associated with glucose intolerance[9–12] and visceral fat accumulation,[13] no previous study has examined the specific relationship between NAFLD pathology and these nutritional parameters.

One aim of the present study was to elucidate whether nutritional status, as estimated by indirect calorimetry, 75-g OGTT and body composition analysis, was related to NAFLD disease progression. The other aim was to elucidate whether these parameters were useful for prediction of the severity of disease.

METHODS

Patients

SUBJECTS WERE 32 patients diagnosed with NAFLD/non-alcoholic steatohepatitis (NASH) by biopsy between April 2009 and March 2011 at our institution. All patients had untreated impaired glucose tolerance (no drug treatment) and present and past alcohol consumption of 20 g or less per week. No patient had been treated with drugs, such as tamoxifen, that can induce NAFLD/NASH. Patients were excluded if they had liver cirrhosis with decreased npRQ accompanied by protein energy malnutrition (PEM).[8] Other exclusion criteria included a history of liver diseases such as primary biliary cirrhosis, autoimmune hepatitis, hepatitis B infection or hepatitis C infection. Hepatocellular carcinoma (HCC) was not detected in any patient.

The study protocol was approved by the institutional review board. Written informed consent was obtained from all patients before trial registration. For all patients, tests were performed in the hospital under resting, fasted conditions in the early morning.

Study design

All subjects were hospitalized for at least 2 days to undergo a liver biopsy. Indirect calorimetry and 75-g OGTT were performed before liver biopsy, as described below. Anthropometric measurements and laboratory analysis were carried out before the indirect calorimetry study. All subjects received nutritional guidance from dieticians and were prescribed medical nutrition therapy (energy 25–30 kcal/kg ideal bodyweight).

Physical examination and serum biochemistry

Anthropometric measurements (body mass index [BMI], body fat percentage, and visceral fat area [VFA]) were performed using a body composition analyzer (InBody 720; BIOSPACE, Tokyo, Japan). We previously determined VFA values using a body composition analyzer and performed abdominal computed tomography using Fat Scan software (E2 system, Osaka, Japan) in 27 NAFLD patients. There was a strong correlation between these two modalities ($n = 27$, $R = 0.9319$, $P < 0.0001$; unpubl. data). Venous blood samples were collected in the early morning after patients had fasted for 12 h. These samples were used for several biochemical tests.

NAFIC score,[14] NAFLD fibrosis score[15] and FIB-4 index[16] were calculated using previously reported formulas.

75-g OGTT

A 75-G OGTT was performed, and plasma glucose and immunoreactive insulin (IRI) were measured at 0, 30, 60, 90 and 120 min after glucose loading. Based on the classification of the Expert Committee on the Diagnosis and Classification of DM,[17] individuals were diagnosed with impaired fasting glucose (IFG) if they had fasting plasma glucose levels of 110 mg/dL or more, but less than 126 mg/dL, and if they had a plasma glucose level less than 140 mg/dL at 120 min after glucose loading. Individuals were diagnosed with impaired glucose tolerance (IGT) if they had plasma glucose levels of less than 110 mg/dL at 0 min after loading and exceeding 140 mg/dL at 120 min after loading. Individuals were diagnosed with diabetes mellitus (DM) if they had plasma glucose levels exceeding 200 mg/dL at 120 min after loading. Homeostasis Model of Assessment – Insulin Resistance (HOMA-IR) was calculated using the following formula:[18] HOMA-IR = fasting insulin (mU/mL) × plasma glucose (mg/dL) / 405. Plasma glucose area under the curve (AUC glucose) and IRI area under the curve (AUC IRI) were calculated using methods reported previously.[19]

Indirect calorimetry

Energy metabolism was measured by indirect calorimetry (Aero Monitor AE-300s; Minato Medical Science, Osaka, Japan). A previously reported method[19] was used to measure oxygen uptake and carbon dioxide exhalation under resting, fasted conditions in the early morning. Twenty-four-hour urine nitrogen levels were also measured. The resulting values were used to calculate npRQ and resting energy expenditure (REE). The basal metabolic rate (BMR) was calculated using the Harris–Benedict formula.[20]

Pathology

All samples were diagnosed by a pathologist who was not notified of subjects' clinical data or course. The

classification of Brunt *et al.*[21] was used for fibrosis staging, and disease activity was assessed using the NAFLD activity score (NAS).[22]

Statistical analyses

Statistical analysis was performed using SPSS ver. 20.0 software (SPSS, Chicago, IL, USA). Results were expressed as mean ±standard deviation or standard error of the mean. A χ^2-test was used for categorical variables. A Student's *t*-test or Mann–Whitney *U*-test was used to compare two groups. One-way ANOVA or Kruskal–Wallis analysis followed by a post-hoc test was used to compare multiple independent groups. Correlation was assessed using Spearman's correlation coefficient. Receiver–operator curves (ROC) were used to assess discrimination ability. Statistical significance was defined as $P < 0.05$.

RESULTS

Patients

THE CLINICAL AND biochemical characteristics of patients enrolled in the study are summarized in Table 1. The 32 subjects (24 male, eight female) had a mean age of 45.4 years (range, 27–75). BMI ranged 22.0–38.8 kg/m^2 and averaged 27.2 kg/m^2. Serum alanine aminotransferase (ALT) levels ranged 22–200 IU/L and averaged 95.6 IU/L. Histological findings are shown also in Table 1. Fibrosis stages were determined according to Brunt *et al.*'s classification,[21] and there were eight patients at stage 0, 10 patients at stage 1, seven patients at stage 2 and seven patients at stage 3. For NAS, there were six patients with scores of less than 3, 20 patients with scores of 3 or 4, and six patients with scores of 5 or more.

Anthropometric measurements

Body mass index, body fat percentage and VFA tended to increase as fibrosis progressed. However, there was no significant difference in these parameters among groups with different Brunt stages (Table 2).

75-g OGTT

Four patients (12.5%) had HbA1c levels of at least 6.1% or fasting glucose of at least 110 mg/dL and in whom impaired glucose tolerance was suspected before the 75-g OGTT. The 75-g OGTT did not reveal a normal glucose tolerance pattern in any patient, and all patients had impaired glucose metabolism. One patient (3.1%) had IFG, 16 patients (50.0%) had IGT and 15 patients (46.9%) had DM.

Table 1 Characteristics of the patient population ($n = 32$)

Variable	
Sex (male/female)	24/8
Age (years)	45.4 ± 12.2
BMI (kg/m^2)	27.2 ± 4.0
AST (IU/L)	65.6 ± 73.4
ALT (IU/L)	95.6 ± 62.7
γ-GT (IU/L)	91.3 ± 76.8
Total cholesterol (mg/dL)	206.7 ± 44.7
Triglyceride (mg/dL)	155.4 ± 85.2
NEFA (μEq/L)	552.1 ± 212.0
Albumin (g/dL)	4.4 ± 0.5
Prothrombin time (%)	104.6 ± 11.2
Platelet count (X 10^4/μL)	23.7 ± 6.2
P-III-P (U/mL)	0.64 ± 0.28
Type IV collagen 7S (ng/mL)	4.4 ± 2.2
Fasting glucose (mg/dL)	94.4 ± 15.6
75-g oral glucose tolerance test	
Normal/IFG/ IGT/DM	0/1/16/15
Histological assessment	
Stage (0/1/2/3)†	8/10/7/7
Grade (0/1/2/3)†	8/6/14/4
NAS (<3/ 3,4/ ≥5)	6/20/6

Results are expressed as mean ± standard deviation.
†Stage and grade on histological assessment were determined using Brunt's classification.[21]
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DM, diabetes mellitus; γ-GT, γ-glutamyl transferase; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NEFA, non-esterified fatty acid.

Fasting glucose, HbA1c and AUC glucose increased as fibrosis progressed, and glucose metabolism was significantly worsened with fibrosis progression (Table 2). The 75-g OGTT revealed a correlation between postprandial hyperglycemia and Brunt stage (Fig. 1a). There were significant differences among fibrosis stages in plasma glucose levels at 0, 30 and 120 min after loading ($P < 0.05$ using Kruskal–Wallis analysis).

In patients with fibrosis stages 1–3, fasting insulin levels were increased and HOMA-IR was elevated, indicating the presence of insulin resistance. This tendency was significantly more pronounced in more advanced fibrosis stages (Table 2). In the 75-g OGTT (Fig. 1b), insulin secretion was delayed and postprandial hyperinsulinemia was observed for all stages compared with healthy controls, as previously reported.[23] In particular, stage 3 patients had significantly greater hyperinsulinemia than patients at the other stages at 0, 90 and 120 min after loading ($P < 0.05$ using Kruskal–Wallis analysis).

Table 2 Clinical features and laboratory data of NAFLD/NASH patients determined using Brunt *et al.*'s classification[21]

Variable	Stage 0 ($n = 8$)	Stage 1 ($n = 10$)	Stage 2 ($n = 7$)	Stage 3 ($n = 7$)	*P*-value†
Sex (male/female)	5/3	8/2	5/2	6/1	0.7348
Age (years)	46.1 ± 11.4	40.3 ± 13.0	46.3 ± 11.9	50.8 ± 11.8	0.3713
BMI (kg/m^2)	25.1 ± 1.9	27.2 ± 5.9	27.6 ± 2.2	29.1 ± 3.1	0.2714
Percent body fat (%)	30.3 ± 6.8	28.7 ± 8.4	33.6 ± 6.7	33.2 ± 7.1	0.5082
VFA (cm^2)	116.7 ± 22.3	122.3 ± 47.5	141.4 ± 26.7	163.5 ± 46.8	0.1029
AST (IU/L)	29.8 ± 5.4	48.6 ± 22.6	78.7 ± 35.5	117.7 ± 142.3	<0.01
ALT (IU/L)	43.3 ± 26.7	91.5 ± 51.8	131.0 ± 43.2	125.7 ± 86.0	<0.05
γ-GT (IU/L)	74.4 ± 29.1	85.1 ± 73.9	75.7 ± 45.1	134.9 ± 127.6	0.5789
Total cholesterol (mg/dL)	200.6 ± 23.0	214.8 ± 59.5	200.9 ± 30.0	188.0 ± 52.1	0.5131
Triglyceride (mg/dL)	116.8 ± 6.5	181.9 ± 109.3	170.8 ± 87.2	145.1 ± 75.6	0.5364
NEFA (μEq/L)	494.0 ± 231.9	571.8 ± 285.0	619.2 ± 31.0	532.9 ± 171.4	0.8629
Type IV collagen 7S (ng/mL)	3.51 ± 0.71	3.46 ± 0.98	3.82 ± 0.54	6.94 ± 3.12	<0.05
Ferritin (ng/mL)	157.0 ± 45.9	225.1 ± 208.9	178.4 ± 67.9	474.4 ± 578.5	<0.05
Fasting glucose (mg/dL)	85.5 ± 8.69	88.6 ± 7.71	109.7 ± 21.5	110.4 ± 34.7	<0.05
Fasting insulin (μU/mL)	8.00 ± 3.62	11.3 ± 4.81	15.2 ± 10.8	26.1 ± 15.3	<0.005
HOMA-IR	1.70 ± 0.80	2.46 ± 1.19	4.11 ± 3.03	6.56 ± 2.78	<0.005
Hemoglobin A1c (%)	5.61 ± 0.55	5.41 ± 0.38	5.97 ± 0.89	6.74 ± 1.75	<0.05
75-g OGTT					
Normal/IFG/IGT/DM	0/0/6/2	0/1/6/3	0/0/4/3	0/0/0/7	
AUC glucose (mg·h/dL)	363.8 ± 64.9	406.4 ± 87.2	450.6 ± 39.0	497.1 ± 45.8	<0.005
AUC IRI (μU·h/mL)	247.4 ± 130.6	247.7 ± 99.1	238.54 ± 96.2	536.2 ± 305.1	<0.05
Indirect calorimetry					
npRQ	0.895 ± 0.068	0.869 ± 0.067	0.808 ± 0.046	0.798 ± 0.026	<0.005
REE/BMR	0.922 ± 0.091	0.922 ± 0.160	1.034 ± 0.069	1.021 ± 0.073	0.2495
NAFIC score[14]	0.125 ± 0.354	0.600 ± 0.699	0.857 ± 0.378	2.143 ± 0.900	<0.0005
NAFLD fibrosis score[15]	−2.77 ± 1.13	−2.55 ± 1.08	−2.06 ± 0.69	−0.73 ± 1.92	<0.05
FIB-4 index[16]	0.880 ± 0.331	0.952 ± 0.472	1.326 ± 0.674	2.564 ± 2.391	<0.05

