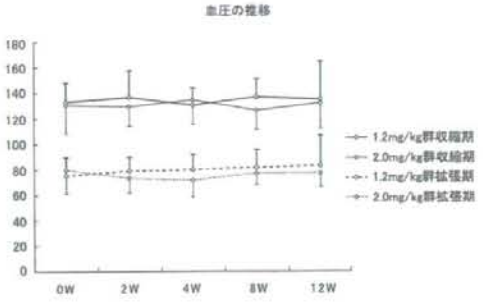

図5　血圧の推移

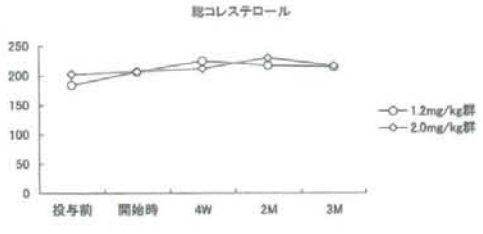

図6　血清総コレステロール値の推移

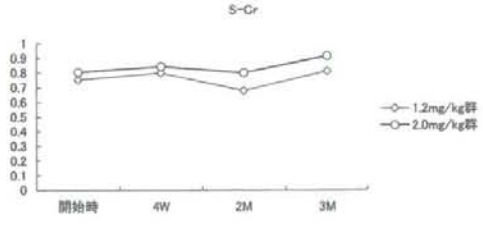

図7　血清クレアチニン値の推移

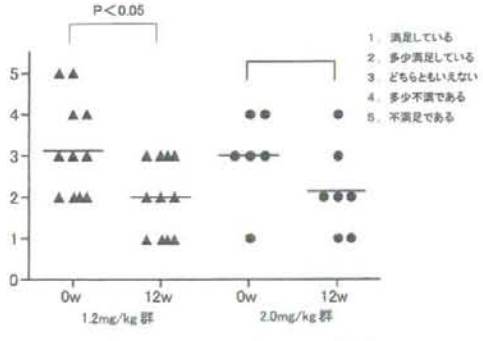

図8　これまでの治療に対する満足度

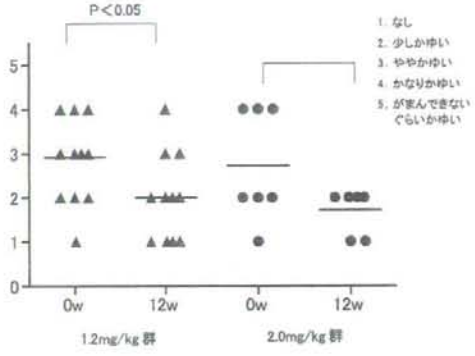

図9　痒み

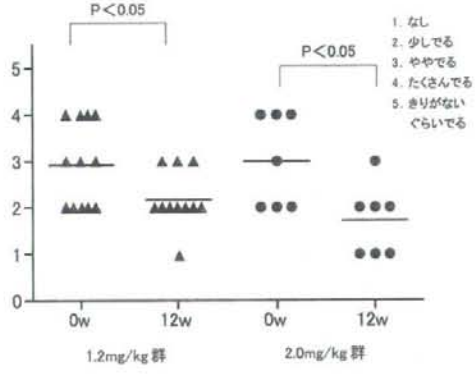

図10　フケの量

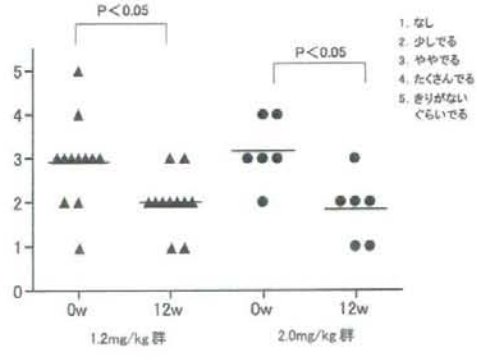

図11　はがれ落ちる皮膚の量

線の診療所の皮膚科で治療されていると思われるが，一般の診療所においても外用療法のみでは難治性の場合により安全で低コストのCyA療法が望まれる。

このような点から中等症以下を対象にした2.0～2.5 mg/kg/day程度の低用量[4)-7)]，あるいは間歇投与[8)9)]での様々なアプローチがなされてきた。近年では機能障害のみならず，生活の質(QOL)の観点から疾病の治療計画を立

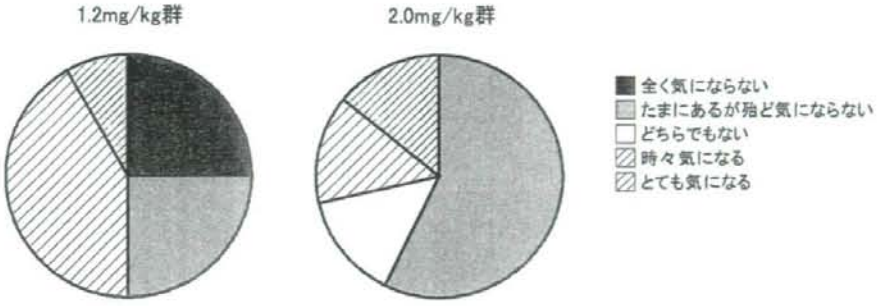

図12 CyA内服併用療法の費用負担について

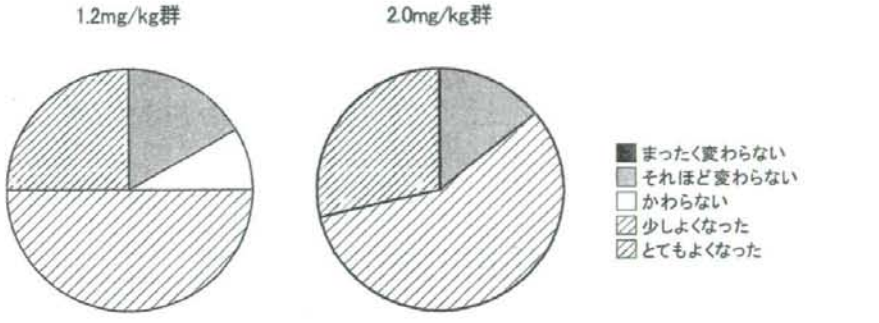

図13 治療の煩わしさについて

てることの重要性が認識され，乾癬に於いてもPDIやself-PASIなどの患者自身が評価するアセスメントツールが普及してきている[10]。

今回，我々は通常の低用量よりさらに少ない投与量が中等症以下の乾癬においてどのような効果を発現するのかを検討した。結果は統計上1.2 mg/kgという低用量でもPASIスコア，瘙痒スコア共に有意な改善が見られた。ただし，PASIスコア，瘙痒スコアともにエントリーの時点で2 mg/kg群と1.2 mg/kg群で有意に差がある結果となり，群間の比較は困難であった。この差は，原則的にA，B各群の交互のエントリーがプロトコール上規定されていたが，主治医の判断でエントリーにバイアスがかかった可能性が考えられた。

これらの結果は，通常の外用療法である程度改善はみられるものの十分な満足が得られない患者に対して，身体的・経済的な負担を最小限にしてさらなる改善が得られる可能性を示唆するものと言える。医師による他覚的な評価も改善がみられているが，アンケートにおけるフケ・鱗屑の量，痒みなどの自覚症状の改善がはっきりとみられたことが満足度の上昇に寄与していると考えられた。

近年CyAの治療効果と薬物動態の関係の研究が進み，乾癬においては血中濃度時間曲線下面積(area under the blood concentration time curve, AUC)がトラフレベルよりも治療効果と相関すると考えられている[11]。1.2 mg/kg投与は，2.0 mg/kgと比較してAUCは小さいと考えられるが，この研究では両者とも十分な臨床効果が得られ，明らかな差がみられなかった。これは，上乗せ効果においては比較的少量でも効果が得られやすいこと，初期のバイアスにより効果が得られやすい軽症例が1.2 mg/kg群に入ったことなどが考えられた。

CyAは副作用の面から欧米ではその連続使用期間を2年と推奨している[12]。しかし，光線治療施設が少なく，生物学的製剤も未だ導入されていない我が国では中等症以上の難治性乾癬患者の外来での管理において，現実的にはCyAの副作用を最少化して安全に長期間投与する方法を工夫する必要があろう。今回の研究から1.2 mg/kg内服療法は安全で低コストで患者満足度を改善する一つの治療法と考えられた。

本研究の要旨は第22回日本乾癬学会学術大会(2007年9月7，8日，志摩市)において発表した。

文　献

1) 阿部浩之ほか：シクロスポリン内服療法施行乾癬患者の高血圧合併例の検討．日皮会誌 115：887-891，2005.
2) シクロスポリンMEPCによる乾癬治療のガイドライン2004年

度版コンセンサス会議報告．日皮会誌　114：1093-1105，2004．
3) 安元慎一郎ほか：乾癬治療におけるシクロスポリンMEPC療法：西部支部会員によるアンケート調査．西日皮膚　67：252-257，2005．
4) 福岡地区乾癬治療研究会：乾癬患者のQOL向上をめざしたサンディミュン低用量併用療法の臨床的研究．西日皮膚　61：224-234，1999．
5) 狩野葉子ほか：乾癬に対するシクロスポリン低用量療法の検討．西日皮膚　61：793-800，1999．
6) 加藤則人ほか：乾癬に対するシクロスポリン低用量寛解導入維持療法の検討．西日皮膚　61：363-368，1999．
7) 荻原啓介ほか：沖縄地区の乾癬に対するシクロスポリン少量長期投与試験：有用性，安全性，至適投与量の検討．西日皮膚　59：275-284，1997．
8) Ohtsuki M et al: Long-term continuous versus intermittent cyclosporin: therapy for psoriasis. J Dermatol **30**: 290-298, 2003.
9) 中山樹一郎，堀　嘉昭：乾癬のシクロスポリン療法　当科における2年間の単独療法と間歇療法との比較．西日皮膚　58：472-478，1996．
10) Kirby B et al: Physical and psychologic measures are necessary to assess overall psoriasis severity. J Am Acad Dermatol **45**: 72-76, 2001.
11) 梅澤慶紀，大井綱郎，古賀道之：尋常性乾癬におけるシクロスポリン1.5 mg/kg 1日1回内服法とステロイド外用療法併用の臨床効果についての検討．日皮会誌　108：1965-1969，1998．
12) Griffiths CE et al: Cyclosporin in psoriasis clinical practice: an international consensus statement. Br J Dermatol (Suppl 67): 11-23, 2004.

