processed in the liver, and high plasma glucose is seen. With four meals including LES, the glucose supplied in meals can be processed in the liver because the amount of glucose per meal is decreased, so elevations in plasma glucose can be kept down. Improvements in the diurnal profile of plasma glucose were thus attributed to decreases in overall plasma glucose with four meals including LES. Therefore, average plasma glucose levels and highest plasma glucose levels were significantly decreased after four meals/day in patients with liver cirrhosis. However, even with four meals including LES, no significant decrease was seen in highest plasma glucose levels and highest plasma glucose levels in patients with chronic hepatitis, but a decreasing trend was seen.

No change in insulin secretion was seen with four meals including LES. In patients with liver cirrhosis, hepatic parenchymal cells decrease, tissue fibrosis occurs, and the glucose absorbed to rebuild lobular structures is not taken up by hepatic cells. As a result, glucose not taken up in the liver is thought to be oxidized in peripheral skeletal muscle and brain by the action of insulin and used as an energy source, so hyperinsulinemia is expressed.[15] Thus, in patients with liver cirrhosis eating three meals/day, the glucose that is not processed in the liver is used in peripheral tissues through the actions of insulin, and this increases insulin secretory capacity. With four meals including LES, plasma glucose is conjectured to decrease because glucose is processed adequately in the liver, so the amount of glucose transported to peripheral tissues is decreases, thereby decreasing insulin secretory capacity. In our investigation, however, no significant difference was seen in insulin secretion. This may have been because glucose was processed in peripheral tissues via insulin, since the amount of glucose supplied in 1 meal with a 4-meal regimen exceeded the glucose-processing capacity of the liver, and as a result no significant difference in insulin secretion was seen. Investigation of increasing the number of meals to more than 4 and decreasing the amount of glucose provided per meal may be warranted.

We investigated the kinds of cases in which four meals a day is effective, but in patients with chronic hepatitis, average and highest plasma glucose levels were not significantly decreased. However, decreasing degrees of average plasma glucose levels and highest plasma glucose levels were significantly greater in proportion to the degree of highest plasma glucose levels. In patients with liver cirrhosis, average plasma glucose levels and highest plasma glucose levels were decreased significantly after LES. In addition, decreasing degrees of average plasma glucose levels and highest plasma glucose levels were significantly greater in proportion to the degree of highest plasma glucose levels. A regimen of four meals including LES should thus be actively used

with chronic liver disease patients showing high plasma glucose especially in patients with liver cirrhosis.

The present investigation examined only a small number of subjects, but was useful as an investigation of dietary measures aimed at lowering plasma glucose in cases of liver cirrhosis with diabetic complications. However, no change in insulin secretion was seen with four meals including LES. The present findings suggest that frequent meals of >four meals/day will need to be investigated in terms of dietary measures that also aim to suppress insulin secretion.

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Hepatology Research

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Original Article

Effect of a late evening snack using branched-chain amino acid-enriched nutrients in patients undergoing hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma

Yohei Harima, Takahiro Yamasaki, Satoe Hamabe, Issei Saeki, Kohsuke Okita, Shuji Terai and Isao Sakaida

Department of Gastroenterology and Hepatology, Yamaguchi University Graduate School of Medicine, Ube, Yamaguchi, Japan

Aim: A late evening snack (LES) is recommended for protein-energy malnutrition in patients with liver cirrhosis. This study investigated energy metabolism in cirrhotic patients with hepatocellular carcinoma (HCC) and the effects of LES using a branched-chain amino acid (BCAA)-enriched nutrient in cirrhotic patients with advanced HCC undergoing hepatic arterial infusion chemotherapy (HAIC).

Methods: Energy metabolism was measured using indirect calorimetry for 10 cirrhotic patients without HCC and 36 patients with various stages of HCC. Next, in 23 cirrhotic patients with advanced HCC undergoing HAIC, 13 patients received LES (LES group), and 10 patients received ordinary food (control group). Changes in energy metabolism and glucose tolerance were examined using indirect calorimetry and 75-g oral glucose tolerance test (OGTT) before and after 1 cycle of treatment.

Results: Non-protein respiratory quotient (npRQ) was significantly lower in patients with advanced HCC than in cirrhotic patients without HCC, or in patients with early-stage HCC. In cirrhotic patients with advanced HCC undergoing HAIC, npRQ, BCAA/tyrosine ratio (BTR), and prealbumin and ALT levels were significantly improved in the LES group, but not in controls. In addition, area under the concentration curve for glucose (AUC glucose) tended to be improved in the LES group.

Conclusions: LES using BCAA-enriched nutrients appears to improve energy metabolism and glucose tolerance in cirrhotic patients with advanced HCC undergoing HAIC.

Key words: advanced hepatocellular carcinoma, branched-chain amino acid, hepatic arterial infusion chemotherapy, late evening snack, nutritional therapy

INTRODUCTION

THE LIVER PLAYS an important role in energy metabolism, and liver diseases lead to abnormalities in nutrient metabolism and subsequent malnutrition.[1] Protein-energy malnutrition (PEM) is a common finding in cirrhotic patients.[2,3] Owen *et al.* reported that patients with cirrhosis show marked decreases in glucose oxidation after an overnight fast, with enhanced fat and protein catabolism similar to that observed in healthy controls after 2–3 days of starvation.[4] PEM is a significant factor in establishing the vital prognosis of liver cirrhosis.[3]

In an attempt to improve the state of energy malnutrition, a late evening snack (LES) has been developed for use by patients with liver cirrhosis, resulting in improved energy substrate metabolism.[5–8] A LES is recommended in the present guidelines of the American Society for Parenteral and Enteral Nutrition[9] and the European Society for Clinical Nutrition and Metabolism.[10] We have also reported that a LES using branched-chain amino acid (BCAA)-enriched nutrients improves energy malnutrition, imbalances in amino acids, and

Correspondence: Dr Takahiro Yamasaki, Department of Gastroenterology and Hepatology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minamikogushi, Ube, Yamaguchi 755-8505, Japan. Email: t.yama@yamaguchi-u.ac.jp
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glucose intolerance in patients with liver cirrhosis.[11–13] However, those studies focused on the effects of LES in patients with liver cirrhosis.