Results are expressed as mean ± standard deviation.
†*P*-value for four-group comparisons.
Differences and correlations among the four groups were determined using one-way ANOVA or Kruskal–Wallis analysis followed by a post-hoc test.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC glucose, plasma glucose area under the curve; AUC IRI, immunoreactive insulin area under the curve; BMI, body mass index; BMR, basal metabolic rate; DM, diabetes mellitus; γ-GT, γ-glutamyl transferase; HOMA-IR, homeostasis model assessment of insulin resistance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NEFA, non-esterified fatty acid; npRQ, non protein respiratory quotient; 75-g OGTT, 75-g oral glucose tolerance test; REE, resting energy expenditure; VFA, visceral fat area.

Indirect calorimetry

Non-protein respiratory quotient values determined using indirect calorimetry data significantly decreased as fibrosis progressed (Table 2, Fig. 2a). In addition, npRQ values significantly decreased as NAS increased (Fig. 2b). There was no relationship between npRQ and disease activity (Fig. 2c).

Resting energy expenditure and BMR predicted using the Harris–Benedict formula[20] did not differ among Brunt stages or NAS classifications (data not shown). The ratio of REE to BMR (REE/BMR) also did not differ among Brunt stages (Table 2) or NAS classifications (data not shown). In addition, this ratio was within the normal range ($0.9 < \text{REE/BMR} < 1.1$),[8] indicating that most subjects were in normal metabolic states, and not hyper- or hypometabolic states.

Blood biochemistry

There were significant differences among stages in AST, ALT, type IV collagen 7S and ferritin levels (Table 2). However, there were no significant differences among stages with respect to other parameters, including γ-glutamyl transferase (γ-GT), total cholesterol, triglyceride and non-esterified fatty acid (NEFA) (Table 2),

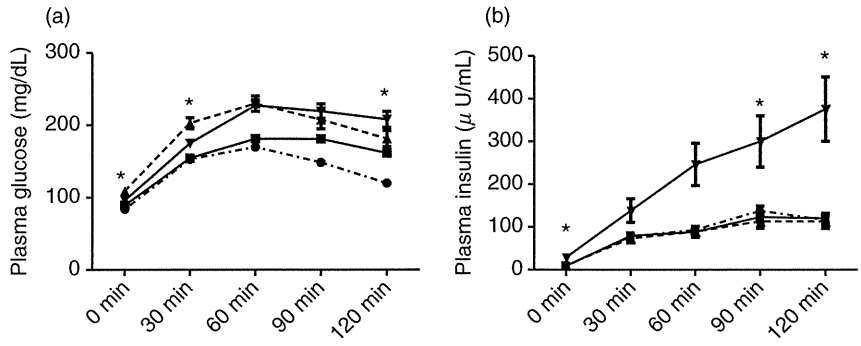

Figure 1 (a) Serum glucose levels during a 75-g oral glucose tolerance test in non-alcoholic fatty liver disease (NAFLD) patients. *There were significant differences among stages in plasma glucose levels at 0, 30 and 120 min ($P < 0.05$) after loading as determined by Kruskal–Wallis analysis. (b) Serum insulin levels during a 75-g oral glucose tolerance test in NAFLD patients. *There were significant differences among stages in plasma glucose levels at 0, 90 and 120 min ($P < 0.05$) after loading as determined by Kruskal–Wallis analysis. Results are expressed as mean ± standard error of the mean. ·●·, Stage 0; —■—, stage 1; -▲-, stage 2; —▼—, stage 3.

AST/ALT ratio, hyaluronic acid, platelet count and prothrombin time (data not shown).

Comparison of patients with mild fibrosis (stages 0–1) and patients with more advanced fibrosis (stages 2–3)

To identify factors correlated with fibrosis in NAFLD patients, we divided subjects into two groups – those with mild fibrosis (stages 0–1) and those with more advanced fibrosis (stages 2–3) – and compared clinical features.

Patients with more advanced fibrosis had significantly higher values than patients with mild fibrosis for the following parameters: VFA, serum AST, ALT, P-III-P, type IV collagen 7S, fasting glucose, fasting insulin, HOMA-IR, HbA1c and AUC glucose (Table 3). Patients having more advanced fibrosis had significantly lower npRQ values than patients with mild fibrosis (Table 3). There were no significant differences between the two groups with respect to other parameters, including γ-GT, total cholesterol, triglyceride and NEFA (data not shown).

Correlation of npRQ with parameters of glucose and fat metabolism and body composition

We next compared npRQ to parameters of glucose and fat metabolism and body composition. There was a negative correlation between npRQ and AUC glucose ($R = -0.6308$, $P < 0.001$ using Spearman's correlation coefficient) (Fig. 3a). There was also a negative correla-

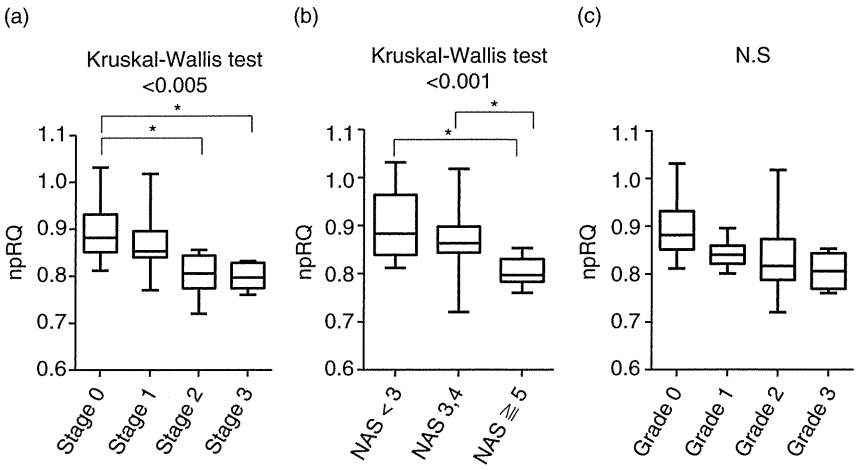

Figure 2 Non-protein respiratory quotient (npRQ) in non-alcoholic fatty liver disease (NAFLD) patients. (a) By stage. (b) By NAFLD activity score (NAS). (c) By grade. There was a significant difference in npRQ among (a) stages and (b) NAS. *Post-hoc test showed a significant difference ($P < 0.05$). N.S., not significant.

Table 3 Comparison of clinical features and laboratory data between stages 0–1 versus stages 2–3 in NAFLD/NASH patients

	Stages 0–1 ($n = 18$)	Stages 2–3 ($n = 14$)	*P*-value†
Sex (male/female)	13/5	11/3	0.6807
Age (years)	42.9 ± 12.35	48.6 ± 11.6	0.1947
BMI (kg/m^2)	26.3 ± 4.6	28.4 ± 2.8	0.1419
Percent body fat (%)	29.4 ± 7.6	33.4 ± 6.7	0.1398
VFA (cm^2)	119.7 ± 36.8	153.3 ± 39.0	<0.05
AST (IU/L)	40.2 ± 19.4	98.2 ± 101.7	<0.05
ALT (IU/L)	70.1 ± 48.2	128.4 ± 65.5	<0.01
P-III-P (U/mL)	0.53 ± 0.10	0.81 ± 0.38	<0.01
Type IV collagen 7S (ng/mL)	3.48 ± 0.84	5.64 ± 2.83	<0.005
Fasting glucose (mg/dL)	87.2 ± 8.1	104.3 ± 18.2	<0.005
Fasting insulin (μU/mL)	9.9 ± 4.5	21.3 ± 14.2	<0.005
HOMA-IR	2.1 ± 1.1	5.2 ± 3.1	<0.0005
Hemoglobin A1c (%)	5.5 ± 0.5	6.0 ± 0.7	<0.05
75-g OGTT			
Normal/IFG/IGT/DM	0/1/12/5	0/0/4/10	
AUC glucose (mg·h/dL)	379.4 ± 64.2	473.4 ± 49.4	<0.0005
AUC IRI (μU·h/mL)	254.2 ± 100.6	371.8 ± 261.6	0.1448
Indirect calorimetry			
npRQ	0.881 ± 0.067	0.803 ± 0.036	<0.0005

Results are expressed as mean ± SD.
†*P*-value for two comparisons.
Differences between two groups were determined using Student's *t*-test, Mann-Whitney's *U*-test, or chi-square test.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC glucose, blood glucose area under the curve; AUC IRI, immunoreactive insulin area under the curve; BMI, body mass index; DM, diabetes mellitus; γ-GT, γ-glutamyl transferase; HOMA-IR, Homeostasis Model of Assessment – Insulin Resistance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; npRQ, non-protein respiratory quotient; 75-g OGTT, 75-g oral glucose tolerance test; VFA, visceral fat area.

tion between npRQ and HOMA-IR ($R = -0.5045$, $P < 0.005$) (Fig. 3b). A weak negative correlation was found between npRQ and fasting glucose ($R = -0.4585$, $P < 0.01$) (Fig. 3c), fasting insulin ($R = -0.4431$, $P < 0.05$) (Fig. 3d), γ-GT ($R = -0.4428$, $P < 0.05$), plasma glucose levels 120 min after loading ($R = -0.3684$, $P < 0.05$) and IRI levels 120 min after loading in the OGTT ($R = -0.3772$, $P < 0.05$). No significant correlation was found between npRQ and AUC IRI ($R = -0.2992$, $P = 0.1021$), total cholesterol ($R = -0.2499$, $P = 0.1678$), triglyceride ($R = -0.0617$, $P = 0.7599$), NEFA ($R = -0.0629$ $P = 0.7367$), BMI ($R = -0.3165$, $P = 0.0776$), body fat percentage ($R = -0.1233$, $P = 0.5088$) or VFA ($R = -0.2308$, $P = 0.2199$). Based on these results, we speculated that low npRQ in NAFLD is associated with impaired glucose tolerance due to insulin resistance.