(2007年12月26日　受付・2008年5月1日　採用決定)

別刷請求先：〒814-0180　福岡市城南区七隈7丁目45-1
福岡大学医学部皮膚科教室
今福　信一

Very Low Dose (1.2 mg/kg) Cyclosporine A Therapy for Psoriasis

Shinichi IMAFUKU, Juichiro NAKAYAMA

Department of Dermatology, Faculty of Medicine, Fukuoka University
Fukuoka 814-0180, Japan (Director: Prof. J. Nakayama)

and Fukuoka-Okinawa Psoriasis Research Group

Cyclosporine A (CyA) was administered at two different low doses (1.2 mg/kg group and 2.0 mg/kg group) orally to psoriasis patients who had not had satisfactory results with topical therapy. Mean PASI scores decreased from 32.7±10.8 to 15.1±10.2 in the 2.0 mg/kg group and from 14.2±12.1 to 6.8±7.9 in the 1.2 mg/kg group. Mean itch scores decreased from 2.1±0.8 to 1.1±0.8 in the 2.0 mg/kg group and from 1.5±0.8 to 0.5±0.7 in the 1.2 mg/kg group. Results of a questionnaire taken before and after the treatment revealed less scale, dandruff and itching in both groups. Approximately half of the patients in both groups answered that the increased cost was of little concern. Very low dose (1.2 mg/kg) CyA therapy improved satisfaction with treatment and may be a new affordable option for patients with mild psoriasis who desire a better quality of life.

Body cell mass is a useful parameter for assessing malnutrition and severity of disease in non-ascitic cirrhotic patients with hepatocellular carcinoma or esophageal varices

TAKUMI KAWAGUCHI[1,2], EITARO TANIGUCHI[2], MINORU ITOU[2], RYOKO IBI[3], TERUYO OKADA[3], MICHIKO MUTOU[3], SATOMI SHIRAISHI[3], YUKI UCHIDA[4], MOMOKA OTSUKA[4], YOUKO UMEKI[5], TETSUHARU ORIISHI[2], HITOMI HAYABUCHI[5], SUIKO TANAKA[4], MACHIKO TAKAKURA[3] and MICHIO SATA[1,2]

[1]Department of Digestive Disease Information and Research, [2]Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Kurume; Departments of [3]Nursing, [4]Nutrition, Kurume University Hospital, Kurume; [5]Department of Nutrition and Health Science, Faculty of Human Environmental Science, Fukuoka Women's University, Fukuoka, Japan

Received May 26, 2008; Accepted July 14, 2008

DOI: 10.3892/ijmm_00000060

Abstract. Body cell mass (BCM) is a nutritional parameter, however, changes in BCM in patients with non-ascitic liver cirrhosis (LC) in comparison to patients with other malnutritional diseases remains unclear. We investigated the difference in BCM between patients with LC and malnourished gastrointestinal disease controls (M.CON), and examined the relationship between BCM and the severity of LC. Results demonstrated that serum nutritional parameters were not significantly different between the LC (n=56) and M.CON groups (n=25), whereas BCM%BW was significantly lower in the LC group than in the M.CON group (50.9±4.6 vs. 54.4±7.1%, P=0.018). Furthermore, BCM%BW negatively correlated with the model for end-stage liver disease (MELD) score (P=0.04). In concluson, BCM showed a significant decrease and a negative correlation with the MELD score in the LC group. BCM may be a useful parameter for assessing malnutrition and severity of LC.

Correspondence to: Dr Takumi Kawaguchi, Department of Digestive Disease Information and Research, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan
E-mail: takumi@med.kurume-u.ac.jp

Abbreviations: BCM, body cell mass; LC, liver cirrhosis; M.CON, malnourished gastrointestinal disease controls; BIA, bioelectrical impedance analyzer; MELD, model for end-stage liver disease; SGA, subjective global assessment; AC, arm circumference; AMC, mid-arm circumference; TSF, tricep skinfold thickness; CV, coefficient of variation; ICF, intracellular fluid; TBW, total body water; ECF, extracellular fluid; FFM, fat free mass

Key words: body composition, eight-polar bioelectrical impedance analyzer, nutritional assessment, liver disease, model for end-stage liver disease score

Introduction

The liver is one of the central organs involved in the metabolism of various nutrients. In patients with liver cirrhosis, malnutrition is a common feature and is associated with mortality and reduced quality of life (1). Therefore, routine nutritional assessment is recommended in patients with liver cirrhosis (2,3). Subjective global assessment (SGA) and anthropometric measurements are useful techniques for nutritional assessment in various diseases. However, these assessments are not suitable for precise assessment of malnutrition and do not predict a poor clinical outcome in patients with liver cirrhosis (4). Thus, malnutrition is often under-diagnosed in patients with liver cirrhosis.

Significant changes in body composition are known to occur before physiological changes (5). Therefore, measurement of body composition is an integral part of nutritional assessment. Among all body compartments, body cell mass (BCM) has been regarded as the most meaningful for the assessment of malnutrition. BCM comprises the metabolically active and protein-rich compartments in the body and is known to be depleted in case of protein-energy malnutrition (6). Precise estimation of BCM can be obtained by isotope dilution or via the total-body potassium count (7); however, these methods are not generally available for clinical use. Despite certain limitations in patients with ascites, bioelectrical impedance analyzer (BIA) is a useful tool for the estimation of BCM in cirrhotic patients (8,9). An eight-polar BIA has been developed (10) and is currently a non-invasive bedside tool for estimating BCM.

Depletion of BCM is associated with post-liver transplantation mortality, and the measurement of BCM provides clinically relevant information in patients with end-stage liver cirrhosis (11). However, in patients with liver cirrhosis without ascites and/or edema, the utility of BCM as a nutritional parameter remains unclear. In addition, comparisons have been made between cirrhotic patients and healthy

control subjects, but not malnourished disease controls, in previous studies. In order to verify the specificity for depletion of BCM, cirrhotic patients should be compared to malnourished disease controls. Moreover, the correlation between BCM and severity of disease remains unknown. The aim of this study was to examine changes in BCM in cirrhotic patients in comparison with other malnourished patients and the association between BCM and severity of disease in cirrhotic patients.

Materials and methods

Subjects. We conducted a cross-sectional study. Via consecutive entry, a total of 56 men with liver cirrhosis (LC) (hepatitis C virus-related, n=36; hepatitis B virus-related, n=13; alcoholic, n=5; cryptogenic, n=2) and 25 age-matched men with chronic gastrointestinal diseases (inflammatory bowel diseases, n=12; pancreatic cancer, n=3; biliary cancer, n=4; colitis, n=3; colon cancer, n=2; chronic pancreatitis, n=1) who served as controls for nutritional status (malnourished disease controls; M.CON) were enrolled in this study during the period from August 2004 to March 2006 at Kurume University Hospital. In the LC group, 28 patients had co-occurring hepatocellular carcinoma. Among them, 20 patients had a single (<3-cm) nodule which had been treated with radiofrequency ablation. Eight patients had advanced tumors which had been treated with chemoembolization. The rest of the 28 patients had co-occurring esophageal varices. Although none of them had episodes of bleeding, they were treated with preventive endoscopic variceal ligation for the esophageal variceal bleeding. The specificity of changes in body composition in cirrhotic patients can be examined by a comparison with malnourished patients. All of the diagnoses were based on clinical, serological, histological, and/or imaging evidence. All patients were hospitalized. For both the LC and M.CON groups, inclusion criteria were: i) Asian men; ii) 20-70 years of age; iii) no edema nor ascites determined by physical examination and ultrasonography; and 4) serum albumin concentration <4.0 g/dl. Patients with chronic renal failure or those who had been taking corticosteroids were excluded. The regular hepatic diet (total energy 30-40 kcal/kg body weight (BW)/day, protein 1.2-1.5 g/kg BW/day) was fed to patients in the LC group. The proper diet (total energy 30-35 kcal/kg BW/day, protein 1.2-1.5 g/kg BW/day) was fed to patients in the M.CON group. In the analysis for an association between BCM and severity of liver disease, subjects were stratified by age (40-60 years) because of the age-related differences in body water compartments (12). None of the subjects were institutionalized. Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the institutional review board.

Laboratory determinations. Venous blood samples were collected in the morning after a 12-h overnight fast. Hemoglobin concentration, total lymphocyte count, international normalized ratio (INR) of prothrombin time, serum albumin, creatinine and total cholesterol concentration were measured using standard clinical methods (Department of Clinical Laboratory, Kurume University Hospital). A prognostic nutritional index was calculated by using the following formula as previously described (13): Prognostic nutritional index = 10 x Serum albumin concentration (g/dl) + 0.005 x Total lymphocyte count (mm^3). Poor prognosis is predicted when the prognostic nutritional index is <40 and total lymphocytes count remains <1000/mm^3.

Table I. Precision of eight-polar BIA in patients with liver cirrhosis.

	Right arm	Left arm	Trunk	Right leg	Left leg
R_5	2.3	2.3	2.9	2.6	2.7
R_{50}	2.0	2.4	2.5	2.3	2.5
R_{250}	2.1	2.0	2.2	2.3	2.3
R_{500}	2.2	2.1	2.4	2.4	2.3

Coefficients of variation were calculated from 2 measurements on 7 of the study subjects (n=14). BIA, direct segmental multifrequency-bioelectrical impedance analyzer; R_x, resistance at x kHz.

Estimations of anthropometry and body composition. Anthropometry and body composition were estimated by an eight-polar BIA at frequencies of 5, 50, 250 and 500 kHz (InBody 3.2, Biospace, Tokyo, Japan). Two electrodes were placed in contact with the palm and thumb of each hand and two electrodes with the anterior and posterior regions of the sole of each foot. Body mass index (BMI) was calculated as BW in kilograms divided by the square of height in meters (kg/m^2). Arm circumference (AC), mid-arm circumference (AMC), tricep skinfold thickness (TSF), waist circumference, hip circumference, and waist-hip ratio were estimated using the software supplied with the eight-polar BIA (Lookin'body 2.0, Biospace) based on a hydration model (14). The accuracy of the eight-polar BIA analyzer has been previously reported (10). In our preliminary study, anthropometic data estimated by the eight-polar BIA analyzer also showed a significant positive correlation with anthropometic data derived by direct measurement in the LC group (AMC, n=43, Spearman rank $r=0.69$, $P<0.0001$; TSF, n=43, Spearman rank $r=0.44$, $P<0.0046$). AC, AMC and TSF were expressed as a percentage of the expected value for age and gender with reference to Japanese Anthropometric Reference Data (JARD, 2001) (15). The validation of BIA as a measure of change in BCM as estimated by comparing whole-body counting of potassium was reported (16); and therefore, BCM was evaluated by an eight-polar BIA in this study. Because absolute values of body composition vary widely, normalization of body compositions including BCM is required. Height is an index for normalization; however, a balance of each body composition is also important, and normalization of BCM by BW is used in the definition of HIV-associated malnutrition (17). Therefore, we normalized BCM by BW and the resulting BCM was expressed as BCM%BW as previously described (11). The precision of InBody 3.2 was determined by measuring resistance two times a day in 7 of the study subjects. The coefficient of variation (CV) [(SD/mean) x 100], calculated from these measurements (n=14) is shown in

Table II. Nutritional status of the patients.