Hepatocellular carcinoma (HCC) is the sixth most common type of cancer in the world.[14] Deaths due to HCC are increasing in almost all countries around the world, including Japan.[15–17] In particular, the prognosis for patients with advanced HCC showing portal vein tumor thrombosis (PVTT) remains poor.[18] Such patients are thus generally treated with hepatic arterial infusion chemotherapy (HAIC).[19–21] Our previous study identified Child-Pugh score[22] as an independent prognostic factor in cirrhotic patients with advanced HCC treated using HAIC.[20,21] In addition, energy expenditure in cirrhotic patients with HCC is reportedly increased compared with that in cirrhotic patient without HCC,[23] and a hypermetabolic rate in patients with gastrointestinal malignancy has been associated with the most advanced stage of the disease.[24]

Given this background, we consider that nutritional support is required for cirrhotic patients with advanced HCC undergoing HAIC. Few reports have examined nutritional support in cirrhotic patients with HCC.[25,26] Furthermore, no clinical studies have evaluated energy metabolism using indirect calorimetry in patients with HCC. We therefore investigated energy metabolism in patients with HCC and the efficacy of nutritional support using LES in patients with advanced HCC undergoing HAIC.

MATERIALS AND METHODS

Energy metabolism in patients with HCC

Patients

WE INVESTIGATED ENERGY metabolism using indirect calorimetry in cirrhotic patients without HCC and with various stages of HCC under the same conditions of liver capacity. Subjects comprised 10 cirrhotic patients without HCC and 36 patients with HCC before treatment ($n = 46$). No patients had received BCAA-enriched nutrients, and all were classified as Child-Pugh A.[22] Table 1 summarizes the clinical profiles of the 46 patients in this study. Tumor stage was determined according to the criteria of the Liver Cancer Study Group of Japan.[27,28] Liver cirrhosis was present in 10 patients, stage I/II HCC in 13 patients, stage III HCC in 13 patients, and stage IV HCC in 10 patients. The 4 groups showed no significant differences in clinical characteristics other than age (HCC stage III group vs. HCC stage IV group, $P = 0.017$). In addition, no significant differences in laboratory parameters, including BCAA/tyrosine ratio (BTR),[29] were identified among the 4 groups.

Energy metabolism was analyzed using indirect calorimetry (Deltatrac II; Detex Ohmeda, Helsinki, Finland). Indirect calorimetry was performed for 30 min after overnight bed rest and fasting. We measured oxygen consumption per minute (VO_2), carbon dioxide

Table 1 Clinical profiles of the 46 patients with and without hepatocellular carcinoma

Clinical characteristics	LC (n = 10)	HCC (n = 36)		
		Stage I/II† (n = 13)	Stage III† (n = 13)	Stage IV† (n = 10)
Age	66.9 ± 9.2	69.1 ± 8.0	73.8 ± 9.4*	62.2 ± 11.2*
Sex (male/female)	4/6	9/4	8/5	8/2
HCV Ab(+)/HBs Ag(+)/others	5/2/3	11/1/1	8/4/1	5/3/2
Child-Pugh A(5)/A(6)	6/4	8/5	10/3	7/3
Total Protein (g/dL)	7.20 ± 0.58	7.25 ± 0.76	7.45 ± 0.57	7.45 ± 0.58
Albumin (g/dL)	3.62 ± 0.46	3.78 ± 0.51	3.88 ± 0.25	3.66 ± 0.28
BTR	4.46 ± 1.16	4.52 ± 1.45	5.19 ± 0.96	4.86 ± 1.06•
NH3	46.6 ± 11.7	45.6 ± 25.0	40.9 ± 16.9	59.6 ± 30.2
Total cholesterol	160.3 ± 30.1	166.5 ± 30.1	176.9 ± 35.1	159.8 ± 13.7
ChE	193.0 ± 73.6	214.9 ± 89.6	255.0 ± 81.4	204.8 ± 60.1
CHI	79.7 ± 16.7	66.2 ± 17.6	68.0 ± 13.4	78.6 ± 18.5
BMI	23.1 ± 1.8	21.4 ± 2.6	22.9 ± 4.8	23.7 ± 3.5

*$P = 0.017$.
†According to the criteria of the Liver Cancer Study Group of Japan.
BMI, body mass index; BTR, branched-chain amino acid/tyrosine ratio; ChE, cholinesterase; CHI, creatinine height index; HCC, hepatocellular carciunoma; HCV, hepatitis C virus; LC, liver cirrhosis; NH3, ammonia.

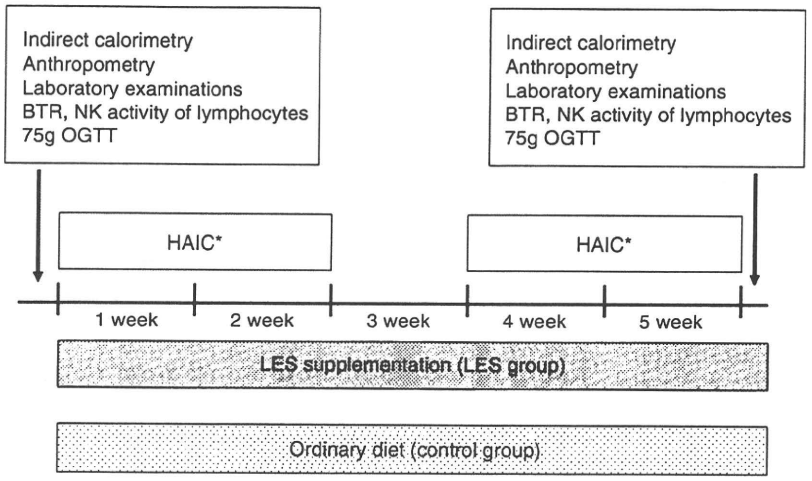

Figure 1 Study protocol. In the late evening snack (LES) group, patients received a LES supplement comprising branched-chain amino acid-enriched nutrients. In the control group, patients received ordinary food to the same amount of calories as the LES group. Before and after 1 cycle of hepatic arterial infusion chemotherapy (HAIC), nutritional evaluation using indirect calorimetry and InBody, laboratory examinations, and glucose tolerance using the 75-g oral glucose tolerance test were measured.

production per minute (VCO_2) and total urine nitrogen (TUN) on the day prior to examination, and the non-protein respiratory quotient (npRQ) was calculated as a measure of energy metabolism for the 4 groups.

