therefore, we may expect a decrease in the $CD4^+/CD8^+$ ratio after splenectomy. A decrease in Treg cells that stimulate TGF-β1 may lead to alleviation of fibrosis.

Because the immune function of $CD4^+$ CTL, $CD8^+$ CTL and the $CD4^+/CD8^+$ ratio is affected by a wide variety of factors including recent exercise, poor nutrition and coincident acute viral infections, it is difficult to evaluate immune function using only $CD4^+$ CTL, $CD8^+$ CTL and the $CD4^+/CD8^+$ ratio. However, in our study, the ratio of $CD4^+$ T cells to all lymphocytes in PB was significantly decreased in cirrhotic patients after splenectomy, while the ratio of $CD8^+$ T cells to all lymphocytes slightly increased, resulting in a significant decrease in the $CD4^+/CD8^+$ ratio. The $CD4^+/CD8^+$ ratios in PB, spleens and livers were significantly higher in patients with hypersplenism and in those in whom liver fibrosis had progressed than in the controls. As a positive correlation was observed between the $CD4^+/CD8^+$ ratios in the spleens, livers and PB, it is possible to expect to predict the immunological state of the liver and spleen from the immunological state of PB. In addition, carcinogenesis was significantly lower in groups in which a large difference in the $CD4^+/CD8^+$ ratio was observed between before and after splenectomy or in those with a high $CD4^+/CD8^+$ ratio before splenectomy though there were few cases that we could observe. The $CD4^+/CD8^+$ ratio is likely to be a key parameter for appropriate tumor-infiltrating lymphocyte function, and was shown to be different in different types of cancer.[2,31–35] Host immune responses to cancer were reported to depend on T lymphocytes, particularly $CD8^+$ lymphocytes.[18,19,24,36–39] An increase in their ratio after splenectomy and the consequent decrease in the $CD4^+/CD8^+$ ratio observed in this study may be a positive change in terms of immunology against HCC. Such a change was particularly marked in patients with a high $CD4^+/CD8^+$ ratio before splenectomy.

In our study, the $CD4^+/CD8^+$ ratio also significantly increased as the fibrosis of non-tumor areas in the liver tissue progressed. These significant differences were observed regardless of the HCC status. Although the cause of these differences is unknown, it appears to depend on the background of histological factors in the liver such as fibrosis. Many studies have investigated the relationship between tumors, Treg and TGF-β.[20–22,25,40] Guo-He *et al.* showed that the expression of TGF-β appeared to be positively correlated with Treg in HCC tissue. The 5-year survival rate was significantly lower in patients with HCC tissues with high Treg cell infiltration than in those with low infiltration.[20,22,36,41] Our study also revealed that Treg cells were positively correlated with TGF-β1 positive cells even in "non-tumor areas" of liver tissue, and that TGF-β1 positive cells were positively correlated with liver fibrosis. There were no significant differences of TGF-β1 before and after splenectomy. The reason for the chronological changes in TGF-β1 levels after splenectomy is unknown because various factors including platelets may be involved in the production of TGF-β1. We also found a slightly higher number of TGF-β1 positive cells in non-tumor areas in the liver tissue of patients with HCC than in those without. Furthermore, the number of TGF-β1 positive cells significantly increased with the progression of liver fibrosis.[4,21,26,42]

In conclusion, splenectomy in cirrhotic patients with hepatitis may be able to improve liver fibrosis, cause beneficial immunological changes and lower the risk of carcinogenesis. It seems necessary to accumulate further cases to establish a convincing conclusion.

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Occlusion of portosystemic shunts improves hyperinsulinemia due to insulin resistance in cirrhotic patients with portal hypertension