	M.CON	LC	P
No. of patients	25	56	
Age (years)	51.9±13.4	55.6±9.1	N.S.
Height (cm)	165.2±7.9	168.1±6.5	N.S.
Total protein (g/dl)	6.71±0.93	7.01±1.42	N.S.
Albumin (g/dl)	3.36±0.43	3.24±0.39	N.S.
Total cholesterol (mg/dl)	157±90	126±79	N.S.
Total lymphocyte count (/mm^3)	1330±796	1280±704	N.S.
Hemoglobin (g/dl)	11.6±1.9	11.9±1.9	N.S.
Creatinine (mg/dl)	0.74±0.26	0.77±0.17	N.S.
Prognostic nutritional index	40.2±5.6	38.8±5.0	N.S.
Accompanying malignancy	9/25	28/56	N.S.

Data are expressed as the mean ± SD. Prognostic nutritional index was calculated by using the following formula as previously described (13): Prognostic nutritional index = 10 x Serum albumin concentration (g/dl) + 0.005 x Total lymphocyte count (mm^3). M.CON, malnourished disease controls; LC, liver cirrhosis.

Table III. Anthropometric assessment.

	M.CON	LC	P
BMI	20.8±3.7	23.1±3.0	0.009
%AC	103.9±17.9	109.6±8.9	N.S.
%AMC	100.3±12.8	107.0±8.4	0.009
%TSF	128.8±80.9	127.4±37.9	N.S.
Waist circumference (cm)	76.7±10.2	83.7±6.6	0.003
Hip circumference (cm)	88.0±6.4	92.9±4.5	0.001
Waist-hip ratio	0.87±0.06	0.90±0.03	0.031

Data are expressed as the mean ± SD. AC, TSF and AMC are expressed as a percentage of the expected value for age and gender according to the Japanese Anthropometric Reference Data (JARD 2001) (15). M.CON, malnourished disease controls; LC, liver cirrhosis; BMI, body mass index; AC, arm circumference; AMC, arm muscle circumference; TSF, tricep skin-fold thickness.

Table I. Similar to a previous report (10), the CV value was <3.0 and comparable to that of a four-polar total-body BIA performed at 50 kHz (CV = 3.0%) (18,19).

Severity of liver disease. The severity of cirrhosis was assessed by the model for end-stage liver disease (MELD) score (20). The MELD score was computed using an on-line worksheet (http://www.mayoclinic.org/gi-rst/mayomodel5.html). To avoid negative scores, laboratory values such as serum creatinine concentrations that were <1 mg/dl were rounded off to 1 as previously described (21).

Statistical analysis. All data are expressed as the mean ± SD. Differences between the two groups were analyzed using the Mann-Whitney U test or the Chi-square test. The Spearman's correlation coefficient was calculated for testing the relationship between different quantities in a bivariate regression model using StatView (version 5.1; SAS Institute, Cary, NC). P-values <0.05 were indicative of significant differences.

Results

Patient characteristics. We enrolled 56 patients with liver cirrhosis and 25 patients with chronic gastrointestinal diseases as malnourished disease controls. Clinical and laboratory data for these subjects are summarized in Table II. Age and height were not significantly different between the LC and M.CON groups. Serum concentrations of total protein, albumin, and total cholesterol, total lymphocyte count, hemoglobin concentration, prognostic nutritional index, and the rate accompanying malignancy were also not significantly different between the two groups.

Comparison of anthropometry between the LC and M.CON groups. In the LC group, BMI and %AMC were significantly higher than in the M.CON group, although %TSF was not significantly different when comparing the two groups (Table III). Waist and hip circumferences and waist-hip ratio were significantly higher in the LC group than in the M.CON group.

Table IV. Assessment of body composition.

	M.CON	LC	P
Fat%BW	17.1±8.4	20.8±5.1	N.S.
TBW%BW	61.1±6.4	58.4±3.8	N.S.
ECF%BW	23.2±2.8	22.8±1.6	N.S.
ICF%BW	37.9±4.8	35.5±2.3	0.017
ECF/TBW	0.334±0.028	0.344±0.007	N.S.
Protein%BW	16.4±2.1	15.4±1.0	0.018
Mineral%BW	5.4±1.0	5.5±0.4	N.S.
BCM%BW	54.3±6.9	50.9±4.5	0.018

Data are expressed as the mean ± SD. M.CON, malnourished disease controls; LC, liver cirrhosis; BW, body weight; TBW, total body water; ICF, intracellular fluid; ECF, extracellular fluid; BCM, body cell mass.

Comparison of body composition between the LC and M.CON groups. There was a significant decrease in both intracellular fluid (ICF)%BW and protein%BW in the LC group compared to those in the M.CON group, although fat%BW, total body water (TBW)%BW, extracellular fluid (ECF)%BW, ICF%TBW, protein%BW and mineral%BW did not differ significantly when comparing the two groups (Table IV). Fig. 1A shows the compositions for BW, fat free mass (FFM), soft lean mass (SLM) and BCM. FFM%BW did not differ between the two groups (Fig. 1B), however, SLM%BW was significantly decreased in the LC group compared to that in the M.CON group (Fig. 1C). The difference was even more significant when comparing BCM%BW between the two groups (Fig. 1D).

Comparison of nutritional parameters between cirrhotic patients with HCC and cirrhotic patients without HCC. There were no significant differences in nutritional status including biochemical tests between cirrhotic patients with HCC and cirrhotic patients without HCC. No significant differences were also found in anthropometric assessment and body composition between cirrhotic patients with HCC and cirrhotic patients without HCC (data not shown).

An association between BCM and severity of disease in the LC group. Although there was no significant correlation between BCM and the MELD score when considering all subjects, an association was evident by stratification of the patients in the LC group according to their age. There was a significant negative correlation between BCM and the MELD score in cirrhotic patients aged 40-60 years (n=29, R^2=0.148, P=0.04) (Fig. 2).

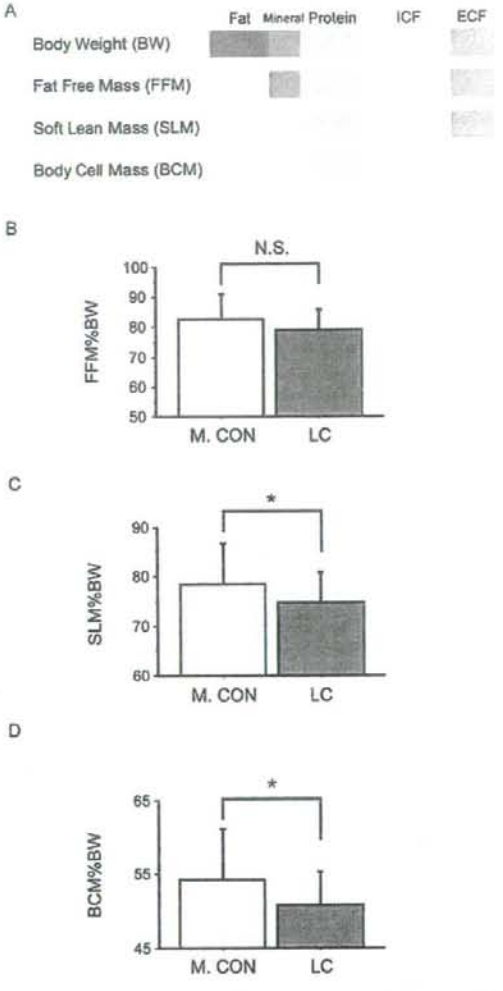

Figure 1. Scheme of body composition (A). Comparison of FFM (B), SLM (C) and BCM (D) between the LC and M.CON groups. Values are expressed as the mean ± SD. Differences between the two groups were analyzed using the Mann-Whitney U test. N.S., not significant. *P<0.05. BW, body weight; ICF, intracellular fluid; ECF, extracellular fluid; M.CON, malnourished disease controls; LC, liver cirrhosis.

Discussion

In this study, we demonstrated a significant decrease in BCM in patients with liver cirrhosis compared to that in malnourished patients with chronic gastrointestinal diseases, although serum or hematological nutritional parameters were not significantly different between the two groups. Moreover, we showed a significant negative correlation between BCM and the MELD score in patients with liver cirrhosis.

Significant changes in body composition are known to occur before physiological changes (5). Similar to previous reports (22,23), an increase in TBW and ECW was also noted in this study when these body compositions were normalized with height (data not shown). In addition, by normalization with BW, we found that ICF%BW and protein%BW were significantly decreased in the LC group compared to those in the M.CON group. In agreement with our data, Figueiredo *et al* recently reported that reduction in ICF, but not fat, occurred

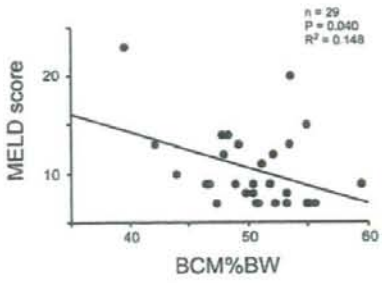

Figure 2. An association between BCM and severity of disease in patients with liver cirrhosis. Subjects was stratified by age because of the age difference in body water compartments (12) (See Materials and methods). An association between BCM and MELD score was analyzed by using the Spearman's correlation coefficient in a bivariate regression model. P<0.05, significant difference. MELD, the model for end-stage liver disease; BCM, body cell mass; BW, body weight.

even before fluid retention was noted clinically (22). We did not investigate the mechanisms for decreases in ICF and protein. However, one would think that loss of body protein leads to a decrease in intracellular osmotic pressure and subsequent decrease in ICF, since the rate of endogenous protein degradation is increased in patients with liver cirrhosis (24).

In this study, both groups manifested a characteristic of protein-energy malnutrition. Although we do not have a normal value for BCM for healthy males in Japan, it is believed to be approximately 60% of BW according to the average value for body compartments (20% of ICF%BW plus 40% of protein%BW). In the M.CON group, BCM%BW was <60% of BW which is considered to be lower than that of normal subjects. In the LC group, BCM%BW was also <60% of BW. In addition, BCM was significantly decreased in the LC group compared to the M.CON group, although serum and hematologic nutritional parameters were not significantly different. These findings may indicate that change in body composition is more evident in the LC group than in the M.CON group.

There were no significant differences in nutritional parameters between cirrhotic patients with HCC and cirrhotic patients without HCC. Occurrence of HCC seems to affect the metabolic course of chronic liver disease; however, our data did not show any difference. One would think that HCC does not affect nutritional parameters, because >70% of cirrhotic patients with HCC showed a single (<3-cm) nodule treated with curative therapy. Alternatively, cirrhotic patients without HCC were admitted for preventive therapy of esophageal varices. Thus, cirrhotic patients without HCC also had the complication of LC and therefore, no significant differences in nutritional parameters could be found.