Effect of LES for advanced HCC during HAIC

Patients

This study was performed on cirrhotic patients with unresectable HCC undergoing HAIC who had been admitted to the Department of Gastroenterology and Hepatology at Yamaguchi University Graduate School of Medicine. Eligibility criteria for this study were as follows: age, 20–80 years; Child-Pugh score A or B;[22] leukocyte count, ≥3000/mm^3; platelet count, ≥50 000/mm^3; serum creatinine, <1.2 mg/dL; unresectable HCC due to extensive, locally advanced disease that did not permit resection, bilobar disease, extrahepatic metastasis, or PVTT; and Eastern Cooperative Oncology Group (ECOG) performance status 0–2.[30]

Between December 2007 and February 2009, 26 patients were enrolled in this study. After randomization using a random number table, 13 patients received LES using a BCAA-enriched nutrient (LES group), and 13 received ordinary food (control group). However, 3 patients dropped out of the control group after withdrawing from the study during treatment. Thus, 13 patients in the LES group and 10 patients in the control group were subjected to analysis.

All patients provided written informed consent prior to enrolment into the study, and all protocols were approved by the Institutional Review Board of Yamaguchi University Hospital.

Table 2 summarizes the clinical profiles of patients in the 2 groups. No significant differences between groups were seen in clinical characteristics.

Table 2 Clinical profiles of the 23 patients with hepatocellular carcinoma

Clinical characteristics	LES group (n = 13)	Control group (n = 10)	*P*-value
Age	64.5 ± 9.5	66.4 ± 12.8	0.69
Sex (male/female)	11/2	8/2	0.78
HCV Ab(+)/HBs Ag(+)/others	7/5/1	8/1/1	0.35
Child-Pugh A/B	6/7	6/4	0.58
Maximum tumor size (mm)	77.7 ± 50.5	88.0 ± 39.7	0.60
Tumor stage II/III/IV A/IV B†	1/3/3/6	1/2/6/1	0.31
CHI	66.7 ± 16.3	72.0 ± 22.7	0.65
BMI	23.3 ± 3.9	21.4 ± 2.8	0.22

†According to the criteria of the Liver Cancer Study Group of Japan.
BMI, body mass index; CHI, creatinine height index; LES, late evening snack.

Study protocol

The intervention schedule is presented in Figure 1. Before this study, nutritional education was presented to all patients by dieticians. Daily nutritional intake for each group was calculated as 25–30 kcal with 1.2–1.3 g of protein per kilogram of ideal body weight per day. In the LES group, actual daily nutritional intake from meals was determined by subtracting the calorie content of LES (210 kcal) and protein (13.5 g) from the aforementioned calculated nutritional intake. One pack of the BCAA-enriched mixture (Aminoleban EN; Otsuka, Tokyo, Japan) used as LES food (at 22:00) contains 210 kcal of energy, 31.05 g of carbohydrate, 13.5 g of protein, 3.5 g of fat, and trace amounts of minerals and vitamins.[31] In the control group, patients received ordinary food with the same calorie content as the LES group.

After insertion of a 5-Fr heparin-coated catheter (Anthron P-U Catheter; Toray Medical, Tokyo, Japan) connected to a subcutaneously implanted reservoir, as described in a previous report,[19] patients received repeated arterial infusion of chemotherapeutic agents via the injection port.[20] One course of chemotherapy comprised 5 consecutive days of daily administration of cisplatin (10 mg/body/day on days 1–5; Randa; Nippon Kayaku, Tokyo, Japan) and isovorin (6.25 mg/body/day on days 1–5; Wyeth, Tokyo, Japan), followed by 5-fluorouracil (250 mg/body/day on days 1–5; Kyowa Hakko, Tokyo, Japan). Days 6 and 7 were rest days. This course was repeated for 2 weeks, followed by a 1-week suspension of chemotherapy. The course was then repeated for 2 weeks.

Nutritional parameters

Energy metabolism was analyzed by indirect calorimetry, and npRQ was calculated. A multi-frequency bioelectrical impedance analysis method (InBody 3.2; BIOSPACE, Tokyo, Japan) was used for anthropometric measurements.

Before and after 1 cycle of treatment, nutritional evaluation by indirect calorimetry and InBody, and changes in laboratory examinations, BTR, natural killer (NK) activity of lymphocytes,[32] plasma glucose and insulin level after 75-g oral glucose tolerance test (OGTT) were measured. The 75-g OGTT was performed at 4 time points: before administration, and at 30, 60 and 120 min. Area under the concentration curve for glucose (AUC glucose) and area under the concentration curve for insulin (AUC insulin) were determined using the above-mentioned 4 points and compared between before and after 1 cycle of treatment. We also divided patients into 3 groups according to blood glucose level 120 min after 75-g OGTT. A normal pattern (normal glucose tolerance [NGT]) was defined as blood glucose level <140 mg/mL at 120 min after 75-g OGTT. In comparison, a diabetic pattern (diabetes mellitus[DM]) was defined as glucose level >200 mg/mL and a borderline pattern (impaired glucose tolerance [IGT]) was defined as 140–200 mg/mL at 120 min after 75-g OGTT. CHI reflects skeletal muscle volume,[33] and was calculated using the following formula: CHI = (urinary creatinine excretion per day (mg))/(ideal body weight × A), where A is 23 for males and 18 for females.

Assessment of therapeutic efficacy

Dynamic computed tomography (CT) was performed before and after treatment. Tumor response was assessed on completion of 1 cycle of treatment. Response was classified according to ECOG criteria.[30] Complete response (CR) was defined as disappearance of all measurable lesions with no remaining signs, symptoms, or biochemical changes related to the tumor, which must have existed for >4 weeks, and appearance of no new lesions. Partial response (PR) was defined as a reduction of >50% in the sum of the products of the greatest perpendicular diameters of all measurable lesions, and appearance of no new lesions. Stable disease (SD) was defined as a reduction of <50% or an increase of <25% in the sum of the products of the greatest perpendicular diameters of all measurable lesions, and appearance of no new lesions. Progressive disease (PD) was defined as an increase of >25% in the sum of the products of the greatest perpendicular diameters of all measurable lesions, or appearance of new lesions.

Statistical analysis

Data are expressed as mean±standard deviation. Statistical analyses were performed using the unpaired *t*-test and the Mann-Whitney *U*-test, as appropriate. Survival period was calculated using the Kaplan-Meier method[34] from the date on which chemotherapy was started until death, and significance was determined by the log-rank test. Survival was confirmed up to 31 October, 2009. Values of $P < 0.05$ were considered statistically significant.