Comparison of npRQ to several parameters and previously established scoring systems

We calculated area under the ROC (AUROC) for npRQ and for several of the parameters shown in Table 2. We compared these AUROC to see if they could differentiate stage 3 from stages 0–2, stages 2–3 from stages 0–1, and stage 0 from stages 1–3. Table 4 summarizes these results. For differentiation of stages 3 from stages 0–2, the calculated AUROC was greatest for NAFIC score (0.9200), followed by HOMA-IR (0.9100), type IV collagen 7S (0.8820), AUC glucose and fasting insulin (0.8743), ferritin (0.8690) and npRQ (0.8343). For differentiation of stages 2–3 from stages 0–1, the AUROC for npRQ was greatest (0.8849), followed by HOMA-IR (0.8846), AUC glucose (0.8690), fasting glucose (0.8651), NAFIC score (0.8373) and fasting insulin (0.8234). For differentiation of stage 0 from stages 1–3, the AUROC for ALT was greatest (0.8568), followed by AST (0.8542), fasting insulin (0.8490), HOMA-IR and AUC glucose (both 0.8478), NAFIC score (0.8281) and npRQ (0.8203).

In each of the three comparisons of stages, AUROC for npRQ, HOMA-IR, AUC glucose, fasting insulin and NAFIC score were all over 0.8000 and showed relatively good results. To differentiate stage 3 from stages 0–2, the AUROC for type IV collagen 7S and ferritin were

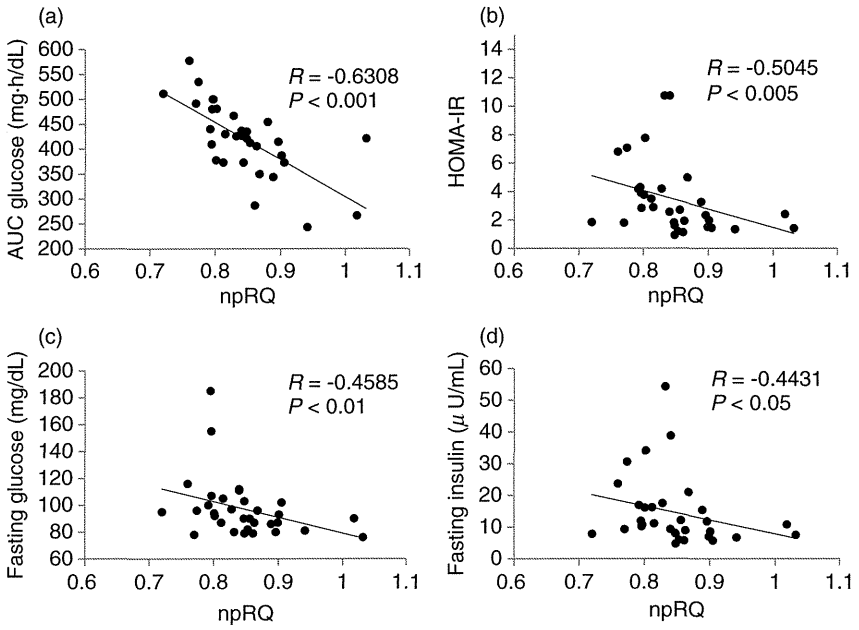

Figure 3 Correlation between non-protein respiratory quotient (npRQ) and other parameters ($n = 32$). (a) With area under the curve (AUC) glucose. (b) With Homeostasis Model of Assessment – Insulin Resistance. (c) With fasting glucose. (d) With fasting insulin.

Table 4 AUROC for npRQ, other biochemical parameters and scoring systems for NAFLD/NASH patients

Variable	AUROC Stage 0 vs. Stages 1–3	AUROC Stages 0–1 vs. Stages 2–3	AUROC Stages 0–2 vs. Stage 3
npRQ	0.8203	0.8849	0.8343
HOMA-IR	0.8478	0.8846	0.9100
AUC glucose	0.8478	0.8690	0.8743
AUC IRI	0.5924	0.6154	0.8133
Fasting glucose	0.7813	0.8651	0.7143
Fasting insulin	0.8490	0.8234	0.8743
Hemoglobin A1c	0.5617	0.7668	0.8080
Type IV collagen 7S	0.5893	0.7870	0.8820
NAFIC score[14]	0.8281	0.8373	0.9200
NAFLD fibrosis score[15]	0.6615	0.7460	0.8057
FIB-4 index[16]	0.6250	0.7222	0.7143
AST	0.8542	0.7976	0.6514
ALT	0.8568	0.7778	0.6343
Ferritin	0.5893	0.6912	0.8690

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC glucose, plasma glucose area under the curve; AUC IRI, immunoreactive insulin area under the curve; AUROC, area under the receiver–operator curve; HOMA-IR, homeostasis model assessment of insulin resistance; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; npRQ, non-protein respiratory quotient.

high; however, AUROC for these parameters were not able to differentiate stage 0 from stages 1–3. This is due to the fact that these two parameters had elevated values in stage 3 and there was no significant difference from stage 0 to stage 2. The AUROC for NAFLD fibrosis score and FIB-4 index were lowest for differentiation of stage 0 from stages 1–3, and increased for differentiation of stages 2–3 from stages 0–1 and differentiation of stage 3 from stages 0–2. This result suggests that these two methods of scoring fibrosis had a relatively high degree of accuracy in distinguishing severe from mild or no fibrosis. AUROC for AST and ALT could be used to differentiate stage 0 from stages 1–3, but were not as accurate in differentiating stage 3 from stages 0–2.

DISCUSSION

NON-ALCOHOLIC FATTY LIVER disease comprises a wide spectrum of conditions ranging from simple steatosis to NASH, which can progress to cirrhosis and HCC. Patients with advanced liver fibrosis are considered to be at high risk for liver failure and HCC.[1–6] Thus, it is important to efficiently identify patients at risk for advanced fibrosis among a large number of NAFLD patients. In addition, differentiation of early-stage NASH allows for early intervention, which can improve patients' outcomes. Liver biopsy is the most reliable method for the diagnosis and determination of fibrosis stage in patients with NASH. However, it is

widely acknowledged that biopsy is costly and runs the risk of sampling error and procedure-related morbidity and mortality. Some guidelines recommend that liver biopsy should be considered in patients who are at risk for NASH with advanced fibrosis,[4–6] but this recommendation is not universally accepted. Therefore, various parameters have been proposed as tools to distinguish NASH from NAFLD, or to determine fibrosis stage. Various serum biochemical markers, including indicators of oxidative stress, insulin resistance, inflammation, and apoptosis, have been used for this purpose.[1–6]

With regard to glucose metabolism and insulin resistance, a 75-g OGTT may help clinicians to identify high-risk patients for more intensive monitoring and treatment because blood glucose and insulin levels in the OGTT are important factors for the diagnosis of NAFLD and prediction of fibrosis.[11,12,24–26] Studies in which a 75-g OGTT was performed have shown that impaired glucose tolerance is common even in NAFLD patients without overt DM,[11,12,24–26] and that postprandial hyperglycemia is associated with advanced fibrosis.[12,24,26] Postprandial hyperinsulinemia is also observed in nearly all NAFLD patients, even those with normal glucose tolerance.[11,12] Kimura *et al.*[11] reported that postprandial hyperinsulinemia, as indicated by an OGTT, became more marked as fibrosis stage advanced.

Indirect calorimetry provides important information about energy expenditure, npRQ, and the rate of oxidation of three major macronutrients (carbohydrates, fat and protein) based on respiratory gas exchange and urinary nitrogen excretion. Indirect calorimetry is considered the gold standard for assessing energy expenditure and aids in the delivery of the highest quality of nutritional care.[7] The advantages of this modality are that it is non-invasive, portable enough to be done at bedside, easy to operate and inexpensive.[7] Many studies have used this modality to estimate the nutritional state of cirrhotic patients with chronic liver disease. Tajika *et al.*[8] have reported that low npRQ derived from PEM is associated with survival in patients with viral liver cirrhosis. We have previously used indirect calorimetry in cirrhotic patients to evaluate the effects of nutritional treatment with branched-chain amino acids.[19,27–29] In diabetic patients, indirect calorimetry is often used to estimate glucose oxidation rate and it has been reported that both glucose oxidation and non-oxidative disposal are impaired during hyperinsulinemic clamping in type 2 DM patients.[30] Although indirect calorimetry is used for assessment in several metabolic diseases, this is the first report to examine indirect calorimetry data from patients with NAFLD. The objective of the present study was to determine how energy metabolism, as estimated by indirect calorimetry, is related to the clinicopathogenesis of NAFLD with glucose intolerance.

We found that npRQ decreased in severity with increased fibrosis stage in NAFLD patients. This observation raised the question of the mechanism underlying decreased npRQ in patients with advanced fibrosis. As fibrosis progressed, npRQ decreased significantly, glucose intolerance worsened and insulin resistance increased (Tables 2,3). In fact, negative correlations were seen between npRQ and several parameters of glucose intolerance: AUC glucose, HOMA-IR, and fasting glucose and insulin levels. Thus, we speculated that decreased npRQ in NAFLD results from glucose intolerance due to insulin resistance, which worsened with fibrosis stage. Decreased npRQ can reflect reduced glucose oxidation and enhanced lipid oxidation.[8,19,27–29] It was reported that peripheral insulin resistance reduces glucose oxidation and glucose uptake in peripheral skeletal muscle.[30] This reduction in glucose uptake may reflect hyperglycemia and decreased glucose oxidation, because the amount of free cellular glucose available for oxidation is reduced.[31] However, it was also speculated that the low glucose oxidation rate seen in viral cirrhosis is a result of reduced glucose production due to decreased hepatic glycogen.[32] In our study, glycogen levels in the liver were not measured directly and thus a definitive statement cannot be made. However, npRQ was low even in the one patient with mild (stage 1) fibrosis, who was found not to be in a state of malnutrition as determined by anthropometry and in whom glycogen storage was likely not decreased to a large extent. Therefore, it is unlikely that the low npRQ in these patients primarily reflects decreased glycogen stores. However, we do not suggest that low npRQ in NAFLD patients is solely due to glucose intolerance because whole-body energy metabolism is a complex process, and thus it is possible that other factors also contribute to low npRQ.[30] Yokoyama *et al.*[33] have reported that the glucose oxidation rate of subjects with type 2 diabetes is inversely correlated with BMI, body fat percentage and plasma fatty acid levels, suggesting that decreased glucose oxidation and increased fat oxidation may be potently affected by adiposity. In our study, npRQ showed no correlation with lipid parameters, including serum total cholesterol, triglyceride and NEFA, or anthropometric parameters such as body fat percentage and VFA. Thus, low npRQ was speculated to be associated with decreased glucose oxidation due to glucose intolerance, but not with increased fat oxidation, which occurs in the maintenance and development

of hyperglycemia during decompensation.[30] Decreased npRQ was correlated with glucose intolerance and fibrosis stage, suggesting that the clinicopathogenesis of NAFLD is closely associated with glucose intolerance, and that early intervention for glucose intolerance is important in clinical practice.