We also showed the significance of BCM in cirrhotic patients. A significant negative correlation between BCM and the MELD score was observed in cirrhotic patients without ascites and/or edema. On the other hand, Muller *et al* reported that there was no significant difference in BCM among patients according to the Child-Pugh classification (25). Although the reason for this discrepancy is unclear, the explanation may be that BCM was evaluated as the absolute value in the previous study. BW increases with increasing severity in liver disease. Therefore, normalization of BCM by BW could be one of the reasons for this discrepancy. In accordance with our data, Figueiredo *et al* reported that normalized BCM is correlated with the Child-Pugh classification (22). Thus, the estimation of BCM is not only non-invasive but also meaningful. However, we have to be cautious in interpreting BCM values when patients exhibit hyperbilirubinemia. In our analysis for an association between BCM and the MELD score, some patient data did not correspond with the regression line, and hyperbilirubinemia (>6 mg/dl of serum bilirubin levels) was a common characteristic in these patients. The relationship between hyperbilirubinemia and impedance is unclear. However, bilirubin is known to modulate cell-cell contact and results in changes in transepithelial electrical resistance (26). Thus, BCM estimated by eight-polar BIA may not predict prognosis in patients with hyperbilirubinemia.

The limitations of this study included the small sample size, the ratio of cases to controls, and the heterogeneity of the malnourished controls. In order to confirm the significance of BCM in patients with LC, a large-scale multicenter clinical study including healthy individuals is required. In addition, the use of the MELD score may not have been suitable for this study. Since the significance of the correlation between BCM and MELD was poor, the real prognostic relevance of BCM should be evaluated in future studies. Moreover, the number of hospitals that are able to measure BCM is still limited. Changes in BCM are known to correlate with energy and protein intake (27). Thus, food consumption should be assessed cautiously in order to prevent underestimations that may occur by using traditional nutritional assessment measures.

In conclusion, we demonstrated a significant decrease in BCM in patients with liver cirrhosis compared to that in malnourished patients with chronic gastrointestinal diseases. We also showed a significant negative correlation between BCM and MELD score in cirrhotic patients without ascites and/or edema. Thus, BCM may be a useful parameter for assessing malnutrition and severity of disease in patients with liver cirrhosis.

Acknowledgements

This study was supported, in part, by a Grant-in-Aid for Young Scientists (B) (no. 19790643 to T.K.).

References

1. Alberino F, Gatta A, Amodio P, *et al*: Nutrition and survival in patients with liver cirrhosis. Nutrition 17: 445-450, 2001.
2. Morgan MY, Madden AM, Soulsby CT and Morris RW: Derivation and validation of a new global method for assessing nutritional status in patients with cirrhosis. Hepatology 44: 823-835, 2006.
3. Plauth M, Cabre E, Riggio O, *et al*: ESPEN Guidelines on Enteral Nutrition: Liver disease. Clin Nutr 25: 285-294, 2006.
4. Alvares-da-Silva MR and Reverbel da Silveira T: Comparison between handgrip strength, subjective global assessment, and prognostic nutritional index in assessing malnutrition and predicting clinical outcome in cirrhotic outpatients. Nutrition 21: 113-117, 2005.

5. Crawford DH, Shepherd RW, Halliday JW, *et al*: Body composition in nonalcoholic cirrhosis: the effect of disease etiology and severity on nutritional compartments. Gastroenterology 106: 1611-1617, 1994.
6. Pirlich M, Schutz T, Spachos T, *et al*: Bioelectrical impedance analysis is a useful bedside technique to assess malnutrition in cirrhotic patients with and without ascites. Hepatology 32: 1208-1215, 2000.
7. Morgan MY and Madden AM: The assessment of body composition in patients with cirrhosis. Eur J Nucl Med 23: 213-225, 1996.
8. Henkel AS and Buchman AL: Nutritional support in patients with chronic liver disease. Nat Clin Pract Gastroenterol Hepatol 3: 202-209, 2006.
9. Pirlich M, Schutz T, Ockenga J, *et al*: Improved assessment of body cell mass by segmental bioimpedance analysis in malnourished subjects and acromegaly. Clin Nutr 22: 167-174, 2003.
10. Bedogni G, Malavolti M, Severi S, *et al*: Accuracy of an eight-point tactile-electrode impedance method in the assessment of total body water. Eur J Clin Nutr 56: 1143-1148, 2002.
11. Selberg O, Bottcher J, Tusch G, Pichlmayr R, Henkel E and Muller MJ: Identification of high- and low-risk patients before liver transplantation: a prospective cohort study of nutritional and metabolic parameters in 150 patients. Hepatology 25: 652-657, 1997.
12. Lesser GT and Markofsky J: Body water compartments with human aging using fat-free mass as the reference standard. Am J Physiol 236: R215-R220, 1979.
13. Buzby GP, Mullen JL, Matthews DC, Hobbs CL and Rosato EF: Prognostic nutritional index in gastrointestinal surgery. Am J Surg 139: 160-167, 1980.
14. Wang Z, Deurenberg P, Wang W, Pietrobelli A, Baumgartner RN and Heymsfield SB: Hydration of fat-free body mass: new physiological modeling approach. Am J Physiol 276: E995-E1003, 1999.
15. Okada T, Muto Y, Yamamori H, *et al*: Japanese Anthropometric Reference Data; JARD 2001. Jpn J Nutr Assess 19: 1-82, 2002.
16. Kotler DP, Rosenbaum K, Allison DB, Wang J and Pierson RN Jr: Validation of bioimpedance analysis as a measure of change in body cell mass as estimated by whole-body counting of potassium in adults. JPEN J Parenter Enteral Nutr 23: 345-349, 1999.
17. Polsky B, Kotler D and Steinhart C: HIV-associated wasting in the HAART era: guidelines for assessment, diagnosis, and treatment. AIDS Patient Care STDS 15: 411-423, 2001.
18. Heitmann BL: Impedance: a valid method in assessment of body composition? Eur J Clin Nutr 48: 228-240, 1994.
19. Kushner RF and Schoeller DA: Estimation of total body water by bioelectrical impedance analysis. Am J Clin Nutr 44: 417-424, 1986.
20. Kamath PS, Wiesner RH, Malinchoc M, *et al*: A model to predict survival in patients with end-stage liver disease. Hepatology 33: 464-470, 2001.
21. Wiesner R, Edwards E, Freeman R, *et al*: Model for end-stage liver disease (MELD) and allocation of donor livers. Gastroenterology 124: 91-96, 2003.
22. Figueiredo FA, De Mello Perez R and Kondo M: Effect of liver cirrhosis on body composition: evidence of significant depletion even in mild disease. J Gastroenterol Hepatol 20: 209-216, 2005.
23. McCullough AJ, Mullen KD and Kalhan SC: Measurements of total body and extracellular water in cirrhotic patients with and without ascites. Hepatology 14: 1102-1111, 1991.
24. Dichi JB, Dichi I, Maio R, *et al*: Whole-body protein turnover in malnourished patients with child class B and C cirrhosis on diets low to high in protein energy. Nutrition 17: 239-242, 2001.
25. Muller MJ, Lautz HU, Plogmann B, Burger M, Korber J and Schmidt FW: Energy expenditure and substrate oxidation in patients with cirrhosis: the impact of cause, clinical staging and nutritional state. Hepatology 15: 782-794, 1992.
26. Raimondi F, Crivaro V, Capasso L, *et al*: Unconjugated bilirubin modulates the intestinal epithelial barrier function in a human-derived in vitro model. Pediatr Res 60: 30-33, 2006.
27. Robert S, Zarowitz BJ, Hyzy R, Eichenhorn M, Peterson EL and Popovich J Jr: Bioelectrical impedance assessment of nutritional status in critically ill patients. Am J Clin Nutr 57: 840-844, 1993.

Hepatology Research 2008; **38**: 1178–1185 doi: 10.1111/j.1872-034X.2008.00390.x

Original Article

Supplement improves nutrition and stresses caused by examination-associated fasting in patients with liver cirrhosis

Takumi Kawaguchi,[1,2] Eitaro Taniguchi,[2] Minoru Itou,[2] Michiko Mutou,[3] Ryoko Ibi,[3] Satomi Shiraishi,[3] Teruyo Okada,[3] Yuki Uchida,[4] Momoka Otsuka,[4] Tatsuyuki Tonan,[5] Kiminori Fujimoto,[5] Tetsuharu Oriishi,[2] Suiko Tanaka,[4] Machiko Takakura[3] and Michio Sata[1,2]

[1]Department of Digestive Disease Information & Research, [2]Department of Medicine, Kurume University School of Medicine, [3]Department of Nursing, [4]Department of Nutrition, Kurume University Hospital, and [5]Department of Radiology, Kurume University School of Medicine, Kurume, Japan

Aim: Cirrhotic patients tend to develop malnutrition by fasting, yet the importance of nutritional care during examination-associated fasting has not been investigated. This study aimed to examine the effects of a nutritional supplement on nutrition and stresses caused by examination-associated fasting in cirrhotic patients.

Methods: Twenty-nine cirrhotic patients were enrolled in this study. No energy was supplied to patients in the fasting group ($n = 11$) prior to computed tomography or magnetic resonance imaging examination. A supplement of 200 kcal was given to the patients in the supplement group ($n = 18$) prior to computed tomography or magnetic resonance imaging examination. The effect of the supplement on stresses was evaluated by self-rating questionnaire. Changes in biochemical parameters were also investigated before and after computed tomography or magnetic resonance imaging examinations.

Results: There were no significant differences in age, sex, body mass index, or liver function tests between the two groups at the start of the study. In the supplement group, stress scores for physical symptoms (thirst and lightheadedness) and mental symptoms (hunger, hypodynamia and fatigue) were significantly lower compared to those in the fasting group. Also in the supplement group, peripheral 3-hydroxybutyric acid and free fatty acids levels were significantly decreased compared to those in the fasting group, to within normal ranges. In addition, a decrease in prothrombin time was significantly inhibited by intake of the supplement.

Conclusion: We demonstrated that a nutritional supplement improved nutrition and reduced both the physical and mental stresses associated with examination-associated fasting in cirrhotic patients.

Key words: mental stress, physical stress, quality of life, starvation, supplementation

INTRODUCTION

MALNUTRITION IS FREQUENTLY seen in patients with liver cirrhosis. Malnutrition is associated with clinical complications of cirrhosis and increases mortality.[1,2] Supplemental enteral nutrition improves nutritional status, liver function, survival, and quality of life (QOL) in cirrhosis patients.[3–11] Thus, nutritional care is an essential element in the management of patients with liver cirrhosis.

The liver has a central role in regulating energy metabolisms. In a fasting state, the liver contributes to about one-half of the body's total caloric requirements by releasing glucose into the blood.[12] In patients with liver cirrhosis, after an overnight fast, hepatic glycogen content is decreased and metabolic profiles are similar to those seen in normal subjects after two to three days fasting.[13] Thus, patients with liver cirrhosis develop a catabolic state of starvation more rapidly than do

Correspondence: Dr Takumi Kawaguchi, Department of Digestive Disease Information & Research, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan.
Email: takumi@med.kurume-u.ac.jp
Received 23 February 2008; revision 22 April 2008; accepted 26 April 2008.

normal subjects and should therefore ensure adequate energy intake and avoid long-term fasting.[1,2,14]

Hepatocellular carcinoma or esophageal varices are major complications in patients with liver cirrhosis.[15–17] For examination and treatment of these complications, abdominal ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), angiography or esophagogastroduodenoscopy (EGD) are commonly used. Nausea and vomiting often occur after the use of ionic high-osmolality contrast media and, thus, patients are fasted before contrast-enhanced examinations. Nonionic low-osmolality contrast media, the current media of choice, are safer than ionic high-osmolality media.[18] Despite this, examination-associated fasting is still used at some institutions and is often required for patients with liver cirrhosis. In addition, frequent examinations are required due to the shortening of hospital stays. However, no study addresses the standard of nutritional care, nor the nutritional status and stresses that exist, for examination-associated fasting in patients with liver cirrhosis. The aim of this study is to examine the effects of supplement on nutrition and stresses caused by examination-associated fasting in patients with liver cirrhosis.