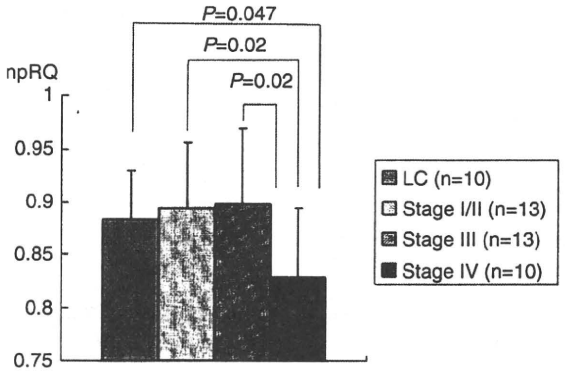

Figure 2 Value of non-protein respiratory quotient (npRQ) in cirrhotic patients without hepatocellular carcinoma (HCC) and with various stages of HCC. No significant difference in npRQ was seen among 3 groups (LC group, HCC stage I/II group, and HCC stage III group). However, npRQ was significantly lower in patients with stage IV HCC than in cirrhotic patients without HCC, or in patients with stage I/II or stage III HCC (LC group vs. HCC stage IV group, $P = 0.047$; HCC stage I/II group vs. HCC stage IV group, $P = 0.02$; HCC stage III group vs. HCC stage IV group, $P = 0.02$).

RESULTS

Energy metabolism in patients with HCC

FIGURE 2 SHOWS npRQ in cirrhotic patients without HCC and with various stages of HCC. Values of npRQ in cirrhotic patients without HCC, with stage I/II HCC, with stage III HCC, and with stage IV HCC were 0.88 ± 0.05, 0.89 ± 0.06, 0.90 ± 0.07, and 0.83 ± 0.07, respectively. No significant differences in npRQ were identified among the 3 groups (LC group, HCC stage I/II group, and HCC stage III group). However, npRQ was significantly lower in patients with stage IV HCC than in cirrhotic patients without HCC, or in patients with stage I/II or stage III HCC (LC group vs. HCC stage IV group, $P = 0.047$; HCC stage I/II group vs. HCC stage IV group, $P = 0.02$; HCC stage III group vs. HCC stage IV group, $P = 0.02$).

Effect of LES for advanced HCC during HAIC

Response to therapy

In the LES group ($n = 13$), 0 (0%), 3 (23%), 8 (62%), and 2 (15%) patients exhibited CR, PR, SD, and PD, respectively (response rate [patients with CR+PR/all patients], 23%). In the control group ($n = 10$), 1 (10%), 2 (20%), 4 (40%), and 3 (30%) patients exhibited CR, PR, SD, and PD, respectively (response rate, 30%). No significant differences in response rates were seen between groups ($P = 0.90$; Mann-Whitney *U*-test). As a result, no significant differences between groups were seen in relation to background.

Energy metabolism

Table 3 shows changes in npRQ before and after 1 cycle of treatment. The value of npRQ increased significantly after 1 cycle of treatment in the LES group (0.81 ± 0.08 vs. 0.88 ± 0.08, $P = 0.01$). However, npRQ did not differ in the control group (0.85 ± 0.08 vs. 0.86 ± 0.06,

Table 3 Changes in energy metabolism and laboratory parameters

	LES group (n = 13)			Control group (n = 10)		
	Before	After	*P*-value	Before	After	*P*-value
npRQ	0.81 ± 0.08	0.88 ± 0.08	0.01	0.85 ± 0.08	0.86 ± 0.06	0.69
BTR	3.76 ± 0.90	4.55 ± 1.38	0.008	4.16 ± 1.03	3.97 ± 1.22	0.47
Total Protein (g/dL)	7.25 ± 0.75	7.46 ± 0.97	0.29	7.32 ± 0.68	7.01 ± 0.59	0.17
Albumin (g/dL)	3.06 ± 0.51	3.08 ± 0.53	0.70	3.26 ± 0.48	3.24 ± 0.42	0.83
Prealbumin (mg/dL)	8.64 ± 4.49	10.17 ± 4.56	0.049	10.42 ± 4.76	11.43 ± 5.24	0.27
Total bilirubin (mg/dL)	0.98 ± 0.55	1.05 ± 0.75	0.57	1.05 ± 0.35	1.00 ± 0.37	0.75
ALT (IU/L)	38.6 ± 31.3	31.3 ± 12.9	0.04	50.9 ± 35.14	36.9 ± 23.22	0.67
PT (%)	76.0 ± 12.5	76.9 ± 7.9	0.72	81.5 ± 9.86	82.6 ± 10.8	0.76
Total cholesterol (mg/dL)	153.2 ± 31.1	153.2 ± 31.1	0.79	162.0 ± 57.4	167.6 ± 68.4	0.58
ChE (IU/L)	140.2 ± 70.4	134.2 ± 73.1	0.20	163.7 ± 68.6	131.1 ± 52.2	0.01
NH3 (μ mol/dL)	58.5 ± 23.1	69.3 ± 27.6	0.07	57.3 ± 23.2	66.0 ± 35.0	0.26
Natural killer cell activity (%)	24.8 ± 11.9	18.2 ± 13.8	0.14	25.5 ± 12.8	18.1 ± 10.9	0.16

ALT, alanine aminotransferase; BTR, branched-chain amino acid/tyrosine ratio; ChE, cholinesterase; LES, late evening snack; NH3, ammonia; PT, prothrombin time; npRQ, non-protein respiratory quotient.

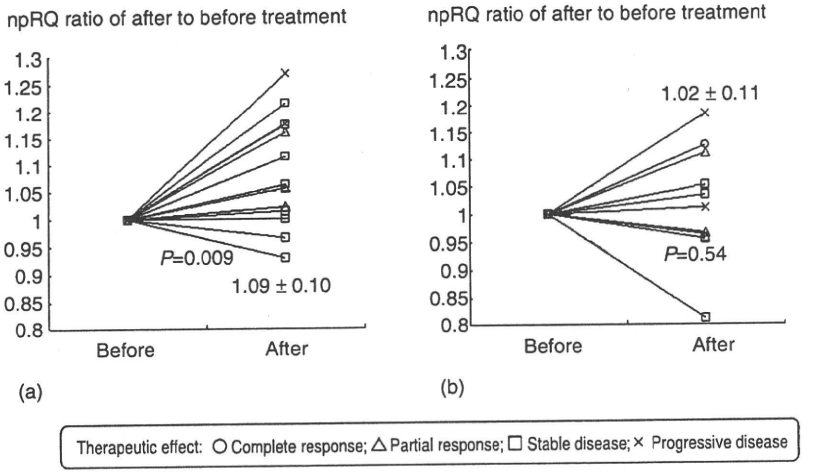

Figure 3 The npRQ ratio after compared to before 1 cycle of HAIC. In the LES group, npRQ improved in 10 patients, was stable in 1 patient, and worsened in 2 patients, regardless of response to therapy ($P = 0.009$) (a). In the control group, npRQ improved in 6 patients and worsened in 4 patients ($P = 0.54$) (b).