To examine the utility of npRQ as a marker of disease progression in NAFLD, we compared AUROC for npRQ with those for various other parameters and scoring systems in three patterns to discriminate NASH from NAFLD (stage 0 vs stages 1–3), significant fibrosis (stages 0–1 vs stages 2–3) and advanced fibrosis (stages 0–2 vs stage 3). As the decrease in npRQ became significant at stage 2 (Fig. 2a), the AUROC for npRQ for differentiation of stages 2–3 from stages 0–1 was superior to other parameters. Therefore, our results indicate that decreased npRQ can be used to detect NASH, including relatively early stage NASH in many NAFLD patients. In addition to npRQ, AUROC for HOMA-IR, AUC glucose, fasting insulin and NAFIC score were each approximately 0.850 and also showed differences for each of the three differentiation patterns. Therefore, these parameters also have the ability to detect NASH from the early stages to the development of severe fibrosis. NAFIC score, the scoring system for fibrosis proposed by Sumida *et al.*,[14] comprises three measurements (serum ferritin, insulin and type IV collagen 7S) and is easy to calculate. A validation study by the Japan Study Group of NAFLD (JSG-NAFLD) reported that NAFIC score was superior to other several previously established scoring systems in detecting NASH with fibrosis among Japanese NAFLD patients, and also for predicting severe fibrosis.[14] In the present study, AUROC for NAFIC score for differentiation of stage 3 from stages 0–2 was 0.9200 and was the highest among the various parameters, supporting the conclusions of the JSG-NAFLD report.[14] NAFLD fibrosis score,[15] which consists of six variables (age, BMI, hyperglycemia, platelet count, albumin and AST/ALT ratio) has been reported to reliably predict advanced fibrosis. In a meta-analysis by Angulo *et al.*,[15] NAFLD fibrosis score had an AUROC of 0.85 for predicting advanced fibrosis (stages 3–4). The fact that subjects with stage 4 were excluded from the present study but were included as "advanced stage" in previously reported studies,[14,15,34] may also contribute to the lower value of AUROC in the present study. In addition, it is uncertain whether a scoring system established using data from Caucasian populations is applicable to Asian patients, because Asian patients tend to develop NASH and other metabolic complications at a lower BMI than Caucasians.[3] The FIB-4 index was developed as a scoring system for estimation of liver fibrosis in subjects with HIV and hepatitis C virus co-infection.[16] It relies on patient age, AST, ALT and platelet count. The advantage of FIB-4 index is that it is easy to calculate and does not require the use of BMI. In a validation study of JSG-NAFLD,[34] FIB-4 index was superior to other fibrosis scoring systems in Japanese NAFLD patients for excluding advanced fibrosis. In our study, the AUROC for FIB-4 index for discrimination of stage 3 from stages 0–2 was not satisfied (0.7143) and was inferior to NAFIC score. As described above, the lower AUROC for FIB-4 index than in previous reports[34] may be due to the fact that stage 4 patients were excluded from the present study, but were included in previous reports. As the number of subjects in the present study was small, we cannot conclude which parameters and scoring system is best for the estimation of severity of NAFLD, nor we can definitively state that npRQ is the best method for differentiation of NASH from NAFLD. However, Table 4 shows that npRQ, HOMA-IR, AUC glucose, fasting glucose, insulin and NAFIC scores all could provide useful information for the detection of NASH, including patients in the early fibrosis stage, whereas NAFLD fibrosis score and type IV collagen were useful for identification of advanced fibrosis. It is important to select these parameters and scoring systems according to the purpose: to differentiate early-stage NASH from NAFLD, or to detect advanced fibrosis.

Although we believed that npRQ is useful for the estimation of disease severity in NAFLD patients, unfortunately, npRQ measurement is not always possible in clinical practice. This is because indirect calorimetry for measurement of npRQ was primarily used in inpatient and research settings[7] and not all clinicians are familiar with the equipment used. In addition, patients must be tested in the early morning after fasting, and 24-h urine specimens must be collected for calculation of npRQ. Okumura *et al.*[35] investigated the use of serum biochemistry to predict npRQ in patients with viral cirrhosis. That report concluded that serum NEFA can be used to predict npRQ. However, in the present study, npRQ was not correlated with NEFA. This may be because the subjects in the present study were NAFLD patients without cirrhosis, and not viral cirrhosis patients with decreased glycogen storage.[35] In NAFLD patients with glucose intolerance, HOMA-IR may be useful for prediction of npRQ without calorimetry, because these two parameters were negatively correlated.

In conclusion, npRQ is useful for the estimation of disease severity in NAFLD patients with glucose intolerance. It enables the detection of NASH with relatively

early-stage fibrosis among NAFLD patients. As this measurement can provide useful information without the burden of blood collection, it should be included in clinical practice in addition to anthropometry and blood analysis. When an NAFLD patient exhibits low npRQ, the patient should undergo further examination such as a liver biopsy to allow for early intervention.

REFERENCES

1 Okanoue T, Umemura A, Yasui K, Itoh Y. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in Japan. *J Gastroenterol Hepatol* 2011; **26** (Suppl 1): 153–62.
2 Hashimoto E, Tokushige K. Prevalence, gender, ethnic variations, and prognosis of NASH. *J Gastroenterol* 2011; **46**: 63–6.
3 Sumida Y, Eguchi Y, Ono M. Current status and agenda in the diagnosis of nonalcoholic steatohepatitis in Japan. *World J Hepatol* 2010; **2**: 374–83.
4 Chalasani N, Younossi Z, Lavine JE *et al.* The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005–23.
5 Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on EASL 2009 special conference. *J Hepatol* 2010; **53**: 372–84.
6 Chitturi S, Farrell GC, Hashimoto E, Saibara T, Lau GK, Sollano JD. Guidlines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pasific region: executive summary. *J Gastroenterol Hepatol* 2007; **22**: 775–77.
7 Haugen HA, Chan LN, Li F. Indirect calorimetry: a practical guide for clinicians. *Nutr Clin Pract* 2007; **22**: 377–88.
8 Tajika M, Kato M, Mohri H *et al.* Prognostic value of energy metabolism in patients with vial liver cirrhosis. *Nutrition* 2002; **18**: 229–34.
9 Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999; **30**: 1356–62.
10 Miyaaki H, Ichikawa T, Nakao K *et al.* Clinicopathological study of nonalcoholic fatty liver disease in Japan: the risk factors for fibrosis. *Liver Int* 2008; **28**: 519–24.
11 Kimura Y, Hyogo H, Ishitobi T, Nabeshima Y, Arihiro K, Chayama K. Postprandial insulin secretion pattern is associated with histological severity in non-alcoholic fatty liver disease patients without prior known diabetes mellitus. *J Gastroenterol Hepatol* 2011; **26**: 517–22.
12 Manchanayake J, Chitturi S, Nolan C, Farell GC. Postparndial hyperinsulinemia is universal in non-diabetic patients with nonalcoholic fatty liver disease. *J Gastroenterol Hepatol* 2011; **26**: 510–6.
13 Eguchi Y, Mizuta T, Sumida Y *et al.* The pathological role of visceral fat accumulation in steatosis, inflammation, and progression of nonalcoholic fatty liver disease. *J Gastroenterol* 2011; **46**: 70–8.
14 Sumida Y, Yoneda M, Hyogo H *et al.* A simple clinical scoring system using ferritin, fasting insulin, and type IV collagen 7S for predicting steatohepatitis in nonalcoholic fatty liver disease. *J Gastroenterol* 2011; **46**: 257–68.
15 Angulo P, Hui JM, Marchesini G *et al.* The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846–54.
16 Sterling RK, Lissen E, Clumeck N *et al.* Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317–25.
17 Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. *Diabet Med* 1998; **15**: 539–53.
18 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia* 1985; **28**: 412–9.
19 Korenaga K, Korenaga M, Uchida K, Yamasaki T, Sakaida I. Effects of a late evening snack combined with alpha-glucosidase inhibitor on liver cirrhosis. *Hepatol Res* 2008; **38**: 1087–97.
20 Harris JA, Benedict FG. A biometric study of basal metabolism in man. *Carnegie Inst* 1919; **279** (3): 48–9.
21 Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Non-alcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467–74.
22 Kleiner DE, Brunt EM, Van Natta M *et al.* Design and validation of a histological scoring system for nonalchoholic fatty liver disease. *Hepatology* 2005; **41**: 1313–21.
23 Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999; **22**: 1462–70.
24 Shiga T, Moriyoshi Y, Nagahara H, Shiratori K. Nonalcoholic fatty liver is a risk factor for postprandial hyperglycemia, but not for impaired fasting glucose. *J Gastroenterol* 2009; **44**: 757–64.
25 Haukeland JW, Konopski Z, Linnestad P *et al.* Abnormal glucose tolerance is a predictor of steatohepatitis and fibrosis in patients with non-alcoholic fatty liver disease. *Scand J Gastroenterol* 2005; **40**: 1469–77.
26 Wong VW, Hui AY, Tsang SW *et al.* Prevalence of undiagnosed diabetes and postchallenge hyperglycemia in Chinese patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2006; **24**: 1215–22.

27 Urata Y, Okita K, Korenaga K, Uchida K, Yamasaki T, Sakaida I. The effect of supplementation with branched-chain amino acids in patients with liver cirrhosis. *Hepatol Res* 2007; **37:** 510–6.
28 Tsuchiya M, Sakaida I, Okamoto M, Okita K. The effect of a late evening snack in patients with liver cirrhosis. *Hepatol Res* 2005; **31:** 95–103.
29 Okamoto M, Sakaida I, Tsuchiya M, Suzuki C, Okita K. Effect of late evening snack on the blood glucose level and energy metabolism in patients with cirrhosis. *Hepatol Res* 2003; **27:** 45–50.
30 Vaag A, Alford F, Henriksen FL, Christopher M, Beck-Nielsen H. Multiple defects of both hepatic and peripheral intracellular glucose processing contribute to the hyperglycemia of NIDDM. *Diabetologia* 1995; **38:** 326–36.
31 Galgani JE, Heilbronn LK, Azuma K *et al.* Metabolic flexibility in response to glucose is not impaired in people with type 2 diabetes after controlling for glucose disposal rate. *Diabetes* 2008; **57:** 841–45.
32 Yokoyama H, Mori K, Emoto M *et al.* Non-oxidative glucose disposal is reduced in type 2 diabetes, but can be restored by aerobic exercise. *Diabetes Obes Metab* 2008; **10:** 400–7.
33 Kabadai UM, Eisenstein AB, Tucchi J, Pellicone J. Hyperglucagonemia in hepatic cirrhosis: its relation to hepatocellular dysfunction and normalization on recovery. *Am J Gastroenterol* 1984; **79:** 143–9.
34 Sumida Y, Yoneda M, Hyogo H *et al.* Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol* 2012; **12:** 2.
35 Okumura H, Yamauchi R, Urano E *et al.* Examination of blood biochemistry index for tailor-made nutritional management of cirrhotic patients. *Jap J Nutr Assesment* 2011; **28:** 18–20 (in Japanese).