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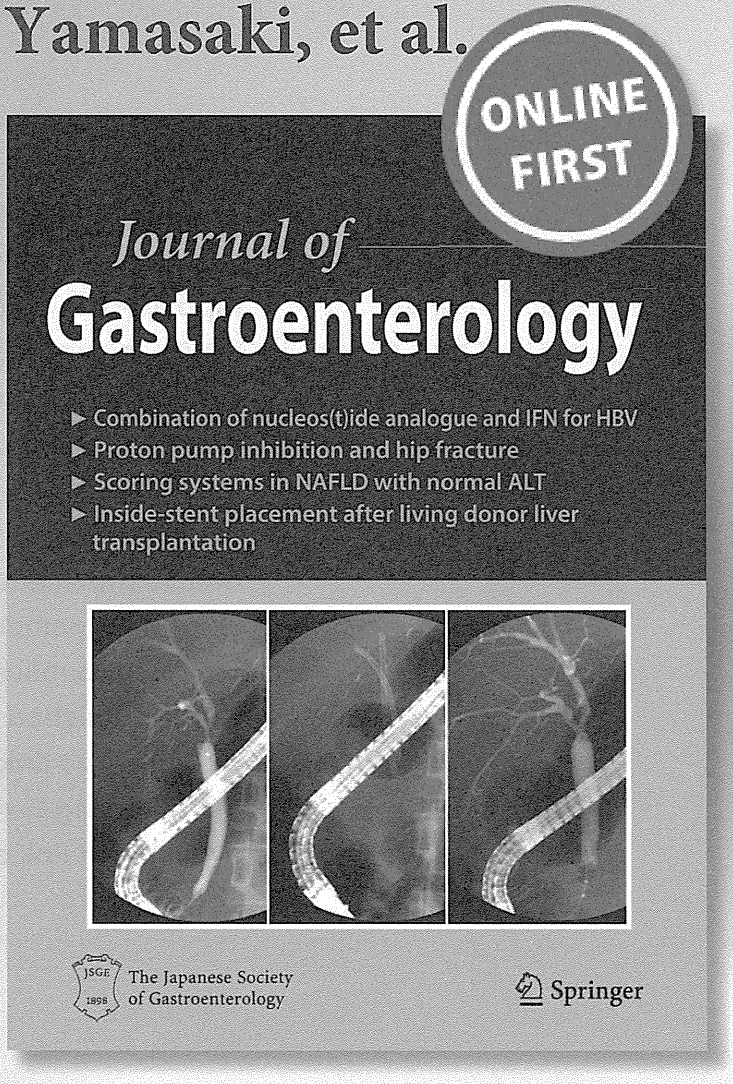

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Occlusion of portosystemic shunts improves hyperinsulinemia due to insulin resistance in cirrhotic patients with portal hypertension

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Abstract
Background Liver cirrhosis (LC) is often complicated by hyperinsulinemia due to insulin resistance (IR), which is considered to be closely related to shunt formation and impaired liver function. This study evaluates whether balloon-occluded retrograde transvenous obliteration (B-RTO) can affect glucose and insulin metabolism in patients with LC.
Methods Twenty-five cirrhotic patients (mean age = 69.6 years; female/male = 12/13; hepatitis C virus/alcohol/nonalcoholic steatohepatitis = 14/6/5; Child-Pugh's class A/B = 10/15) with gastric varices and/or hepatic encephalopathy caused by portosystemic shunts (PSS) due to portal hypertension (PH) underwent B-RTO at our hospital. Testing was performed before and at 1 month after the procedure.
Results Shunt occlusion resulted in a decrease in extrahepatic collateral blood flow and an increase in portal venous flow, as well as a dramatic improvement in hepatic function markers. In addition, B-RTO significantly decreased homeostasis model assessment (HOMA) of IR without a statistical decline of HOMA of β-cell function. The 75-g oral glucose tolerance test (75-OGTT) revealed that occlusion of PSS reduced both fasting immunoreactive insulin (IRI) levels and the area under the curve for IRI. However, no significant change in preprandial or postprandial plasma glucose levels was observed. Furthermore, according to the criteria of the American Diabetes Association, B-RTO led to an improved 75-OGTT profile in 58.3 % of patients who had impaired glucose tolerance or diabetes mellitus before the procedure.
Conclusions Shunt occlusion improves IR-related hyperinsulinemia through increased portal venous flow, ameliorated liver function, and consequent augmented hepatic insulin clearance in cirrhotic patients with PH.