$P = 0.69$). Figure 3 shows the npRQ ratio of after compared to before 1 cycle of HAIC. In the LES group, npRQ improved in 10 patients, was stable in 1 patient, and worsened in 2 patients, regardless of response to therapy ($P = 0.009$). In the control group, npRQ improved in 6 patients and worsened in 4 patients ($P = 0.54$).

Blood biochemistry

Significant improvements in BTR, prealbumin and ALT levels were observed after 1 cycle of treatment in the LES group, but not in the control group. Cholinesterase levels were significantly decreased after 1 cycle of treatment in the control group, but did not differ in the LES group (Table 3). Figure 4 shows the BTR ratio of after to before 1 cycle of HAIC. In the LES group, BTR improved in 10 patients and worsened in 3 patients ($P = 0.005$). Conversely, BTR worsened in 7 patients in the control group ($P = 0.46$).

Anthropometry

No significant differences in anthropometric measurements (weight; skeletal muscle mass; body fat mass; fat-free mass; mid-upper arm muscle circumference (AMC); midarm circumference (AC); and body cell mass (BCM)) as measured using InBody were observed between groups (data not shown).

Changes in glucose tolerance

We examined the effects of LES using a BCAA-enriched nutrient on glucose tolerance using the 75-g OGTT in 21

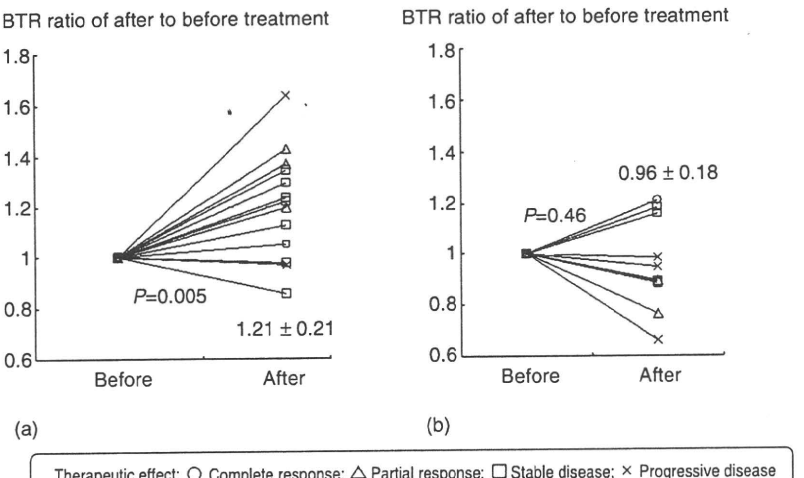

Figure 4 Branched-chain amino acid/tyrosine ratio (BTR) after compared to before 1 cycle of HAIC. In the LES group, BTR improved in 10 patients and worsened in 3 patients ($P = 0.005$) (a). Conversely, BTR worsened in 7 patients in the control group ($P = 0.46$) (b).

Table 4 Changes in glucose tolerance

	LES group (n = 12)			Control group (n = 9)		
	Before	After	*P*-value	Before	After	*P*-value
AUC glucose	396.1 ± 117.0	363.2 ± 135.6	0.055	355.3 ± 62.9	321.1 ± 108.6	0.17
AUC insulin	181.8 ± 123.3	223.2 ± 169.0	0.10	155.4 ± 85.3	117.7 ± 71.4	0.15
Fasting glucose (mg/dL)	99.5 ± 27.9	98.5 ± 32.6	0.71	100.7 ± 13.9	99.6 ± 18.7	0.89
Fasting insulin (μ U/mL)	11.3 ± 10.2	12.8 ± 7.1	0.38	14.5 ± 15.6	8.2 ± 1.8	0.28
HOMA-IR	2.9 ± 2.7	3.3 ± 2.5	0.47	4.0 ± 5.2	2.1 ± 0.8	0.32

AUC, area under the concentration curve; HOMA-IR, homeostasis model assessment method for insulin resistance; LES, late evening snack.

of 23 patients. One patient with DM in the LES group did not undergo the 75-g OGTT after treatment due to markedly high glucose levels, while the remaining patient in the control group declined to undergo the 75-g OGTT after treatment.

Table 4 shows changes in glucose tolerance before and after 1 cycle of treatment. In the LES group ($n = 12$), 1, 2, and 9 patients exhibited NGT, IGT, and DM, respectively. In the control group, 3, 2, and 4 patients exhibited NGT, IGT, and DM, respectively. No significant differences at baseline were seen between groups with regard to NGT, IGT, or DM using the 75-g OGTT, fasting glucose, fasting insulin, homeostasis model assessment method for insulin resistance (HOMA-IR), AUC glucose, and AUC insulin. AUC glucose tended to improve after 1 cycle of treatment in the LES group ($P = 0.055$). However, no significant differences in other parameters were apparent.

Prognosis

No significant differences in survival rates were seen between groups ($P = 0.667$; log-rank test) (Fig. 5a). On the other hand, survival in patients assessed as SD or PD according to the response criteria[30] tended to improve in the LES group ($n = 10$ in the LES group, $n = 7$ in the control group; $P = 0.156$; log-rank test) (Fig. 5b). In addition, no significant differences between groups assessed as SD or PD were seen with relation to background (data not shown).

By final follow-up, 2 patients remained alive (LES group, $n = 1$; control group, $n = 1$), while the other 21 patients had died. In the LES group, cause of death was cancer progression in 12 patients. In the control group, cause of death was cancer progression in 8 patients and hepatic failure in 1 patient.