METHODS

Patients

A TOTAL OF 29 patients with hepatitis C virus (HCV)-related liver cirrhosis ($n = 25$), hepatitis B virus (HBV)-related liver cirrhosis ($n = 3$), or alcoholic liver cirrhosis ($n = 1$) were enrolled in this study during the period from April 2005 to July 2006 at Kurume University Hospital, Japan. The diagnosis of liver cirrhosis was based on clinical, serological, imaging, and/or histological evidence. All of the patients were hospitalized for the treatment of hepatocellular carcinoma or esophageal varices. Patients with ascites or renal failure were excluded because the effects of nutritional supplements on body composition cannot be precisely evaluated under conditions of abnormal body water distribution. Informed consent for participation in the study was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki (1975) and was approved by the Ethics Committee of the Kurume University School of Medicine, Japan.

Study design

We conducted a randomized controlled trial on 29 patients with liver cirrhosis. Enrolled patients were randomly assigned to the fasting group or the supplement group. The time schedule of examination and supplement administration are summarized in Figure 1. In both groups, contrast-enhanced CT or contrast-enhanced MRI was conducted between 10.00–11.30 hours. Blood tests, measurement of body weight and body composition analysis were performed a day before examination and after CT or MRI examination. Urine was collected between 06:00–10:00 hours for both groups. There was no energy supply between supper and lunch in the fasting group ($n = 11$), while 200 kcal of a nutritional supplement (Calorie Mate JELLY; Otsuka Pharmaceutical, Japan) was given to the patients in the supplement group ($n = 18$), between 06:00–06:10 hours. The composition of the supplement is shown in Table 1. The effects of the supplement on the CT or MRI

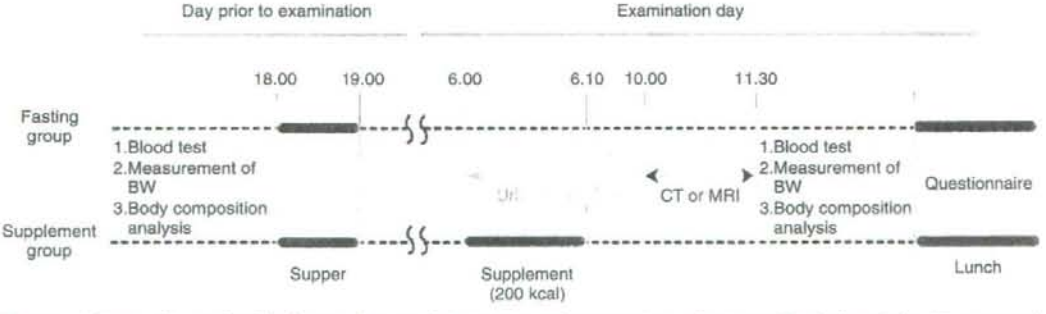

Figure 1 Scheme of time schedule for supplement administration and examination of patients. BW, body weight; CT, computed tomography; MRI, magnetic resonance imaging. (—) Fasting duration; (**—**) feeding duration.

Table 1 The composition of supplement (Calorie Mate JELLY; Otsuka Pharmaceutical, Japan)

Component	Content
Serving size (g)	215
Energy (kcal)	200
Protein (g)	7.6
BCAAs	1.7
Fat (g)	4.4
Carbohydrate (g)	32.5
Dietary fiber (g)	2
Sodium (mg)	30
Potassium (mg)	60
Calcium (mg)	200
Magnesium (mg)	50
Phosphorus (mg)	218
Vitamin A (μg)	225
Vitamin B1 (mg)	0.5
Vitamin B2 (mg)	0.6
Vitamin B6 (mg)	0.5
Vitamin B12 (μg)	1
Niacin (mg)	5.5
Pantothenic acid (mg)	2.8
Folate (μg)	100
Vitamin D (μg)	2.5
Vitamin E (mg)	4

BCAAs, branched-chain amino acids.

imaging diagnoses were evaluated by radiologists blinded to which group the patients belonged.

Contrast medium for CT or MRI examination

All patients underwent contrast-enhanced CT or MRI examinations using Iopamidol (Bayer Yakuhin, Japan), Iohexol (Bayer Yakuhin), or Iotrolan (Daiichi Sankyo, Japan).

Effects of supplement on mental and physical stresses

Physical symptoms (thirst, light-headedness, nausea, headache, palpitations, cold sweat) and mental symptoms (hunger, hypodynamia, fatigue, poor thinking, poor concentration, irritability) were evaluated by self-rating questionnaire accoding to the following scale: 1 = none; 2 = mild; 3 = moderate; and 4 = severe.

Effects of supplement on biochemical parameters, body weight and composition

Platelet count, prothrombin time, serum albumin, total bilirubin, total cholesterol, creatinine level, osmolality, blood glucose level, blood urea nitrogen concentration and urine specific gravity were measured using standard clinical methods (Department of Clinical Laboratory, Kurume University Hospital, Japan). Body weight and composition were measured using an eight-polar direct segmental multifrequency-bioelectrical impedance analyzer (DSM-BIA; InBody 3.2, Biospace, Japan). The changes in body composition were assessed by the differences between values obtained a day before and a day after CT or MRI examination and are expressed as Δbody composition.

Statistical analysis

All data are expressed as mean ± SE. Differences between the two groups were analyzed using the Mann-Whitney *U*-test. P values < 0.05 were considered significant.

RESULTS

Patient characteristics

THE CHARACTERISTICS OF all patients are summarized in Table 2. There were no significant differences in etiology of cirrhosis, age, sex, body mass index and liver function tests between patients in the fasting group and those in the supplement group (Table 2). Fasting duration was significantly shortened, by about 4 h, with the administration of supplement (Table 2).

Interference of supplement on CT or MRI imaging

There was no evidence of retention of the supplement in the gut or the contraction of gallbladder in CT or MRI imaging (Fig. 2A,B). In addition, the intake of the supplement did not induce vomiting and there was no interference with the diagnostic CT or MRI imaging by intake of the supplement (Table 2).

Effects of supplement on mental or physical symptoms

Among physical symptoms, stress scores for thirst and light-headedness were significantly lower in the supplement group compared to those in the fasting group (Table 3). Among mental symptoms, stress scores for hunger, hypodynamia and fatigue were significantly lower in the supplement group compared to those in the fasting group (Table 3).

Effects of supplement on biochemical parameters and body weight and compositions

Changes in body weight and body compositions were expressed by the difference between values obtained a

Table 2 Characteristics of all patients

	Normal range	Fasting group ($n = 11$)	Supplement group ($n = 28$)	P value
HCV:HBV:Alcohol	–	9:2:0	16:1:1	0.43
Age (years)	–	59.9 ± 3.4	66.6 ± 2.5	0.13
Sex (M:F)	–	6:5	12:6	0.51
BMI	–	22.7 ± 0.8	21.2 ± 0.4	0.13
Albumin (g/dL)	4.0–5.0	3.34 ± 0.15	3.42 ± 0.12	0.71
Total bilirubin (mg/dL)	0.61–1.04	1.85 ± 0.35	1.41 ± 0.26	0.11
Prothrombin time (%)	4.0–5.0	70.5 ± 4.9	79.2 ± 2.7	0.08
Total cholesterol (mg/dL)	150–219	136 ± 36	145 ± 29	0.56
Platelet count (/μL)	70–139	8.6 ± 0.9	11.3 ± 1.7	0.33
Fasting duration (h)	–	13.8 ± 0.3	4.7 ± 0.1	<0.0001
Number of patients that vomited	–	0	0	>0.99
Interference of supplement on imaging	–	NA	0	–

Data expressed as mean ± SE. Differences between the two groups were analyzed using the Mann–Whitney *U*-test. BMI, body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus; NA, not applicable.

day before and after examination. There was no significant difference noted in body weight changes between the fasting group and the supplement group (Table 4). Similarly, changes in body composition showed no significant differences between the fasting group and the supplement group (Table 4). Intake of the supplement did not affect levels of blood glucose, plasma osmolality, blood urea nitrogen, and serum creatinine (Table 4). No significant changes occurred in urine volume and urine specific gravity when comparing the two groups (Table 4).

Effects of supplement on serum 3-hydroxybutyric acid, plasma free fatty acids levels and prothrombin time

Intake of the supplement resulted in significant decreases in serum 3-hydroxybutyric acid and plasma free fatty acids levels. Further, these levels reached to within in normal ranges (Fig. 3A,B). Decreases in prothrombin time were significantly inhibited by the intake of the supplement compared to that in the fasting group after examination (Fig. 3C).

DISCUSSION

IN THIS STUDY, we first demonstrated that the nutritional supplement improved malnutrition and both physical symptoms and mental stresses caused by fasting before examinations in patients with liver cirrhosis.

Nausea and vomiting occur frequently after the use of ionic high-osmolality contrast media and, therefore, patients are fasted before contrast-enhanced examina-

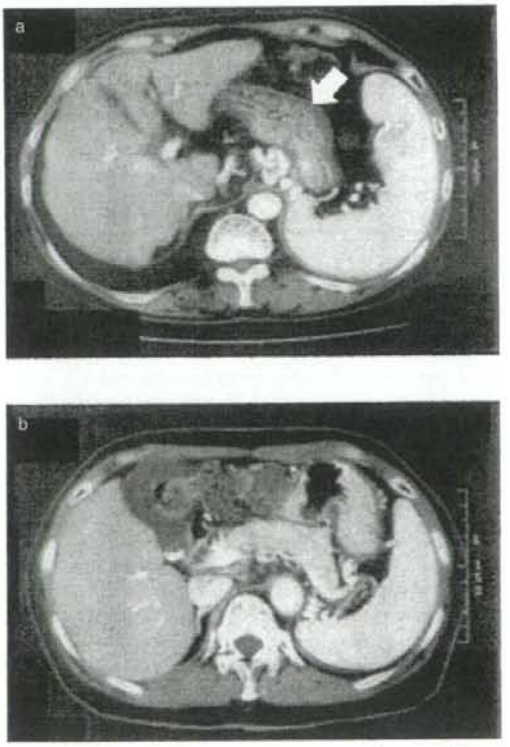

Figure 2 Representative CT images from the supplement group. No retention of the supplement in the (a) gut was detected, or contraction of the (b) gallbladder in CT imaging. Arrows indicate stomach.