J Gastroenterol
DOI 10.1007/s00535-012-0697-6

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

First jejunal vein oriented mesenteric excision for pancreatoduodenectomy

Masafumi Nakamura · Hiroshi Nakashima · Kosuke Tsutsumi · Hideo Matsumoto · Yu Muta · Daisuke Ueno · Koji Yoshida · Keisuke Hino · Atsushi Urakami · Masao Tanaka

Received: 19 July 2012 / Accepted: 26 September 2012

Abstract
Background Dissection of the pancreatic head from the superior mesenteric vein (SMV) and artery (SMA) are major points of bleeding in pancreaticoduodenectomy (PD) because of congestion of the pancreatic head. The "SMA-first" approach, which involves ligating the artery from the SMA first, can be used to solve this problem. However, the SMA-first approach has problematic anatomical issues. We applied a new surgical approach, first jejunal vein oriented mesenteric excision (FME), for PD. This study aimed to clarify the effect of FME on reduction of bleeding during PD.
Methods The jejunal vein, the most frequent source of bleeding during dissection of the mesoduodenum, was identified at the beginning of dissection of the pancreatic head from SMV and SMA. The mesoduodenum, including plural IPDAs, was completely divided before dissection of the pancreatic head from the SMV. The perioperative outcomes of two groups, patients who underwent FME-based PD and patients who underwent standard PD, were compared. Additionally, the spatial characteristics of the first jejunal vein (FJV) were analyzed using computed tomography.
Results FME-based PD significantly reduced intraoperative blood loss compared with conventional PD (569 vs. 1094 ml, $P = 0.0315$). The median distance of the FJV was 0 mm from the middle colic artery and 0 mm from the third portion of the duodenum. The FJV was posterior to the SMA in the majority of the patients but was anterior to the SMA in 16.7 % of patients.
Conclusions FME is useful for reducing intraoperative bleeding.

Keywords First jejunal vein · pancreaticoduodenectomy · Mesenteric excision

M. Nakamura (✉) · H. Nakashima · K. Tsutsumi · H. Matsumoto · Y. Muta · D. Ueno · A. Urakami
Department of Digestive Surgery, Kawasaki Medical School, 577 Matsushima, Kurashiki 701-0192, Japan
e-mail: mnaka56@gmail.com; mnakamura@med.kawasakim.ac.jp

K. Yoshida · K. Hino
Division of Hepatology and Pancreatology, Kawasaki Medical College, Kurashiki, Japan

M. Tanaka
Department of Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Introduction

Radical resection of pancreatic cancer is the only method that can be used to achieve a complete cure. Pancreatoduodenectomy (PD) is an invasive surgery and sometimes causes massive intraoperative bleeding, especially during dissection of the pancreatic head from the superior mesenteric artery and vein (SMA and SMV) [1]. The SMV is located anterior to the SMA, and dissection of the pancreatic head from the SMV is conventionally performed earlier than that from the SMA. However, conventional SMV-first PD causes congestion of the pancreatic head, which often leads to bleeding during dissection of the pancreatic head. Several "SMA-first" techniques, where the inferior pancreaticoduodenal artery (IPDA) is ligated first, have been reported [1–3]. However, there are several issues with the IPDA in the SMA-first approach. The IPDA forms a common trunk with the first jejunal artery (FJA) or arises separately from the SMA [4]. In both cases, the IPDA often arises from the posterior wall of the SMA and is embedded in the posterior side of the mesoduodenum

Published online: 18 October 2012

[5]. Two branches of the IPDA, the anterior and posterior IPDA (AIPDA and PIPDA), sometimes arise separately from the SMA without having trunks in common with the IPDA and FJA [4]. Furthermore, the first jejunal vein (FJV), the first major branch of the SMV, is usually embedded in the dorsal side of the mesoduodenum [6–9], where the IPDA is located. Therefore, identification of the IPDA sometimes causes injury to the FJV and increases blood loss. The current study describes our method of FJV-oriented mesenteric excision (FME), in which we first identify the FJV and completely divide the mesoduodenum. We aimed to clarify whether FME can reduce intraoperative bleeding.

Materials and methods

Patients

We retrospectively analyzed the perioperative outcomes of 36 patients who underwent resection of the pancreatic head, PD, and total pancreatectomy (TP) from 2010 through June 2012 in Kawasaki Medical College. All patients gave their informed consent for surgical treatment. FME was introduced to Kawasaki Medical College in September 2011. Therefore, 18 patients underwent conventional PD up to August 2011, and 16 patients underwent FME from September 2011 through June 2012. However, two patients underwent conventional PD between September 2011 and June 2012 because we suspected that FME was difficult to perform on these two patients. Of them, one patient had anatomical abnormality that SMA was placed just behind SMV, and the other patient developed a severe pancreatic pseudocyst between the SMA and SMV. The procedures followed were in accordance with the Declaration of Helsinki (1964, and amended in 1975, 1983, 1989, 1996, and 2000) of the World Medical Association.

FME

The greatest difference between FME-based PD and conventional PD is the early detection of the FJV and complete division of the mesoduodenum prior to the separation of the pancreatic head from the SMV. The mesoduodenum between the SMA and SMV was pushed from the dorsal to the ventral direction and stretched (Fig. 1). The stretched mesentery was cut layer by layer. The layer before the SMA did not contain the IPDA. Therefore, the layer was cut by a bipolar sealing system without ligation. The layer deeper than the SMA contained the IPDA and FJV. We identified the FJV prior to IPDA, and restarted cutting the dorsal half of the mesoduodenum layer by layer. The IPDA was often visible while cutting the mesentery layer by layer. This process was continued until the mesoduodenum was completely divided.

Analysis of anatomical findings for the FJV

Computed tomography (CT) images of 56 patients who underwent surgery for the treatment of hepatobiliary–pancreatic disease were analyzed to determine the anatomy of the FJV. Two out of 56 patients had massive invasions of pancreatic cancer into the SMV, so anatomical analysis of the SMV was impossible. CT images used in this study were scanned at the Kawasaki Medical School and other hospitals. The thickness of the CT images was 5 mm or less.

Patient characteristics and statistical analysis

Clinically relevant pancreatic fistulae—equivalent to grades B and C as defined by the International Study Group of Pancreatic Fistula (ISGPF)—were diagnosed based on the following criteria: persistent drainage (>3 weeks); signs of infection; re-admission (<1 month) or fluid collection with elevated drain amylase levels (>3 × normal

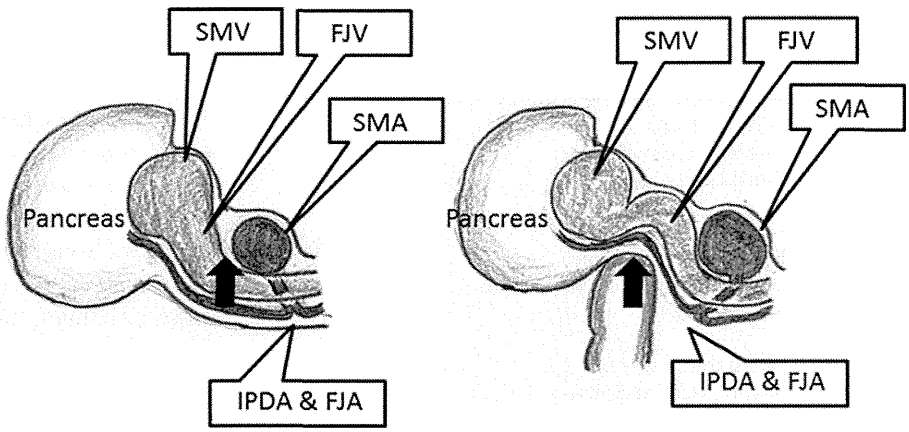

Fig. 1 Schema of FJV-oriented excision of the mesoduodenum. The mesoduodenum between the SMA and SMV is pushed from the dorsal to the ventral side, and the mesoduodenum is retracted to the right. Therefore, the common trunk of the IPDA and FJA is also retracted to the right

serum amylase), as described previously [10]. Statistical analysis was performed using JMP 8.01 software (SAS Institute, Cary, NC, USA). Data were analyzed using the Mann–Whitney U test and χ^2 test. Statistical significance was defined as a P value of <0.05.

Results

Surgical anatomy of the FJV

The anatomical characteristics of the FJV were analyzed from CT images (Table 1). The FJV was adjacent to the middle colic artery and the third portion of the duodenum, which are caudal borders of the dissection along the SMA in PD (Fig. 2). The position of the FJV relative to the SMA was posterior in 83.3 % of the patients and anterior in 16.7 % of the patients. We usually transected the FJV, which was anterior to the SMA, to make dissection easier (Fig. 3).

Patient characteristics

Table 2 shows the patients' characteristics. Most patients had pancreatic cancer or bile duct cancer, while the others had pancreatic metastasis of renal cancer and schwannoma. Preoperative comorbidities are listed in Table 2. Nine patients (56 %) in the FME group and ten patients (50 %) in the conventional group had preoperative comorbidities. There was no significant difference in the rate of preoperative comorbidities among these two groups.

Perioperative outcomes

Table 3 shows the perioperative factors and postoperative outcomes of the patients. There was no difference in the type of resection, including combined resection of the portal vein and/or other organs, between the two groups. The amount of intraoperative bleeding was significantly less in the FME group than that in the conventional group (569 vs. 1094 g, $P = 0.0315$). Consistent with intraoperative bleeding, the rate of transfusion of red blood cells was significantly less in the FME group than that in the conventional group (31 vs. 70 %, $P = 0.0192$). Other perioperative outcomes, including operation time, morbidity, period of hospital stay, and mortality, showed no difference between the groups.

Four patients in the FME group suffered from postoperative liver abscess, acute myocardial infarction, abdominal abscess without amylase-rich fluid, and colitis caused by *Clostridium difficile*. These patients had preoperatively suffered from cholangitis, an old myocardial infarction, acute cholecystitis, and had undergone antibacterial treatment of nontuberculous mycobacterial infection for 20 years, respectively.

The status of the surgical margin was compared between the groups, except for three patients who underwent a palliative operation (Table 4). There was no difference in the status of the surgical margin between the two groups.