Case presentation

Figure 6 shows a patient from the LES group. This 49-year-old man showed multiple HCCs in both lobes (stage III).[27,28] Mild ascites was identified, but no hepatic encephalopathy was present. On admission, hepatic reserve function was defined as Child-Pugh B (9 points). Prior to starting LES, npRQ was 0.71, and BTR value was low, at 2.2. After 1 cycle of HAIC, laboratory investigations were improved, and no ascites was apparent. Hepatic reserve function had improved to Child-Pugh A (6 points). Values of npRQ and BTR increased to 0.75 and 2.68, respectively, after 1 cycle of treatment. The patient exhibited DM on the 75-g OGTT. AUC glucose improved after 1 cycle of treatment (before, 414.75;

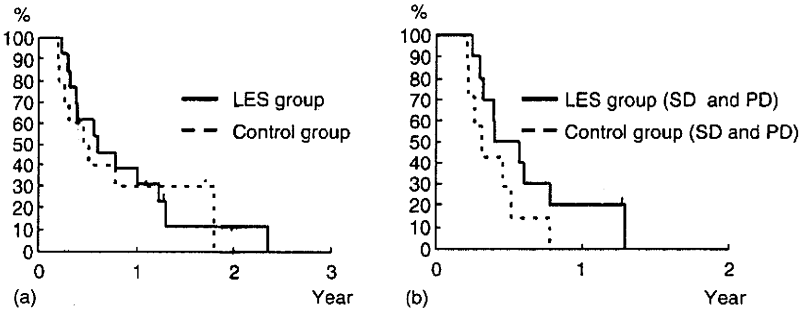

Figure 5 No significant differences in survival rates were seen between groups ($P = 0.667$; log-rank test) (a). On the other hand, survival in patients assessed as stable disease (SD) or progressive disease (PD) according to the response criteria tended to improve in the LES group ($n = 10$ in the LES group, $n = 7$ in the control group; $P = 0.156$; log-rank test) (b).

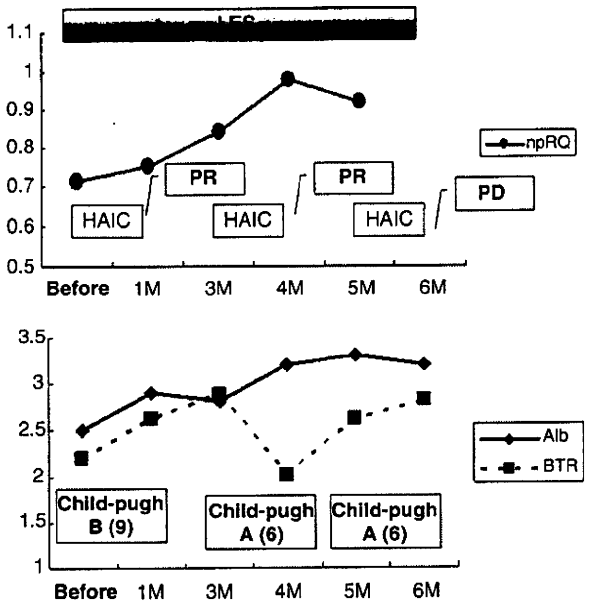

Figure 6 A case in the LES group. This 49-year-old man had multiple HCCs in both lobes (stage III). On admission, hepatic reserve function was Child-Pugh B (9 points). Prior to starting LES, npRQ was 0.71, and BTR was low at 2.2. After 1 cycle of HAIC, hepatic reserve function was improved to Child-Pugh A (6 points) from Child-Pugh B (9 points). Values of npRQ and BTR increased to 0.75 and 2.68, respectively, after 1 cycle of the treatment. The patient exhibited PR according to the response criteria. Thereafter, he received 3 courses of HAIC, and npRQ, serum albumin and BTR values improved. After the third course of HAIC, he exhibited PD. One year after the first course of HAIC, he died of tumor progression.

after, 369.75). PR was exhibited according to the response criteria.[30] Thereafter, the patient received 3 courses of HAIC, and npRQ, serum albumin and BTR value improved. After the third course of HAIC, he exhibited PD. One year after the first course of HAIC, he died of tumor progression.

Figure 7 shows a patient from the control group. This 73-year-old woman presented with massive HCC in the right lobe with tumor thrombus in the main trunk of the portal vein (Vp4) (stage IV A).[27,28] No ascites or hepatic encephalopathy was identified. On admission, hepatic reserve function was defined as Child-Pugh A (6 points). Prior to starting HAIC, npRQ was 0.91. However, BTR was low, at 2.34. After 1 cycle of HAIC, laboratory investigations showed slight deterioration. Values for npRQ and BTR decreased to 0.87 and 2.06, respectively, after 1 treatment cycle. The patient exhibited DM on the 75-g OGTT. AUC glucose remained almost unchanged (before, 446.25; after, 437.75). She exhibited SD according to the response criteria.[30] Although she received a second course of HAIC, npRQ, serum albumin and BTR values worsened. In addition, she showed moderate ascites during the second course of treatment. After the second course of treatment, hepatic reserve function was classified as Child-Pugh C (10 points). Thereafter, she developed hepatic encephalopathy and died of hepatic failure 6 months after the first course of HAIC.

DISCUSSION

PEM IS OFTEN observed in cirrhotic patients,[2,3] and this malnutrition adversely affects prognosis.[3] When energy metabolism of cirrhotic patients is measured using indirect calorimetry, npRQ decreases as the severity of liver cirrhosis increases.[3] However, no clinical studies have evaluated energy metabolism in patients with HCC. We therefore investigated energy metabolism

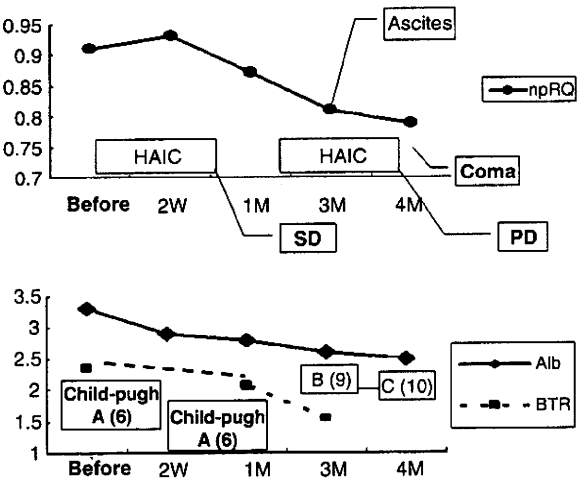

Figure 7 A case in the control group. This 73-year-old woman presented with massive HCC in the right lobe with tumor thrombus in the main trunk of the portal vein (Vp4) (stage IV A). On admission, hepatic reserve function was Child-Pugh A (6 points). Prior to starting HAIC, npRQ was 0.91. However, BTR was low at 2.34. After 1 cycle of HAIC, npRQ and the BTR value decreased to 0.87 and 2.06, respectively. She exhibited SD according to the response criteria. Although she received a second course of HAIC, npRQ, serum albumin and BTR values all worsened. After the second course of treatment, hepatic reserve function was Child-Pugh C (10 points). Thereafter, she developed hepatic encephalopathy and died of hepatic failure 6 months after the first course of HAIC.