Table 3 Effects of the supplement on physical and mental symptoms as evaluated by a self-rating questionnaire (1 = none; 2 = mild, 3 = moderate, 4 = severe)

	Fasting group	Supplement group	*P* value
Physical symptoms			
Thirst	2.8 ± 0.4	1.6 ± 0.2	0.01
Light-headedness	2.0 ± 0.4	1.2 ± 0.1	0.04
Nausea	1.5 ± 0.3	1.0 ± 0.0	0.07
Headache	1.3 ± 0.1	1.1 ± 0.1	0.11
Palpitation	1.1 ± 0.1	1.1 ± 0.1	0.72
Cold sweat	1.0 ± 0.0	1.1 ± 0.1	0.43
Mental symptoms			
Hunger	2.6 ± 0.4	1.3 ± 0.1	0.003
Hypodynamia	2.1 ± 0.3	1.2 ± 0.1	0.01
Fatigue	2.2 ± 0.4	1.2 ± 0.1	0.01
Poor thinking	1.7 ± 0.3	1.2 ± 0.1	0.25
Poor concentration	1.7 ± 0.3	1.3 ± 0.1	0.29
Irritability	1.5 ± 0.2	1.2 ± 0.1	0.45

Data expressed as mean ± SE. Differences between the two groups were analyzed using the Mann–Whitney *U*-test.

tions. However, nonionic low-osmolality contrast media is safer than ionic high-osmolality media and is the media of choice today.[18] Although the supplement was given approximately 4 h before examination, none of the patients experienced nausea or vomiting. Further, there was no interference with diagnostic imaging. Thus, the intake of the supplement had no adverse effects on the examination and diagnostic imaging in this study.

Although nutritional supplements vary in form and shape, we chose a jelly-type supplement for this study. Sensory-specific satiety has an important influence on QOL.[19,20] In particular, texture-specific satiety has a significant effect.[21] The decision to use a jelly-type supplement was based on the fact that the texture of jelly is more like solid food. In addition, high viscosity formula-diet accelerates gastric empting, resulting in prevention of gastro-oesphageal reflux and aspiration pneumonia.[22] We also selected a branched-chain amino acids (BCAAs)-rich supplement because serum BCAAs levels are decreased in patients with liver cirrhosis and treatment with BCAAs is known to improve nutritional status and QOL.[9,23]

With intake of the supplement, physical symptoms such as thirst and light-headedness improved signifi-

Table 4 Effects of supplement on biochemical parameters, body weight and composition

	Fasting group	Supplement group	*P* value
Biochemical parameters			
Blood glucose (mg/dL)	106.5 ± 6.4	105.1 ± 9.4	0.33
Plasma osmolality (mOsm/L)	285.5 ± 1.7	286.0 ± 1.0	0.77
Blood urea nitrogen (mg/dL)	16.9 ± 2.1	17.9 ± 2.3	0.72
Creatinine (mg/dL)	0.74 ± 0.05	0.90 ± 0.08	0.30
Urine volume (mL)	190 ± 41	200 ± 33	0.93
Urine specific gravity	1.023 ± 0.06	1.016 ± 0.02	0.50
ΔBody weight (kg)	−0.95 ± 0.34	−0.80 ± 0.37	0.70
Body composition			
ΔIntracellular fluid (kg)	−0.26 ± 0.28	−0.57 ± 0.07	0.59
ΔExtracellular fluid (kg)	−0.68 ± 0.14	−0.43 ± 0.07	0.14
ΔProtein (kg)	−0.11 ± 0.12	−0.24 ± 0.03	0.59
ΔMineral (kg)	−0.26 ± 0.13	−0.09 ± 0.14	0.07
ΔFat (kg)	−0.34 ± 0.37	−0.54 ± 0.17	0.44

Data are expressed as mean ± SE. Differences between the two groups were analyzed using the Mann–Whitney *U*-test.

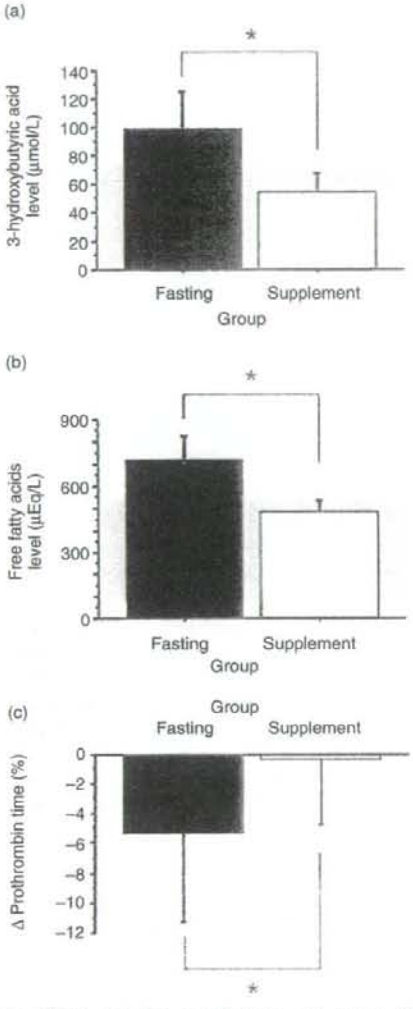

Figure 3 Effects of the supplement on serum (a) 3-hydroxyburyric acid level, (b) plasma free fatty acids level, and changes in (c) prothrombin time. Shaded regions in (a-b) indicate the normal ranges for serum 3-hydroxyburyric acid and plasma free fatty acids, respectively. Values are expressed as mean ± SE. Differences between the two groups were analyzed using the Mann–Whitney *U*-test. $^*P < 0.05$.

cantly. Biochemical examination showed that blood glucose concentration, plasma osmolality and urine specific gravity were all within normal ranges, even in the fasting group. In addition, there were no significant changes in these parameters in the supplement group. Similarly, bioelectrical impedance analysis showed no changes in body weight or composition. Thus, changes in glucose levels or body water distribution do not seem to be the main causative factors for the improvement of thirst and light-headedness. Long-term fasting itself causes stress due to a dissatisfied demand for food and reduction of such stress is known to improve physical symptoms.[24,25] Thus, relief from long-term examination-associated fasting might be responsible for the stress reduction and subsequent improvement of physical symptoms.

Patients with liver cirrhosis develop a catabolic state of starvation more rapidly than do normal subjects. Approximately 80% of the daily non-protein caloric requirement is supplied from fat even after overnight fasting.[13] Peripheral 3-hydroxybutyric acid and free fatty acids are metabolites derived from fat and are used as indicators of starvation. In this study, levels of peripheral 3-hydroxybutyric acid and free fatty acids were significantly higher in the fasting group. Peripheral 3-hydroxybutyric acid stimulates appetite and elevated plasma free fatty acids levels cause hypodynamia and fatigue through increased serotonin release in the brain.[26,27] In the present study, serum 3-hydroxybutyric acid and plasma free fatty acids levels were significantly increased in patients with examination-associated fasting compare to those in age-, BMI-, and liver function tests-matched patients with only overnight fasting (51.6 ± 10.5 vs 99.4 ± 25.0 μmol/L, $P < 0.04$; 436 ± 48 vs 686 ± 93 μEq/L, $P < 0.03$), indicating the importance of supplementation for the examination-associated fasting. With the intake of the supplement, both 3-hydroxybutyric acid and plasma free fatty acids levels were reduced to within normal range, and hunger, hypodynamia and fatigue were significantly improved. Although it is not clear why the supplement improves mental symptoms, it is possible that glucose supplementation changes the fuel source from fat to glucose and results in a decrease of 3-hydroxybutyric acid and plasma free fatty acids levels, leading to improvement in mental symptoms. Alternatively, hunger is also regulated by gut hormones and food intake stimulates the satiety center through regulation of gut hormones such as ghrelin, polypeptide YY, and glucagon-like peptide-1.[28,29] Thus, intake of the supplement might improve mental symptoms through regulation of gut hormones.

A decrease in prothrombin time was significantly inhibited by intake of the supplement. The half-life of prothrombin is several hours and prothrombin is a rapid turnover protein, used as not only as a prognostic

parameter[30,31] but also as a nutritional parameter.[32] Protein turnover is increased in patients with liver cirrhosis and treatment with glucose is reported to reduce the protein turnover rate and improve the nitrogen balance.[33] The supplement used in this study contains 33.0 g of glucose. Thus, glucose supplementation might be responsible for improved nitrogen balance, leading to a subsequent increase in prothrombin time. In addition, it has been shown that treatment with BCAAs improves nutritional status in patients with liver cirrhosis.[9,23] The supplement used in this study contains 1.7 g of BCAAs, BCAAs might up-regulate the mammalian target of rapamycin, which is a key molecule in protein synthesis.[34]

There are several limitations in this study. First, nutritional validity of this supplement for outcome, survival rate, and long-term QOL is unclear in patients with liver cirrhosis. Second, the supplement was administrated for only CT or MRI examination. In order to improve long-term nutritional status, outcome and QOL in liver cirrhosis a supplement verified by scientific research and supplementation should be given in other examinations such as blood test, US, EGD, angiography. Third, even in the supplement group there was an approximately 1 kg decrease in body weight within a day, mainly attributable to the loss of extracellular fluid. Intake of the supplement did not prevent significant changes in body weight or composition. Hypermetabolism is a characteristic of patients with liver cirrhosis,[35] and these patients should ensure energy intake is adequate.[1,2] The principal etiology for liver disease among the patients in this study was HCV infection. A decrease in hepatic glycogen storage and malnutrition are more severe in HCV-related chronic liver disease than in other hepato-biliary disorders.[36,37] A nutritional supplement of 200 kcal may not be a sufficient energy source to prevent loss of body weight in patients with HCV-related liver cirrhosis.

We demonstrate that a nutritional supplement improves nutrition and both physical and mental stresses caused by fasting before contrast-enhanced CT or MRI examination, without inducing any adverse effects in patients with liver cirrhosis.

ACKNOWLEDGMENTS

THE AUTHORS WISH to thank Kiyomi Yoshida, Mariko Suga, Aimi Yoshiyama, Yuko Adachi and Mari Torii for collecting clinical data. This study was supported, in part, by grants from the Ministry of Education, Culture, Sports, Science and Technology, Japan (Grant-in-Aid for Young Scientists, B, No. 19790643 to T.K.), the Vehicle Racing Commemorative Foundation, Japan, and the Ishibashi Foundation for the Promotion of Science, Japan.