Keywords Portal hypertension · Liver cirrhosis · Portosystemic shunt · Hyperinsulinemia · Insulin resistance

Introduction

Hepatogenous diabetes is characterized by hyperinsulinemia [1–4] and insulin resistance (IR) [5]. The pathogenesis of IR is thought to result from decreased degradation of insulin by the liver due to impaired hepatic parenchymal function [6, 7]. It is also possible that the presence of both intrahepatic and extrahepatic portosystemic shunts (PSS) may contribute to decreased hepatic insulin clearance [8, 9]. Recently, the transjugular intrahepatic portosystemic shunt (TIPS), which is a shunting created within the liver parenchyma between the hepatic vein and the portal vein, has been reported to exacerbate glycemic control through deteriorated IR [10–13]. However, the direct effect of balloon-occluded retrograde transvenous obliteration (B-RTO), which is a transcatheter procedure occluding PSS by using sclerosants [14], on glucose and insulin metabolism in patients with liver cirrhosis (LC) is poorly understood. B-RTO is a minimally invasive, highly effective therapy for isolated gastric varices due to PSS, which are difficult to be controlled by other interventional procedures, including endoscopic injection sclerotherapy,

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percutaneous transhepatic obliteration, partial splenic embolization, and surgical treatment [14–16]. B-RTO is also useful in the management of refractory hepatic encephalopathy caused by giant collateral vessels, which are resistant to dietary restriction and drug medication [17–21]. Occlusion of PSS via B-RTO results in hemodynamic changes that are completely opposite of those induced by the TIPS procedure. Therefore, we hypothesized that shunt occlusion might improve glycemic control in patients with LC. The goal of the present study is to assess changes in hemodynamics and glycometabolism after B-RTO to determine whether the procedure can affect hyperinsulinemia and IR in cirrhotic patients with portal hypertension (PH).

Methods

Patients

Between May 2009 and December 2012, 25 cirrhotic patients with gastric varices and/or hepatic encephalopathy caused by PSS due to PH underwent B-RTO at our hospital. A diagnosis of cirrhosis was established by a combination of biochemical, clinical, and ultrasonographic findings. Patients who were taking oral antidiabetic drugs or receiving subcutaneous insulin therapy were excluded from this study. Their clinical characteristics are shown in Table 1. The mean age was 69.6 years, and 12 females and 13 males were included. The average body mass index of them was 24.8. According to the Child-Pugh's classification, 10 and 15 patients were classified as class A and class B, respectively. The cause of cirrhosis was hepatitis C virus in 14 patients, alcohol in six patients, and nonalcoholic steatohepatitis in five patients. Four patients had complicating hepatocellular carcinoma (HCC). The indication for B-RTO was gastric varices in 20 cases and portosystemic encephalopathy in five cases.

Table 1 Clinical characteristics of 25 patients

Age	69.6 ± 1.9 years (median 66.0 years, range 53–86 years)
Sex	Female/male = 12/13
BMI	24.8 ± 0.9 (median 23.8, range 17.1–34.2)
Child-Pugh's score	6.8 ± 0.3 points (median 7.0 points, class A/B/C = 10/15/0)
Etiology	HCV/alcohol/NASH = 14/6/5
Indication	GV/HE = 20/5

Data represent mean ± standard error

BMI body mass index, *HCV* hepatitis C virus, *NASH* nonalcoholic steatohepatitis, *GV* gastric varices, *HE* hepatic encephalopathy

This retrospective clinical study was performed according to the Declaration of Helsinki and the clinical research guidelines in Japan. The protocol of the present study was approved by the ethical committee on human research of Yamaguchi University Hospital (H25-47, Institutional Review Board of Yamaguchi University Hospital).

B-RTO procedure

The B-RTO procedure was performed, on the whole, according to the method described by Kanagawa et al. [14]. Briefly, after an 8-Fr catheter sheath introducer (Terumo Corp., Tokyo, Japan) was inserted from the right femoral vein under local anesthesia, a 6-Fr occlusive balloon catheter (20 mm in diameter; Terumo Clinical Supply Co., Ltd., Gifu, Japan) was advanced into the left renal vein through the sheath introducer. After advancing it to the periphery of the gastrorenal (GR) or splenorenal (SR) shunt as far as possible, the shunt vessel was occluded with a balloon containing saline mixed with contrast medium. Retrograde venography was carried out to identify the PSS and to evaluate the degree of collateral vessels other than the GR or SR shunt. If necessary, downgrading procedures (e.g., embolization with 50 % glucose solution or microcoils) were performed through a selectively catheterized microcatheter system, and a 5 % solution of ethanolamine oleate with iopamidol as a sclerosing agent was then infused into the shunt vessels. The entire setup was left in place until the next morning, and the balloon catheter was removed after checking for stable thrombus on the second day. During these procedures, 4000 U of human haptoglobin were given intravenously to prevent renal failure related to hemolysis. The procedure was defined as being successful, if dynamic computed tomography at 1 week after B-RTO revealed complete thrombosis of major shunt such as the GR or SR shunt. In consequence, B-RTO procedures were successfully performed in all 25 cases enrolled in this study.