using indirect calorimetry in cirrhotic patients without HCC and with various stages of HCC under the same conditions of liver capacity, namely Child-Pugh A. In our study, no significant differences were seen among 3 groups (LC group, HCC stage I/II group, and HCC stage III group), but npRQ was significantly lower in patients with stage IV HCC than in cirrhotic patients without HCC, or in patients with stage I/II or stage III HCC. Although there was a significant difference in age between the HCC stage III group and the HCC stage IV group, there was no significant correlation between the value of npRQ before treatment and age (data not shown). These findings suggest that patients with advanced HCC remained under conditions of severe energy malnutrition even if liver capacity was good. A hypermetabolic rate in patients with gastrointestinal malignancy is reportedly usually associated with the most advanced stage of the disease.[24] The p53 tumor suppressor gene regulates glucose metabolism, and loss of p53 upregulates energy metabolism.[35] Mutation of the p53 gene is associated with poor tumor differentiation and advanced stage of HCC.[36] We speculate that reductions in npRQ among patients with advanced HCC may be related to the upregulation of glucose metabolism in cancer cells, but the underlying mechanisms remain unclear. Our results suggest that nutritional support is warranted for cirrhotic patients with advanced HCC, even if liver capacity is good.

In an attempt to improve the state of energy malnutrition, LES has been developed and improved energy metabolism has been reported.[5–8,11–13] However, those studies focused on the effects of LES in patients with cirrhosis.

Poon *et al.* reported that nutritional supplementation with oral BCAAs is beneficial for increasing serum albumin level, reducing morbidity and improving quality of life in patients undergoing transarterial chemoembolization for HCC,[25] but the nutritional supplementation did not use LES. Takeshita *et al.* only reported that LES using BCAA-enriched nutrients prevents suppression of liver function in patients with HCC undergoing transarterial chemoembolization.[26] That study did not evaluate energy metabolism before and after LES. We therefore investigated the efficacy of nutritional support using LES in patients with advanced HCC undergoing HAIC using indirect calorimetry.

The present findings showed that LES using BCAA-enriched nutrients improves npRQ, BTR, ALT, and prealbumin significantly before and after 1 cycle of HAIC compared with the control group. Nakaya *et al.* reported that LES using BCAA-enriched nutrients improved npRQ, BTR, and serum albumin before and 3 months after in cirrhotic patients compared with LES using ordinary food.[8] Although no significant difference in serum albumin was identified in our study, prealbumin (a rapid turnover protein with a half-life in plasma of 2 days) was significantly increased by LES. Prealbumin is more sensitive to changes in protein-energy status than albumin.[37] We thus consider that improvement of npRQ and prealbumin reflects energy metabolism in cirrhotic patients with advanced HCC undergoing HAIC. Unfortunately, we could not evaluate nutritional parameters in the long term, as some patients died in the short term. Despite the small sample size, of the 12 patients for whom npRQ was evaluated at 3 months after HAIC, patients treated with LES ($n = 6$) tended to show improved npRQ ($P = 0.10$), and npRQ was not significantly difference before and 3 months after HAIC in control patients ($n = 6$; $P = 0.91$; data not shown).

BCAAs reportedly improve glucose intolerance.[11–13,38] The present study evaluated glucose tolerance before and at the end of 1 cycle of treatment. AUC glucose in the LES group tended to improve ($P = 0.055$). In addition, AUC glucose in patients who showed glucose intolerance (IGT and DM; $n = 11$) in the LES group tended to improve (before, 412.3 ± 107.7; after, 376.1 ± 134.3; $P = 0.052$) and AUC glucose in patients who had glucose intolerance ($n = 6$) in the control group showed no significant difference (before, 376.5 ± 67.8; after, 338.3 ± 132.7; $P = 0.30$). One reason might be the effect of LES itself. A LES improves postprandial hyperglycemia, because the glucose load per meal is decreased by fractionated meals including a LES, and glucose is properly oxidized in the tissues. Another reason might be the effects of the leucine and isoleucine contained among the BCAAs. Leucine and isoleucine promote glucose uptake in skeletal muscle under insulin-free conditions.[39] Leucine also increases the activity of p70S6 kinase via the mammalian target of rapamycine pathway, and the ability to synthesize glycogen is improved.[39] However, we have previously reported that glucose tolerance worsened after 3 months of LES administration in cirrhotic patients with DM according to the 75-g OGTT.[40] In 12 patients (LES group, $n = 6$; control group, $n = 6$) for whom glucose tolerance was evaluated using the 75-g OGTT at 3 months after HAIC, AUC glucose was significantly worsened in both groups in this study (data not shown). We reported that LES combined with an alpha-glucosidase inhibitor to slow glucose absorption into the blood and ameliorate postprandial hyperglycemia improved glucose tolerance (AUC glucose) over the long term (3 months) in

patients with liver cirrhosis,[41] and concomitant use of an alpha-glucosidase inhibitor with LES might be a useful nutritional therapy in patients with advanced HCC who show glucose intolerance.

Unfortunately, no significant differences in survival rate were identified between groups ($P = 0.667$; log-rank test). Poon *et al.* also reported no difference in survival between patients who received BCAAs and those receiving ordinary food.[25] However, survival in patients assessed as SD or PD tended to improve in the LES group ($P = 0.156$; log-rank test), although no significant differences between groups assessed as SD or PD were seen with relation to background. Significant improvement in npRQ was observed in the LES group, and significant reductions in cholinesterase and natural killer cell activity were observed in the control group, for groups assessed as SD or PD (data not shown). In addition, the frequency of HCC treatment tended to be increased in the LES group (data not shown). We speculate that life prolongation in patients assessed as SD or PD may be related to improvements in energy metabolism and immune defense,[32] and the continuation of HCC treatment, by means of LES using BCAA-enriched nutrients. Our previous study identified therapeutic effect as an independent prognostic factor in cirrhotic patients with advanced HCC treated using HAIC.[20] In this study, patients exhibited CR or PR showed good prognosis without relation to LES (data not shown). Therefore, we consider that patients exhibited SD or PD may be suitable candidates for LES using BCAA-enriched nutrients because of life prolongation. As our study examined only a small population, further investigations are necessary.