REFERENCES

1 Plauth M, Cabre E, Riggio O *et al.* ESPEN guidelines on enteral nutrition: liver disease. *Clin Nutr* 2006; 25: 285–94.
2 Force ABoDatCGT. Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients. *JPEN J Parenter Enteral Nutr* 2002; 26: 1SA–138SA.
3 Kawamura N, Nakajima H, Takashi SI. Administration of granulated BCAA and quality of life. *Hepatol Res* 2004; 30S: 42–5.
4 Yoshida T, Muto Y, Moriwaki H, Yamato M. Effect of long-term oral supplementation with branched-chain amino acid granules on the prognosis of liver cirrhosis. *Gastroenterol Jpn* 1989; 24: 692–8.
5 Cabre E, Gonzalez-Huix F, Abad-Lacruz A *et al.* Effect of total enteral nutrition on the short-term outcome of severely malnourished cirrhotics. A randomized controlled trial. *Gastroenterology* 1990; 98: 715–20.
6 Kearns PJ, Young H, Garcia G *et al.* Accelerated improvement of alcoholic liver disease with enteral nutrition. *Gastroenterology* 1992; 102: 200–5.
7 Le Cornu KA, McKiernan FJ, Kapadia SA, Neuberger JM. A prospective randomized study of preoperative nutritional supplementation in patients awaiting elective orthotopic liver transplantation. *Transplantation* 2000; 69: 1364–9.
8 Suzuki K, Kato A, Iwai M. Branched-chain amino acid treatment in patients with liver cirrhosis. *Hepatol Res* 2004; 30S: 25–9.
9 Muto Y, Sato S, Watanabe A *et al.* Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. *Clin Gastroenterol Hepatol* 2005; 3: 705–13.
10 Sato S, Watanabe A, Muto Y *et al.* Clinical comparison of branched-chain amino acid (l-Leucine, l-Isoleucine, l-Valine) granules and oral nutrition for hepatic insufficiency in patients with decompensated liver cirrhosis (LIV-EN study). *Hepatol Res* 2005; 31: 232–40.
11 Muto Y, Sato S, Watanabe A *et al.* Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. *Hepatol Res* 2006; 35: 204–14.
12 Owen OE, Reichle FA, Mozzoli MA *et al.* Hepatic, gut, and renal substrate flux rates in patients with hepatic cirrhosis. *J Clin Invest* 1981; 68: 240–52.
13 Owen OE, Trapp VE, Reichard GA Jr *et al.* Nature and quantity of fuels consumed in patients with alcoholic cirrhosis. *J Clin Invest* 1983; 72: 1821–32.

14 Swart GR, Zillikens MC, van Vuure JK, van den Berg JW. Effect of a late evening meal on nitrogen balance in patients with cirrhosis of the liver. *BMJ* 1989; **299**: 1202–3.
15 Gores GJ, Wiesner RH, Dickson ER, Zinsmeister AR, Jorgensen RA, Langworthy A. Prospective evaluation of esophageal varices in primary biliary cirrhosis: development, natural history, and influence on survival. *Gastroenterology* 1989; **96**: 1552–9.
16 Tong MJ, el-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. *N Engl J Med* 1995; **332**: 1463–6.
17 Lai CL, Yuen MF. The natural history and treatment of chronic hepatitis B: a critical evaluation of standard treatment criteria and end points. *Ann Intern Med* 2007; **147**: 58–61.
18 Barrett BJ, Parfrey PS, Vavasour HM, O'Dea F, Kent G, Stone E. A comparison of nonionic, low-osmolality radiocontrast agents with ionic, high-osmolality agents during cardiac catheterization. *N Engl J Med* 1992; **326**: 431–6.
19 Rolls BJ, Rolls ET, Rowe EA, Sweeney K. Sensory specific satiety in man. *Physiol Behav* 1981; **27**: 137–42.
20 Kawaguchi T, Taniguchi E, Itou M *et al.* Appearance-specific satiety increases appetite and quality of life in patients with metastatic liver tumor: a case report. *Kurume Med J* 2006; **53**: 41–6.
21 Rolls BJ, Rowe EA, Rolls ET, Kingston B, Megson A, Gunary R. Variety in a meal enhances food intake in man. *Physiol Behav* 1981; **26**: 215–21.
22 Shimoyama Y, Kusano M, Kuwamura O *et al.* High-viscosity liquid meal accelerates gastric emptying. *Neurogastroenterol Motil* 2007; 879–86.
23 Nakaya Y, Okita K, Suzuki K *et al.* BCAA-enriched snack improves nutritional state of cirrhosis. *Nutrition* 2007; **23**: 113–20.
24 Reibel DK, Greeson JM, Brainard GC, Rosenzweig S. Mindfulness-based stress reduction and health-related quality of life in a heterogeneous patient population. *Gen Hosp Psychiatry* 2001; **23**: 183–92.
25 Greco CM, Rudy TE, Manzi S. Effects of a stress-reduction program on psychological function, pain, and physical function of systemic lupus erythematosus patients: a randomized controlled trial. *Arthritis Rheum* 2004; **51**: 625–34.
26 Fernstrom JD, Fernstrom MH. Exercise, serum free tryptophan, and central fatigue. *J Nutr* 2006; **136**: 553S–9S.
27 Davis JM, Alderson NL, Welsh RS. Serotonin and central nervous system fatigue: nutritional considerations. *Am J Clin Nutr* 2000; **72**: 573S–8S.
28 Martins C, Morgan LM, Bloom SR, Robertson MD. Effects of exercise on gut peptides, energy intake and appetite. *J Endocrinol* 2007; **193**: 251–8.
29 Kojima M, Kangawa K. Drug insight: the functions of ghrelin and its potential as a multitherapeutic hormone. *Nat Clin Pract Endocrinol Metab* 2006; **2**: 80–8.
30 Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; **60**: 646–9.
31 Kamath PS, Wiesner RH, Malinchoc M *et al.* A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464–70.
32 Gunsar F, Raimondo ML, Jones S *et al.* Nutritional status and prognosis in cirrhotic patients. *Aliment Pharmacol Ther* 2006; **24**: 563–72.
33 Swart GR, van den Berg JW, Wattimena JL, Rietveld T, van Vuure JK, Frenkel M. Elevated protein requirements in cirrhosis of the liver investigated by whole body protein turnover studies. *Clin Sci (Lond.)* 1988; **75**: 101–7.
34 Ijichi C, Matsumura T, Tsuji T, Eto Y. Branched-chain amino acids promote albumin synthesis in rat primary hepatocytes through the mTOR signal transduction system. *Biochem Biophys Res Commun* 2003; **303**: 59–64.
35 Muller MJ, Bottcher J, Selberg O *et al.* Hypermetabolism in clinically stable patients with liver cirrhosis. *Am J Clin Nutr* 1999; **69**: 1194–201.
36 Kawaguchi T, Yoshida T, Harada M *et al.* Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. *Am J Pathol* 2004; **165**: 1499–508.
37 Itou M, Kawaguchi T, Taniguchi E *et al.* Altered Expression of Glucagon-Like Peptide-1 and Dipeptidyl Peptidase IV in Patients with HCV-related Glucose Intolerance. *J Gastroenterol Hepatol* 2008; **23**: 224–51.

doi:10.1111/j.1440-1746.2007.05183.x

HEPATOLOGY

Altered expression of glucagon-like peptide-1 and dipeptidyl peptidase IV in patients with HCV-related glucose intolerance

Minoru Itou,* Takumi Kawaguchi,*† Eitaro Taniguchi,*‡ Shuji Sumie,* Tetsuharu Oriishi,* Keiichi Mitsuyama,* Osamu Tsuruta,* Takato Ueno*‡ and Michio Sata*†‡

Departments of *Medicine and †Digestive Disease Information and Research, Kurume University School of Medicine and ‡Research Center for Innovative Cancer Therapy, and the 21st Century Center of Excellence Program for Medical Science, Kurume University, Kurume, Japan

Key words
dipeptidyl peptidase IV, glucagon-like peptide-1, glucose intolerance, hepatitis C virus, insulin resistance.

Accepted for publication 5 June 2007.

Correspondence
Dr Takumi Kawaguchi, Department of Digestive Disease Information and Research, and Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, 67 Asahi-machi, 830-0011, Japan.
Email: takumi@med.kurume-u.ac.jp

Abstract

Background and Aim: The pathogenesis of hepatitis C virus (HCV)-associated glucose intolerance remains unclear. Glucagon-like peptide-1 (GLP-1), a gut hormone, synthesizes hepatic glycogen and is inactivated by dipeptidyl peptidase IV (DPPIV). The aims of this study were to investigate the alterations in the expression of GLP-1 and DPPIV in HCV-associated glucose intolerance.

Methods: We enrolled patients with HCV- or hepatitis B virus (HBV)-related liver disease ($n = 94$ and 37, respectively), patients with inflammatory bowel disease (IBD; $n = 14$) as disease controls, and healthy controls ($n = 48$). The serum or tissue GLP-1 and DPPIV expression levels were determined by enzyme immunoassay, immunoblotting, or immunostaining. The hepatic glycogen content was assayed by periodic acid–Schiff staining.

Results: The serum GLP-1 levels were significantly decreased in the HCV group (4.9 ± 0.3 ng/mL) than those in the controls (7.5 ± 0.6 ng/mL), the HBV group (7.0 ± 0.5 ng/mL), or the IBD group (10.8 ± 1.0 ng/mL, $P < 0.01$). Although the ileum GLP-1 expression was not significantly different between the controls and the HCV group, the DPPIV expression was significantly increased in the ileum, liver, and serum in the HCV group. Hepatic glycogen content was decreased to a greater extent in the HCV group than that in the HBV group (127.5 ± 5.3 *vs* 187.7 ± 6.6 arbitrary units; $n = 19$, $P < 0.01$).

Conclusion: We demonstrated the altered expressions of GLP-1 and DPPIV in patients with HCV-associated glucose intolerance. Since hepatic glycogen synthesis, a GLP-1 action, was impaired, the altered expressions of GLP-1 and DPPIV may be involved in the development of HCV-associated glucose intolerance.

Introduction

The liver plays crucial roles in glucose metabolism,[1] and chronic liver diseases are associated with glucose intolerance.[2] Several epidemiological studies have revealed an association between hepatitis C virus (HCV) infection and impaired glucose metabolism.[3–5] In patients with HCV infection, glucose intolerance decreases the sustained response rate to antivirus therapy and is an important risk factor for the development of hepatocellular carcinoma as well as long-term survival in patients with cirrhosis.[6–8] Therefore, it is important to investigate the mechanisms for HCV-related glucose intolerance. Increased body mass index (BMI) and decreased insulin secretion are typical characteristics of type 2 diabetes mellitus.[9] In patients with HCV infection, however, glucose intolerance is independent of BMI, and insulin secretion is increased in patients with HCV infection.[3,10] These findings suggest that other factors mediate HCV-related glucose intolerance.

In patients with HCV infection, the homeostasis model assessment method for insulin resistance (HOMA–IR) value is significantly higher than in other hepatobiliary disorders.[1] Recently, we found that the HCV core directly downregulates the intracellular insulin signaling cascade in hepatocytes.[11] Thus, increased hepatic insulin resistance is one of the pathogenesis for HCV-associated glucose intolerance.