One patient who developed a severe pancreatic pseudocyst between the SMA and SMV underwent conventional PD after September 2011. The severe pseudocyst may have influenced perioperative outcome, so we calculated perioperative outcomes without this patient, as indicated in Table 5. The perioperative outcomes showed significantly less blood loss and a significantly lower rate of transfusion in the FME group than in the conventional group, consistent with the results of the analysis including the patient with the pancreatic pseudocyst (Table 3).

Discussion

We showed that the FJV was situated in the close vicinity of the root of the middle colic artery and the third portion of the duodenum, and was posterior to the SMA in more than 80 % of the patients. The third portion of the duodenum is located at the caudal margin of the mesoduodenum, where IPDA is often located [4]. Furthermore, the middle colic artery is at the caudal margin of the dissection area for pancreatoduodenectomy [5]. These anatomical findings suggest that the FJV was important when dissecting the IPDA and mesoduodenum.

Our method of FME did not involve total excision of the mesentery of the pancreatic head and duodenum. The

Table 1 Anatomical characteristics of the FJV (54 patients)

					Median distance
Distance from the MCA (mm)[a]	25 to 21	20 to 11	10 to 0	−1 to −20	0
Number of patients	1	6	45	2	
Distance from the third part of duodenum (mm)	>20	20 to 1	0	−1 to −10	0
Number of patients	1	8	44	1	
FJV: anterior/posterior to SMA	Anterior		Posterior		
Number of patients (%)	9 (16.7)		45 (83.3)		

[a] MCA is cranial (+)/caudal (−) to FJV

FJV first jejunal vein, *MCA* middle colic artery, *SMA* superior mesenteric vein

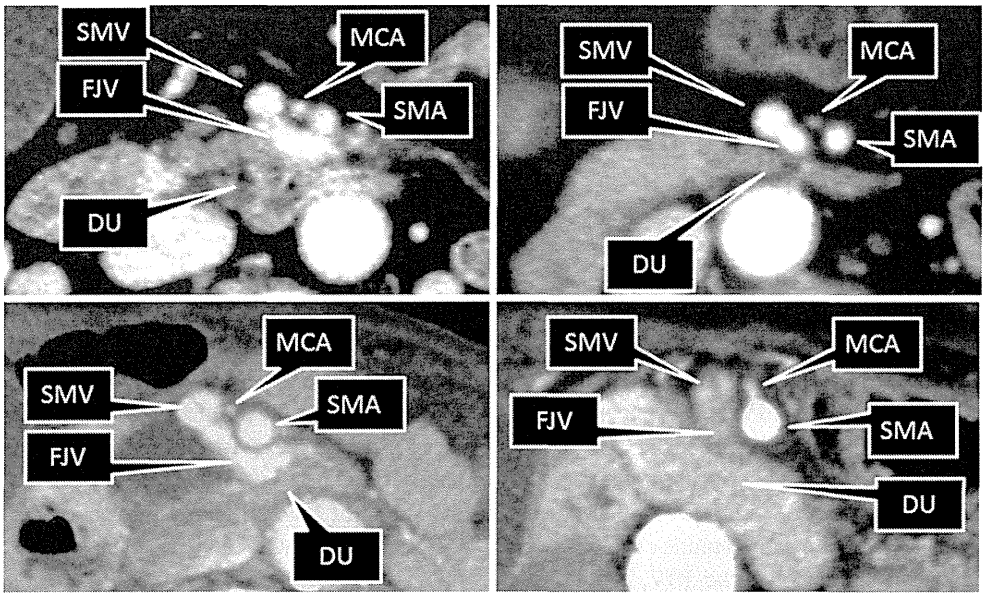

Fig. 2 CT images displaying the vessels around the mesoduodenum in patients whose FJV was posterior to the SMA. Each *panel* corresponds to an individual patient

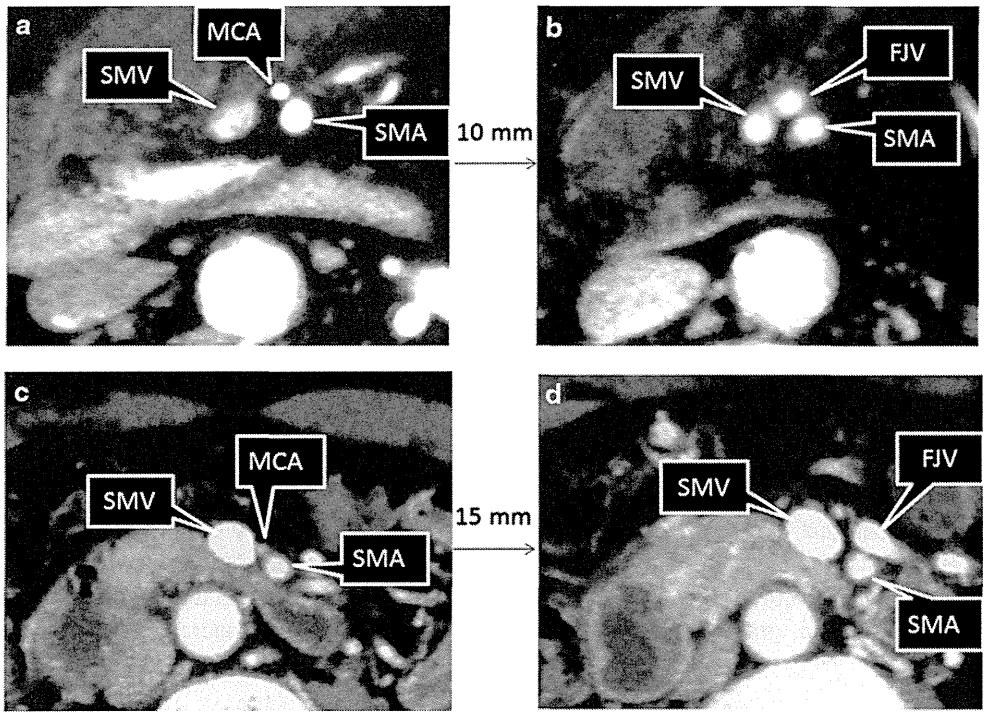

Fig. 3 CT images displaying the vessels around the mesoduodenum in patients whose FJV was anterior to the SMA. Each *pair of panels* (**a** and **b**), (**c** and **d**) are images of the same person. (**a**, **c**) show sections cranial to (**b**, **d**). The distance between each pair of panels is indicated (from **a** to **b**: 10 mm; **c** to **d**: 15 mm)

mesenteric root of the pancreas is typically a trunk shared with the IPDA and FJA [5, 11, 12]. This common trunk arises from the SMA to the left side. Therefore, we needed to perform extensive dissection of the SMA nerve plexus to achieve complete excision of the mesentery. During the FME method, the mesoduodenum is retracted to the right. Therefore, we can detect and ligate the common trunk by FME. However, extensive retroperitoneal dissection might

Table 2 Patient characteristics ($n = 36$)

	FME (16)	Conventional method (20)	*P* value
Age (years), mean ± SD	71.7 ± 8.3	68.3 ± 11.6	0.503
Sex (male/female)	10/6	13/7	0.877
Diagnosis (PC/BDC/other disease)	13/3/0	12/6/2	0.269
Comorbidities (%)	9 (56)	10 (50)	0.790
Arrhythmia (%)	0 (0)	1 (5)	
Hypertension (%)	0 (0)	1 (5)	
Diabetes mellitus (%)	2 (13)	4 (20)	
Ischemic heart disease (%)	3 (19)	0 (0)	
COPD (%)	0 (0)	1 (5)	
Cholangitis (%)	1 (6)	0 (0)	
Cholecystitis (%)	1 (6)	0 (0)	
Liver abscess (%)	1 (6)	2 (10)	
Liver cirrhosis (%)	1 (6)	1 (5)	
NTM (%)	1 (6)	0 (0)	
Turner syndrome (%)	0 (0)	1 (5)	
Severe pancreatitis (%)	0 (0)	1 (5)	
Chronic renal failure (%)	1 (6)	0 (0)	
Other cancers (%)	1 (6)	2 (10)	

FME first jejunal vein oriented mesenteric excision, *SD* standard deviation, *PC* pancreatic cancer, *BDC* bile duct cancer, *COPD* chronic obstructive pulmonary disease, *NTM* nontuberculous mycobacterial infection

Table 3 Perioperative outcomes

	FME ($n = 16$)	Conventional method ($n = 20$)	*P* value
Type of resection (PD/TP)	15/1	20/0	0.9097
Portal vein resection (%)	5 (31)	4 (25)	0.6985
Other organ resection (%)	1 (6)	2 (10)	0.8397
Operation time (min), median (range)	374 (240–718)	414 (310–585)	0.4169
Blood loss (g), median (range)	569 (100–1339)	1094 (200–3522)	0.0315*
RBC transfusion (%)	5 (31)	14 (70)	0.0192*
Hospital stay (days), median (range)	28 (20–53)	31 (11–98)	0.6485
Mortality (%)	0 (0)	1 (5)	0.9097
Postoperative complications (%)	4 (25)	4 (20)	0.9642
Pancreatic fistula (%)	0 (0)	2 (10)	0.569
Intra-abdominal abscess (%)	1 (6)	0 (0)	0.9097
Liver abscess (%)	1 (6)	0 (0)	0.9097
Intestinal bleeding (%)	0 (0)	1 (5)	0.9097
Pneumonia (%)	0 (0)	1 (5)	0.9097
Ischemic heart disease (%)	1 (6)	0 (0)	0.9097
Colitis (%)	1 (6)	0 (0)	0.9097
Diarrhea (%)	1 (6)	0 (0)	0.9097

FME first jejunal vein oriented mesenteric excision, *PD* pancreatoduodenectomy, *TP* total pancreatectomy

Statistical significance was defined as $P < 0.05$. * Indicates statistical significance. *RBC transfusion* indicates transfusion of red blood cells during the operation and within 24 h after the operation

Table 4 Resection margins of curatively operated patients

	FME (15)	Conventional (18)	*P* value
Positive (%)	2 (13.3)	6 (33.3)	0.35391

FME first jejunal vein oriented mesenteric excision

not contribute to the survival of pancreatic cancer patients [13–18]. The primary purpose of FME is not extensive dissection of the mesentery, but to reduce intraoperative bleeding. Therefore, we usually do not insist on dissecting the common trunk, and are careful not to dissect too much of the nerve plexus around the SMA to prevent intractable diarrhea. Similarly, the nerve plexus was not completely cleared up to the root of the first jejunal artery in the conventional method. Thus, both FME and the conventional method showed no statistical difference in the rate of postoperative diarrhea, as indicated in Table 3.

Large amounts of bleeding are reported to be associated with perioperative complications [19–22]. Our study results showed no difference in morbidity between the FME and conventional groups, although intraoperative bleeding was significantly reduced in the FME compared to the conventional method. Patients in both the FME and conventional groups had high rates of preoperative comorbidities—56 and 50 %, respectively—as listed in Table 2. Four patients in the FME group suffered from postoperative liver abscess, acute myocardial infarction, abdominal abscess without amylase-rich fluid, and colitis caused by *Clostridium difficile*. These patients had preoperatively suffered from cholangitis, an old myocardial infarction, acute cholecystitis, and had undergone antibacterial treatment of nontuberculous mycobacterial infection for 20 years, respectively. Taking these facts into consideration, we suspected that the high comorbidity rate might affect the rate of perioperative complications.