In conclusion, LES using BCAA-enriched nutrients offers the possibility of improving energy metabolism and glucose tolerance in cirrhotic patients with advanced HCC undergoing HAIC. Although our study design shows limitations in the comparison between ordinary food and both LES and BCAA, we speculate that these results are caused by effects from both LES and BCAA. We consider that tailored nutritional support, such as tumor staging, is required in patients with HCC.

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Research Article

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Possible involvement and the mechanisms of excess *trans*-fatty acid consumption in severe NAFLD in mice

Noriyuki Obara[1], Koji Fukushima[1], Yoshiyuki Ueno[1,*], Yuta Wakui[1], Osamu Kimura[1], Keiichi Tamai[1], Eiji Kakazu[1], Jun Inoue[1], Yasuteru Kondo[1], Norihiko Ogawa[2], Kenta Sato[3], Tsuyoshi Tsuduki[3], Kazuyuki Ishida[4], Tooru Shimosegawa[1]

[1]*Division of Gastroenterology, Tohoku University Graduate School of Medicine, 1-1 Seiryo, Aobaku, Sendai 980-8574, Japan;* [2]*Division of Advanced Surgical Science and Technology, Graduate School of Medicine, Tohoku University, Sendai, Japan;* [3]*Laboratory of Food and Biomolecular Science, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan;* [4]*Department of Pathology, Tohoku University Hospital, Sendai, Japan*

Background & Aims: Excessive *trans*-fatty acids (TFA) consumption has been thought to be a risk factor mainly for coronary artery diseases while less attention has been paid to liver disease. We aimed to clarify the impact of TFA-rich oil consumption on the hepatic pathophysiology compared to natural oil.

Methods: Mice were fed either a low-fat (LF) or high-fat (HF) diet made of either natural oil as control (LF-C or HF-C) or partially hydrogenated oil, TFA-rich oil (LF-T or HF-T) for 24 weeks. We evaluated the liver and body weight, serological features, liver lipid content and composition, liver histology and hepatic lipid metabolism-related gene expression profile. In addition, primary cultures of mice Kupffer cells (KCs) were evaluated for cytokine secretion and phagocytotic ability after incubation in *cis*- or *trans*-fatty acid-containing medium.

Results: The HF-T-fed mice showed significant increases of the liver and body weights, plasma alanine-aminotransferase, free fatty acid and hepatic triglyceride content compared to the HF-C group, whereas the LF-T group did not differ from the LF-C group. HF-T-fed mice developed severe steatosis, along with increased lipogenic gene expression and hepatic TFA accumulation. KCs showed increased tumor necrosis factor secretion and attenuated phagocytotic ability in the TFA-containing medium compared to its *cis*-isomer.

Conclusions: Excessive consumption of the TFA-rich oil up-regulated the lipogenic gene expression along with marked hepatic lipid accumulation. TFA might be pathogenic through causing severe steatosis and modulating the function of KCs. The quantity and composition of dietary lipids could be responsible for the pathogenesis of non-alcoholic steatohepatitis.

Keywords: *trans*-Fatty acid; NASH; NAFLD; Metabolic syndrome; Kupffer cell.
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*Corresponding author. Tel.: +81 22 717 7171; fax: +81 22 717 7177.
E-mail address: yueno@mail.tains.tohoku.ac.jp (Y. Ueno).

Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; FFA, free fatty acid; LPS, lipopolysaccharide; TFA, *trans*-fatty acid; ALT, alanine-aminotransferase; LF(-C or -T), low-fat (control or TFA-rich) diet; HF(-C or -T), high-fat (control or TFA-rich) diet; KCs, Kupffer cells (KCs); AST, aspartate-aminotransferase; TG, triglyceride; ELISA, Enzyme-Linked ImmunoSorbent Assay; HDL, high density lipoprotein; (V)LDL, (very) low density lipoprotein; NAS, NAFLD activity score; TBARS, thiobarbituric acid reactive substances; TNFα, tumor necrosis factor α; IL-6, interleukin-6; SD, standard deviation; iNOS, inducible nitric oxide synthase; TGF-β, transforming growth factor-β; SREBP-1, sterol regulatory element-binding protein-1; FAS, fatty acid synthase; ACC, acetyl CoA carboxylase; PPAR, peroxisome proliferator activated receptor; PGC-1β, PPARγ coactivator-1β; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid.

Introduction

In concordance with the prevalence of obesity, the incidence of non-alcoholic fatty liver disease (NAFLD) has increased and is nowadays recognized as the most common liver disease [2]. It is known that a part of NAFLD can progress to non-alcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis and hepatocellular carcinoma [9]. Nevertheless, the mechanisms of NAFLD-to-NASH transition remain to be clarified; NAFLD appears to originate from the dysregulation of hepatic lipid metabolism as a part of the metabolic syndrome accompanied by visceral obesity, dyslipidemia, atherosclerosis, and insulin resistance [25]. According to the hypothetical theory named the 2-hit theory [5], the secondary hit to NAFLD that can be due to free fatty acid (FFA)s, oxidative stress, lipopolysaccharide (LPS) and inflammatory cytokines, causes NASH as a consequence.

In terms of the "first hit", the lipid accumulation in the liver is induced by high-fat diets [6,23] that include various lipid species. Such dietary lipid species uniquely affect the obesity phenotype, liver histology and gene expression pattern in the rat liver [3]. In this context, lipid species could play a potential role in the pathogenesis of NAFLD and/or NASH.

trans-Fatty acid (TFA) is produced through the industrial hardening of the vegetable oils to make the products more stable and robust, and thus easier to handle or store. Excess consumption of TFA is known as a risk factor for coronary artery diseases, insulin resistance and obesity accompanied by systemic inflammation, the features of metabolic syndrome [20,29]. Nevertheless, little is known about the effects on the liver induced by lipids.