The gut has currently been recognized as an endocrine system that contributes to glucose metabolism.[12] Among several gut hormones, glucagon-like peptide-1 (GLP-1) is well known to be involved in glucose metabolism. GLP-1 is secreted from L-cells in the ileum and exerts a direct insulinotropic effect on the pancreatic β-cell.[13,14] GLP-1 activates adenylate cyclase and subsequently enhances insulin secretion from pancreatic β-cells.[15] GLP-1 also has insulin-independent effects on glucose disposal in extra-pancreatic tissues including liver.[16] In hepatocytes, GLP-1 activates glycogen synthesis through activation of glycogen

Journal of Gastroenterology and Hepatology **23** (2008) 244–251

synthesis α.[17] The presence of GLP-1 receptors on the hepatocytes is consistent with the insulin-independent effect of GLP-1.[16] Thus, GLP-1 increases insulin secretion and hepatic glycogen production.

GLP-1 has an extremely short duration of action due to rapid inactivation by the enzyme dipeptidyl peptidase IV (DPPIV, enzyme code number 3.4.14.5).[13,14,18] DPPIV is a membrane-associated peptidase and is widely distributed in numerous tissues. Increased serum DPPIV activity has been reported in diabetic mice and patients with type 2 diabetes mellitus.[19,20] Thus, DPPIV is involved in glucose metabolism through the regulation of GLP-1 activity.

The aim of this study was to investigate alterations of GLP-1 and DPPIV expressions in HCV-associated glucose intolerance.

Materials and methods

Materials

All reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan) unless otherwise indicated.

Patients

In total, 145 patients with liver or bowel disease, HCV-related liver disease (HCV; $n = 94$), hepatitis B virus-related liver disease (HBV; $n = 37$), or inflammatory bowel disease (IBD; $n = 14$), and 48 healthy controls (CON) were enrolled. All of the patients and healthy controls were Asian men. All of the diagnoses were based on clinical, serological, and/or histological evidence. Demographic data were collected on the same day as collecting blood. The BMI was calculated as body weight in kilograms divided by the square of height in meters (kg/m^2). To reduce the selection bias, age, BMI, aspartate aminotransferase (AST), alanine aminotransaminase (ALT), albumin, and total bilirubin were not matched among the groups. Since it is possible that treatments for diabetes mellitus affect GLP-1 levels, patients who had been taking oral hypoglycemic agents or insulin injections were excluded. In addition, patients with a history of and evidence of pancreatitis or a pancreatic tumor were excluded, as were those with other causes of liver disease, in particular those known to be involved in the pathogenesis of diabetes mellitus, such as hemochromatosis or alcoholic liver disease.

The study protocol was approved by the institutional review board, and informed consent for participation in the study was obtained from each patient. None of the patients was institutionalized. The study protocol was approved by the Ethics Committee of the Kurume University School of Medicine. All of the experiments were carried out in accordance with the Declaration of Helsinki.

Laboratory determinations

Venous blood samples were taken in the morning after a 12-h overnight fast. Plasma glucose, serum aspartate, AST, ALT, albumin, total bilirubin, and immunoreactive insulin (IRI) levels were measured using standard clinical methods (Department of Clinical Laboratory, Kurume University Hospital, Kurume, Japan). Insulin resistance and pancreatic β-cell function were calculated on the basis of the fasting levels of plasma glucose and IRI, according to the HOMA equation: Insulin resistance (HOMA–IR) = fasting glucose (mg/dL) × fasting IRI (μU/mL)/405; pancreatic β-cell function (HOMA-β) = fasting IRI (μU/mL) × 360/(fasting glucose (mg/dL) − 63).[21] The stage of liver fibrosis was assessed by AST to the platelet ratio index (APRI): the serum AST level (U/L)/upper limit of normal AST (33 U/L) × 100/platelet count (×10^9/L).[22] APRI is one of the models for predicting the stage of liver fibrosis. Patients with APRI values of 0.5 or less were diagnosed as chronic hepatitis (CH) patients, and patients with APRI values above 0.5 were diagnosed as having liver cirrhosis.[22]

The degree of liver cirrhosis was categorized based on Child–Turcotte criteria.[23]

GLP-1 assay

The quantification of GLP-1 in the serum samples was accomplished by a GLP-1 (7–36) amide enzyme immunoassay (EIA) kit (YK 160; Yanaihara, Fujinomiya, Japan) that specifically quantifies the biologically active form of GLP-1, GLP-1 (7–36) amide.[24] This EIA kit is based on a competitive enzyme immunoassay using a combination of highly specific antibodies to GLP-1 (7–36) amide with a biotin-avidin affinity system. The absorbance (492 nm) of each well was then measured with a Bio-Rad Model 550 microplate reader (Bio-Rad, Hercules, CA, USA). Each serum sample was assayed in duplicate and the values were averaged.

Immunohistochemistry

Frozen samples of the ileum and paraffin-embedded liver sections were deparaffinized and subjected to immunochemical staining using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) with an antihuman GLP-1 antibody (Santa Cruz, Santa Cruz, CA, USA) or an antihuman DPPIV antibody (Santa Cruz, USA). The primary antibodies for GLP-1 or DPPIV were used at a 1:100 dilution. After washing, the sections were incubated with a fluorescein isothiocyanate-conjugated goat antimouse immunoglobulin M (Cappel, Aurora, OH, USA) or tetramethylrhodamine isomer R-conjugated swine antirabbit immunoglobulin (Dako, Kyoto, Japan) diluted at 1:100 in phosphate-buffered saline (PBS). Subsequently, the sections were washed with PBS and mounted in VECTASHIELD (Vector Laboratories, USA).

Immunoblotting liver or ileum specimens

The immunoblotting of DPPIV was performed as previously described.[25] The liver and ileum tissues were dissolved in a minimum volume of radioimmunoprecipitation buffer and were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis on a 10% acrylamide gel. The resolved proteins were transferred electrophoretically to polyvinylidene difluoride membranes (FLUOROTRANS; Pall Life Sciences, Ann Arbor, MI, USA). The membranes were incubated with an anti-DPPIV polyclonal rabbit antibody (Santa Cruz, USA) or an anti-actin rabbit antibody (Sigma, St Louis, MO, USA), and then incubated with horseradish peroxidase-conjugated goat antirabbit immunoglobulin G (Vector Laboratories, USA). The membranes were then incubated with chemiluminescent reagents (ECL kit; GE Health-

Table 1 Baseline characteristics of patients

	CON	HBV	HCV	IBD
No. patients	48	37	94	14
Age (years)	60.1 ± 2.2	54.6 ± 1.4	61.2 ± 1.1*	41.1 ± 3.9
Body mass index (kg/m^2)	22.3 ± 0.3	22.8 ± 0.4	23.1 ± 0.3**	20.2 ± 0.7
Aspartate aminotransferase (U/L)	24.2 ± 1.7	55.6 ± 9.2	69.1 ± 4.0***	21.3 ± 2.8
Alanine aminotransaminase (U/L)	21.1 ± 1.4	56.9 ± 11.2	72.1 ± 4.7***	22.4 ± 3.3
Albumin (g/dL)	4.1 ± 0.1	3.8 ± 0.1	3.7 ± 0.1****	3.9 ± 0.1
Total bilirubin (mg/dL)	0.7 ± 0.1	1.3 ± 0.2	1.1 ± 0.1***	0.6 ± 0.1
Glucose (mg/dL)	89.6 ± 3.9	82.1 ± 2.8	92.4 ± 3.9	82.5 ± 4.2
Immunoreactive insulin (μU/mL)	7.8 ± 1.1	8.3 ± 0.7	17.1 ± 3.4****	9.8 ± 2.5
HOMA-IR	1.7 ± 0.3	1.7 ± 0.2	2.9 ± 0.4*****	2.1 ± 0.6

Note: Data are expressed as mean ± SEM or number of patients. All of the patients were Asian men. *$P < 0.05$ compared to that of hepatitis B virus (HBV) or inflammatory bowel disease (IBD) group; **$P < 0.05$ compared to that of IBD group; ***$P < 0.05$ compared to that of the healthy controls (CON) or IBD groups; ****$P < 0.05$ compared to that of CON group; *****$P < 0.05$ compared to that of the CON or HBV groups. HCV, hepatitis C virus; HOMA-IR, homeostasis model assessment method for insulin resistance. Statistical comparisons among multiple groups were performed by ANOVA followed by Fisher's protected least significant difference.

care, Little Chalfont, Buckinghamshire, UK) and immediately exposed in a LAS-1000 plus lumino-image analyzer (Fuji Photo Film, Tokyo, Japan).

Quantitation

The immunoblotting intensities were determined using NIH-Image J (developed at the National Institutes of Health [NIH], Bethesda, MA, USA) available on the Internet at http://rsb.info.nih.gov/ij/download.html). In preliminary studies, the band intensity showed a linear correlation with the sample concentration over the range analyzed.

Serum DPPIV assay

The quantification of DPPIV in the serum samples was accomplished using a soluble human DPPIV ELISA according to the manufacturer's instructions (Chemicon, Temecula, CA, USA). The absorbance (492 nm) of each well was then measured with a Bio-Rad Model 550 microplate reader (Bio-Rad, USA). Each serum sample was assayed in duplicate and the values were averaged.

Oral glucose tolerance test and insulinogenic index

Twenty-one patients with HCV infection underwent oral glucose tolerance tests (OGTT) with 75 g of glucose according to the recommendations of the National Diabetes Data Group of National Institutes of Health.[26] After overnight fasting, blood samples were drawn for the determination of glucose and IRI at 0, 30, and 120 min after glucose loading. Pancreatic β-cell function was evaluated by the insulinogenic index, which represents the capacity of insulin secretion. The formula is as follows: Insulinogenic index = (30-min IRI − fasting IRI [μU/mL])/(30-min glucose − fasting plasma glucose [mg/dL]).[21] Pancreatic β-cell function was also evaluated by HOMA-β.[21]

Hepatic glycogen content

The liver specimens were stained with periodic acid–Schiff reagent to evaluate the hepatic glycogen content.[27] The hepatic glycogen content was quantitated by the public domain NIH Image-J program by an investigator without any information.

Statistical analysis

All data are expressed as mean ± SEM. Differences between groups were analyzed by the Mann–Whitney *U*-test and the Spearman correlation test. Statistical comparisons among multiple groups were performed by ANOVA followed by Fisher's protected least significant difference using StatView Power PC version for Macintosh (version 5.1; SAS, Cary, NC, USA). *P*-values < 0.05 were considered significant.

Results

Baseline characteristics of all patients

Baseline clinical and laboratory data for all patients and healthy controls are summarized in Table 1. There were no significant differences in age and BMI between the control and HCV groups. Although the HBV group was significantly younger than the HCV group ($P < 0.05$), there were no significant differences in BMI, aspartate aminotransferase, alanine aminotransferase, albumin, and total bilirubin levels between the HBV and HCV groups. Fasting glucose levels were within the normal range in all of the groups and there were no significant differences among the groups (Table 1). However, the fasting insulin levels were significantly increased in the HCV group compared to those of the controls, but were not significantly different compared to the HBV or the IBD groups (Table 1). The HOMA-IR values in the HCV group were significantly higher than those in the controls ($P < 0.05$) or the HBV group ($P < 0.05$) (Table 1).