We found that the FJV was an important and major vein during PD in this study. The FJV was dorsal to the SMA in 83.3 % of the patients in our study. This frequency is consistent with previous reports [6–9]. Before we adopted FME, we frequently used to experience a large amount of bleeding

Table 5 Perioperative outcomes of 35 selected cases

	FME ($n = 16$)	Conventional method ($n = 19$)	P value
Operation time (min), median (range)	374 (240–718)	397 (310–585)	0.4972
Blood loss (g), median (range)	569 (100–1339)	1087 (200–3522)	0.0487*
RBC transfusion (%)	5 (31)	13 (68)	0.0266*
Postoperative complications (%)	4 (25)	4 (21)	0.7820
Pancreatic fistula (%)	0 (0)	2 (11)	0.1106
Hospital stay (days), median (range)	28 (20–53)	31 (11–98)	0.6880
Mortality (%)	0 (0)	1 (5)	>0.9999

RBC transfusion indicates transfusion of red blood cells during the operation and within 24 h after the operation. Statistical significance was defined as a P value of <0.05. * Indicates statistical significance

from the FJV when dividing the mesoduodenum, as we did not pay adequate attention to the FJV. This massive bleeding from the FJV that occurs when insufficient attention is paid to the FJV may be the reason why prior division of the mesoduodenum was not popular previously.

The FJV was anterior to the SMA in 16.3 % of the patients in our study. Prior division of the mesentery is easier in these patients than in patients who have the FJV posterior to the SMA. When the FJV is anterior to the SMA it is easily ligated and cut, so the mesentery can be easily and safely divided.

We dissect the mesoduodenum layer by layer in the FME technique. Therefore, the root of the replaced right hepatic artery arising from the SMA is easy to detect. The frequency of any type of hepatic artery replacement has been reported to be as high as 24.3 % [23, 24]. In cases where the replaced right hepatic artery is involved by cancer, ligation of its root is also easily performed during FME [24].

In this report, we have shown only the short-term outcome of FME-based PD. We need to accumulate more patients using this procedure and perform a longer follow-up to determine a more precise long-term outcome, including survival rate.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kawai M, Tani M, Ina S, et al. CLIP method (preoperative CT image-assessed ligation of inferior pancreaticoduodenal artery) reduces intraoperative bleeding during pancreaticoduodenectomy. World J Surg. 2008;32:82–7.
2. Shrikhande SV, Barreto SG, Bodhankar YD, et al. Superior mesenteric artery first combined with uncinate process approach versus uncinate process first approach in pancreatoduodenectomy: a comparative study evaluating perioperative outcomes. Langenbecks Arch Surg. 2011;396:1205–12.
3. Xu YF, Liu ZJ, Gong JP. Pancreaticoduodenectomy with early superior mesenteric artery dissection. Hepatobiliary Pancreat Dis Int. 2010;9:579–83.
4. Horiguchi A, Ishihara S, Ito M, et al. Three-dimensional models of arteries constructed using multidetector-row CT images to perform pancreatoduodenectomy safely following dissection of the inferior pancreaticoduodenal artery. J Hepatobiliary Pancreat Sci. 2010;17:523–6.
5. Kawabata Y, Tanaka T, Nishi T, et al. Appraisal of a total mesopancreatoduodenum excision with pancreaticoduodenectomy for pancreatic head carcinoma. Eur J Surg Oncol. 2012;38:574–9.
6. Katz MH, Fleming JB, Pisters PW, et al. Anatomy of the superior mesenteric vein with special reference to the surgical management of first-order branch involvement at pancreaticoduodenectomy. Ann Surg. 2008;248:1098–102.
7. Ibukuro K, Tsukiyama T, Mori K, et al. Peripancreatic veins on thin-section (3 mm) helical CT. AJR Am J Roentgenol. 1996;167:1003–8.
8. Ito K, Blasbalg R, Hussain SM, et al. Portal vein and its tributaries: evaluation with thin-section three-dimensional contrast-enhanced dynamic fat-suppressed MR imaging. Radiology. 2000;215:381–6.
9. Graf O, Boland GW, Kaufman JA, et al. Anatomic variants of mesenteric veins: depiction with helical CT venography. AJR Am J Roentgenol. 1997;168:1209–13.
10. Bassi C, Dervenis C, Butturini G, et al. Postoperative pancreatic fistula: an international study group (ISGPF) definition. Surgery. 2005;138:8–13.
11. Kayahara M, Nakagawara H, Kitagawa H, et al. The nature of neural invasion by pancreatic cancer. Pancreas. 2007;35:218–23.
12. Makino I, Kitagawa H, Ohta T, et al. Nerve plexus invasion in pancreatic cancer: spread patterns on histopathologic and embryological analyses. Pancreas. 2008;37:358–65.
13. Raut CP, Tseng JF, Sun CC, et al. Impact of resection status on pattern of failure and survival after pancreaticoduodenectomy for pancreatic adenocarcinoma. Ann Surg. 2007;246:52–60.
14. Nimura Y, Nagino M, Takao S, et al. Standard versus extended lymphadenectomy in radical pancreatoduodenectomy for ductal adenocarcinoma of the head of the pancreas: long-term results of a Japanese multicenter randomized controlled trial. J Hepatobiliary Pancreat Sci. 2012;19:230–41.
15. Yeo CJ, Cameron JL, Sohn TA, et al. Pancreaticoduodenectomy with or without extended retroperitoneal lymphadenectomy for periampullary adenocarcinoma: comparison of morbidity and mortality and short-term outcome. Ann Surg. 1999;229:613–22 (discussion 622–14).
16. Yeo CJ, Cameron JL, Lillemoe KD, et al. Pancreaticoduodenectomy with or without distal gastrectomy and extended retroperitoneal lymphadenectomy for periampullary adenocarcinoma, part 2: randomized controlled trial evaluating survival, morbidity, and mortality. Ann Surg. 2002;236:355–66 (discussion 366–58).
17. Yeo CJ. The Johns Hopkins experience with pancreaticoduodenectomy with or without extended retroperitoneal lymphadenectomy for periampullary adenocarcinoma. J Gastrointest Surg. 2000;4:231–2.
18. Reddy SK, Tyler DS, Pappas TN, et al. Extended resection for pancreatic adenocarcinoma. Oncologist. 2007;12:654–63.

19. Miedema BW, Sarr MG, van Heerden JA, et al. Complications following pancreaticoduodenectomy. Current management. Arch Surg. 1992;127:945–9 (discussion 949–50).
20. Yeh TS, Jan YY, Jeng LB, et al. Pancreaticojejunal anastomotic leak after pancreaticoduodenectomy–multivariate analysis of perioperative risk factors. J Surg Res. 1997;67:119–25.
21. Gouma DJ, van Geenen RC, van Gulik TM, et al. Rates of complications and death after pancreaticoduodenectomy: risk factors and the impact of hospital volume. Ann Surg. 2000;232: 786–95.
22. Kawai M, Tani M, Terasawa H, et al. Early removal of prophylactic drains reduces the risk of intra-abdominal infections in patients with pancreatic head resection: prospective study for 104 consecutive patients. Ann Surg. 2006;244:1–7.
23. Hiatt JR, Gabbay J, Busuttil RW. Surgical anatomy of the hepatic arteries in 1000 cases. Ann Surg. 1994;220:50–2.
24. Yamamoto S, Kubota K, Rokkaku K, et al. Disposal of replaced common hepatic artery coursing within the pancreas during pancreatoduodenectomy: report of a case. Surg Today. 2005; 35:984–7.

Hepatic Medicine: Evidence and Research

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Treatment of nonalcoholic steatohepatitis with vitamins E and C: a pilot study

Miwa Kawanaka[1]
Ken Nishino[1]
Jun Nakamura[1]
Mitsuhiko Suehiro[1]
Daisuke Goto[1]
Noriyo Urata[1]
Takahito Oka[1]
Hirofumi Kawamoto[1]
Hajime Nakamura[2]
Junji Yodoi[3]
Keisuke Hino[4]
Gotaro Yamada[1]

[1]Department of Internal Medicine 2, Kawasaki Hospital, Kawasaki Medical School, Okayama, [2]Department of Experimental Therapeutics, Translational Research Center, Kyoto University Hospital, Kyoto, [3]Department of Biological Responses, Institute for Virus Research, Kyoto University, Kyoto, [4]Department of Hepatology and Pancreatology, Kawasaki Medical School, Okayama, Japan

Correspondence: Miwa Kawanaka
Department of Internal Medicine 2, Kawasaki Hospital, Kawasaki Medical School, 2-1-80 Nakasange, Kita-ku, Okayama City, Okayama 700-8505, Japan
Tel +81 86 225 211
Fax +81 86 232 8343
Email m.kawanaka@med.kawasaki-m.ac.jp

Background: Nonalcoholic steatohepatitis (NASH) is a common liver disease that can progress to cirrhosis. Oxidative stress is one of the central mechanisms causing hepatocellular injury in the disease. In this study, antioxidant therapy using both vitamins C and E was conducted in patients with NASH.

Methods: Vitamin E 300 mg/day and vitamin C 300 mg/day were administered orally to 23 patients with NASH for 12 months. Body mass index was measured during therapy. Serum levels of alanine aminotransferase, thioredoxin (an oxidative stress marker), and high-sensitivity C-reactive protein were measured before treatment and after 12 months in all patients. Ten of the 23 patients underwent liver biopsy before and after treatment.

Results: Body mass index remained unchanged during treatment with vitamins C and E. Serum alanine aminotransferase, thioredoxin, and high-sensitivity C-reactive protein levels were decreased significantly at 12 months compared with pretreatment. Liver biopsies showed improved necroinflammatory activity in eight cases and improved fibrosis staging in 4.

Conclusion: Serum alanine aminotransferase, thioredoxin, and high-sensitivity C-reactive protein levels, and liver histology were clearly improved with vitamin C and E therapy. These findings suggest that combination therapy using these vitamins may be useful in patients with NASH to minimize damage from oxidative stress and slow the processes leading to cirrhosis.

Keywords: vitamin E, vitamin C, nonalcoholic steatohepatitis, oxidative stress

Introduction

Nonalcoholic steatohepatitis (NASH) is a very common chronic liver disease that resembles alcoholic liver disease clinically and histologically, but occurs in individuals in the absence of a history of significant alcohol consumption. NASH is frequently associated with clinical conditions such as obesity, type 2 diabetes mellitus, hyperlipidemia, and hypertension. These background characteristics indicate that NASH may be part of the spectrum of "metabolic syndrome". More recently, NASH has been proposed as a possible cause of cryptogenic cirrhosis.[1] The pathogenesis of NASH is multifactorial, involving abnormal lipid metabolism, production of reactive oxygen species, increased hepatic lipid peroxidation, activated stellate cells, and abnormal patterns of cytokine production. Oxidative stress appears to be a key factor in the progression from steatosis to NASH and potentially to cirrhosis.[2–5]

No universally effective treatment for NASH has been identified. Vitamin E refers to a group of naturally occurring compounds with antioxidant properties. A pilot trial of vitamin E aimed at decreasing oxidative stress in pediatric patients with presumed

submit your manuscript | www.dovepress.com
Dovepress
http://dx.doi.org/10.2147/HMER.S41258

NASH showed that serum alanine aminotransferase and aspartate aminotransferase levels normalized during this treatment.[6] We have previously evaluated the efficacy of vitamin E therapy in patients with NASH.[7] Significant improvements in serum alanine aminotransferase and gamma glutamyl transferase were observed during treatment. At the same time, levels of the oxidative stress markers, thioredoxin and thiobarbituric acid, were significantly decreased. Furthermore, Harrison et al recently reported that treatment with vitamins C and E improves liver fibrosis in patients with NASH.[8] In the present study, vitamins C and E were administered for 12 months at lower doses than those used by Harrison et al, and changes in levels of alanine aminotransferase, oxidative stress markers, and high-sensitivity C-reactive protein were measured before and after treatment to clarify the efficacy and mechanisms of action underlying vitamin C and E therapy. Thioredoxin is a stress-inducible thiol-containing protein that has been shown to be an indicator of oxidative stress in a variety of diseases. Weight reduction is reported to improve liver enzyme abnormalities and liver histology in obese patients with NASH.[9,10] However, many patients find it virtually impossible to maintain body weight loss. Therefore, this study evaluated the effects of vitamin C and E on serum alanine aminotransferase, thioredoxin, high-sensitivity C-reactive protein, and liver histology in patients with NASH in association with changes in body mass index during treatment.

Materials and methods

Patients

Twenty-three patients with NASH (ten men and 13 women, Matteoni classification 3 or 4, mean age 53.1 ± 14.9 years) were included in this study. Twenty patients had a body mass index > 25 kg/m^2. Nineteen patients had hypertension and 18 patients had hyperinsulinemia and dyslipidemia (Table 1). Before pretreatment, all patients had been diagnosed as having NASH by liver biopsy.

Table 1 Demographic feature of patients at baseline

Feature	n
N	23
Age	53.1 ± 14.9
Male/female	10/13
BMI (kg/m^2)	20
Hypertension	19
Hyperinsulinemia	18
Dyslipidemia	18
Stage 0/1/2/3	0/14/6/3
Grade 0/1/2/3	0/15/5/3

Diagnostic criteria

NASH was diagnosed based on the following criteria: negative results for hepatitis B surface antigen and hepatitis C virus RNA, and exclusion of autoimmune liver disease, drug-induced hepatic disorder, or metabolic liver disease (eg, Wilson's disease, hemochromatosis); alcohol intake ≤ 30 g/week; and presence of steatosis (>30%) or steatohepatitis. The pathological classification proposed by Matteoni et al[11] was used to diagnose histological types 1 and 2 as simple steatosis and types 3 and 4 as NASH. Fibrosis was graded from stage 0 to stage 4 in accordance with the staging system, and inflammatory activity in the liver was graded from grade 0 to grade 3, also according to the grading system proposed by Brunt et al.[12] Steatosis was defined according to the percent of hepatocytes affected, and divided into four grades: grade 0, 0%; grade 1, >0% but <33%; grade 2, 33%–66%; and grade 3, >66%. Some pathologists read the liver biopsy blinded. Diabetes mellitus was diagnosed based on the following criteria proposed by the Japan Diabetic Society: fasting blood glucose ≥ 126 mg/dL; two-hour post-75 g oral glucose tolerance test result ≥ 200 mg/dL; casual blood glucose ≥ 200 mg/dL; and glycosylated hemoglobin ≥ 6.1 or treatment with one or more antidiabetic agents. Dyslipidemia was defined as triglyceride > 150 mg/dL and/or a low-density lipoprotein cholesterol level > 140 mg/dL or treatment with one or more lipid-lowering drugs. Hypertension was defined as systolic/diastolic blood pressure ≥ 140/90 mmHg or treatment with one or more antihypertensives.

Changes in body mass index were observed monthly in all patients during vitamin C and E therapy. Serum thioredoxin[13] and high-sensitivity C-reactive protein[14] levels were determined using enzyme-linked immunosorbent assays (Mitsubishi Kagaku Yatoron, Tokyo, Japan). Serum thioredoxin levels were also estimated using an enzyme-linked immunosorbent assay kit (Ledox BioScience, Kyoto, Japan). Vitamin E (α-tocopherol, Eisai, Tokyo, Japan) and vitamin C (ascorbic acid, Sionogi, Tokyo, Japan) were each administered orally at 300 mg/day for 12 months.

During therapy, the lifestyle of all patients remained unchanged. Body mass index and serum alanine aminotransferase, thioredoxin, and high-sensitivity C-reactive protein levels were measured before and after treatment. Liver biopsy was performed for all patients before treatment, and ten patients from whom consent was able to be obtained underwent liver biopsy after treatment. Liver histology was evaluated by staging of fibrosis and grading of necroinflammatory activity and steatosis.

Immunostaining for 8-hydroxydeoxyguanosine, an oxidative stress marker, was also done before and after treatment.

The study was approved by the ethics committee at our institution and complied with the Declaration of Helsinki and internationally recognized guidelines. Informed consent was obtained.

Statistical analysis

The analysis was performed using JMP version 9.0.1 software (SAS Institute Japan, Tokyo, Japan). Univariate and multivariate analyses of predictors of survival were assessed using the Cox proportional hazards model. A P value less than 0.05 was considered to be statistically significant.

Results

During the 12 months of treatment with vitamins C and E, body mass index remained unchanged (26.6 ± 3.1 kg/m^2 at baseline and 26.8 ± 3.1 kg/m^2 after treatment). Serum alanine aminotransferase and high-sensitivity C-reactive protein levels decreased significantly during the 12 months, respectively, from 96.5 ± 45.9 IU/L to 40.3 ± 17.6 IU/L ($P < 0.0001$, Figure 1) and from 133.5 ± 59.8 mg/L to 67.5 ± 47.5 mg/L ($P < 0.005$, Figure 1). Before therapy, serum thioredoxin levels were 43.5 ± 17.2 ng/mL, but remained below 35.7 ± 11.5 ng/mL by 12 months following vitamin C and E therapy ($P = 0.08$, Figure 1). In the liver, staging of fibrosis, grading of necroinflammatory activity, and steatosis were compared before and after treatment. Staging of fibrosis improved in four of 10 cases, grade of necroinflammatory activity decreased in eight of 10 cases, and grade of steatosis decreased in six of 10 cases. In four patients, the grade of steatosis remained unchanged, but the grade of necroinflammatory activity improved in all cases, and staging of fibrosis also improved in two cases (Table 2). Table 3 compares histological data for case 3 following treatment with vitamins C and E. Fibrosis stage improved from F3 to F1, while the grading of necroinflammatory activity decreased from A3 to A1. However, no change in fatty deposition was evident. Treatment with vitamins C and E also resulted in a decrease in 8-hydroxydeoxyguanosine levels (Table 4).

Discussion

The pathogenesis of NASH remains poorly understood. Obesity, type 2 diabetes mellitus, hyperinsulinemia, increased triglyceride levels, toxins, and medical conditions can lead to increased serum fatty acid levels, which are then presented to the liver. The multihit theory suggests that the first hit involves accumulation of excess fat in the hepatic parenchyma. The second hit involves oxidative stress resulting from an imbalance between pro-oxidant and antioxidant processes in the liver, which may result from induction of microsomal cytochrome P450 2E1, mitochondrial release of reactive oxygen species, release of hydrogen peroxide from peroxisomal-beta oxidation of fatty acids, release of cytokines from activated inflammatory cells, and insulin resistance.[1–5]

Optimal therapies for NASH have yet to be established. Maintenance of weight loss results in significant clinical and histological improvement.[9,10] However, adverse effects on liver histology, such as progression of fibrosis, have also been noted.[15]

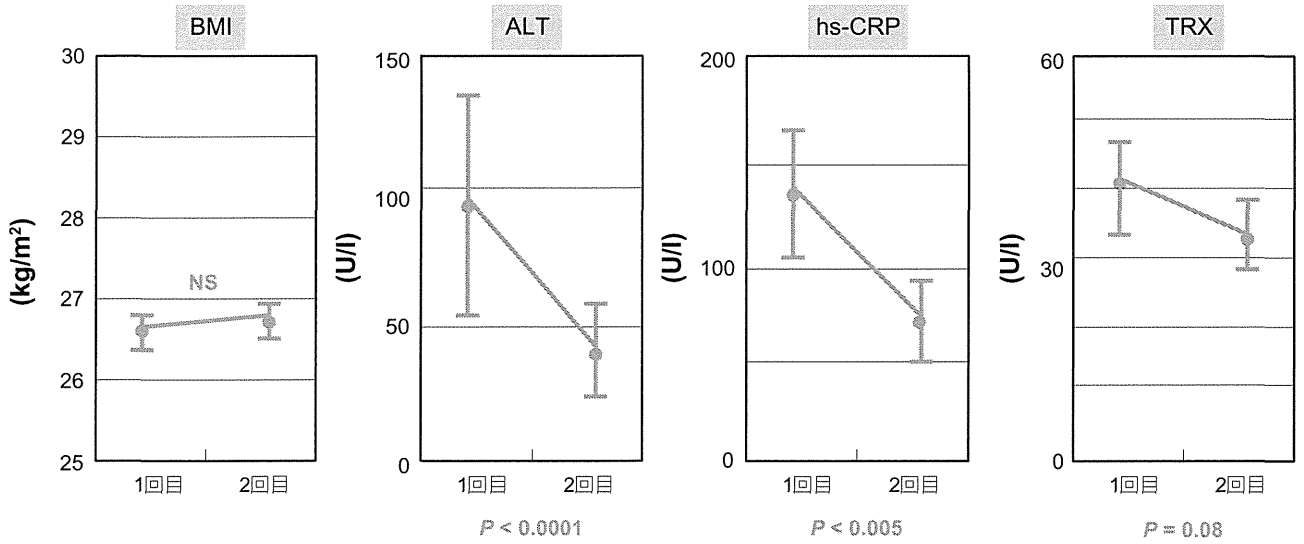

Figure 1 Body mass index remained unchanged, but changes were seen in serum alanine aminotransferase, thioredoxin, and high-sensitivity C-reactive protein levels in patients with nonalcoholic steatohepatitis before and 12 months following treatment with vitamins C and E.
Abbreviations: BMI, body mass index; ALT, alanine aminotransferase; hs-CRP, high-sensitivity C-reactive protein; TRX, thioredoxin.