Laboratory test

Hepatic function markers, including total bilirubin (T-Bil), direct bilirubin (D-Bil), albumin, cholinesterase (ChE), prothrombin time (PT) activity, PT-international normalized ratio (INR), anti-thrombin (AT) III activity, the branched chain amino acids/tyrosine molar ratio (BTR), ammonia, and indocyanine green retention rate at 15 min (ICG-15), were evaluated before and at 1 month (1 M) after the procedure. Furthermore, indices of glucose metabolism, such as fasting plasma glucose (PG) level, fasting immunoreactive insulin (IRI) level, hemoglobin A1c (HbA1c), and glycoalbumin (GA) concentration, were

assessed and homeostasis model assessment of IR/β-cell function (HOMA-IR/-β) was also measured pre-B-RTO and post-B-RTO (1 M). Patients who had fasting PG >140 mg/dl were excluded from assessment of HOMA-IR, because HOMA-IR is not a useful index in the setting of severe hyperglycemia. The equations for calculating HOMA-IR and HOMA-β were as follows:

$$\text{HOMA} - \text{IR} = \text{fasting IRI}\ (\mu\text{U/ml}) \times \text{fasting PG}\ (\text{mg/dl})/405$$

$$\text{HOMA} - \beta = \text{fasting IRI}\ (\mu\text{U/ml}) \times 360/\{\text{fasting PG}\ (\text{mg/dl}) - 63\}.$$

HOMA-IR ≥2.5 was regarded as being insulin resistant [22]. Fasting IRI level was also assessed as a surrogate for IR.

Oral glucose tolerance test

A 75-g oral glucose tolerance test (75-OGTT) was performed in 18 patients of all before and at 1 M after the procedure. Glucose intolerance was evaluated based on a 75-OGTT according to the criteria of the American Diabetes Association [23]: normal glucose tolerance (NGT) = fasting PG <110 mg/dl and 2-hour (2-h) PG <140 mg/dl, impaired glucose tolerance (IGT) = 2-h PG between 140 and 200 mg/dl, and diabetes mellitus (DM) = fasting PG ≥126 mg/dl or 2-h PG ≥200 mg/dl. On the morning prior to B-RTO and at 1 M after the treatment, blood samples were collected from fasting patients before and at 30, 60, and 120 min after the oral administration of a 75-g dose of glucose. To quantify the response of PG and IRI to the 75-OGTT, the area under the curve (AUC) was calculated [24]. Insulinogenic index (II), (IRI at 30 min − IRI at 0 min)/(PG at 30 min − PG at 0 min), was also calculated to evaluate post-load insulin secretion as β-cell function.

Measurement of wedged hepatic venous pressure and hepatic venous pressure gradient

The right hepatic venous branch was catheterized, and the free and wedged hepatic venous pressures (fHVP and wHVP) were measured before and after occluding the vein by inflating the balloon catheter (Terumo Clinical Supply Co., Ltd., Gifu, Japan) with diluted contrast medium. The hepatic venous pressure gradient (HVPG) was defined as the pressure difference between the portal and hepatic veins and was calculated by subtracting fHVP from wHVP.

Measurement of portal flow velocity and portal flow volume

Portal flow velocity (PFVe) measurements were performed in fasting patients before and after the procedure using Doppler ultrasonography (US), as described previously [17]. The Doppler sample volume was placed fully within the main portal vein, and the angle of insonation was kept at less than 60°. Portal flow volume (PFVo) was calculated using PFVe and the diameter of the main portal vein, as measured by Doppler US.

Statistical analysis

All data are expressed as mean ± standard error. Paired *t* tests were used for statistical analyses. A p value of <0.05 was considered statistically significant.

Results

Preoperative glucose intolerance and insulin resistance

Before the procedure, only one of 25 patients (4.0 %) was diagnosed with DM according to the fasting PG levels (Fig. 1a). However, 68.0 % of patients with LC enrolled in

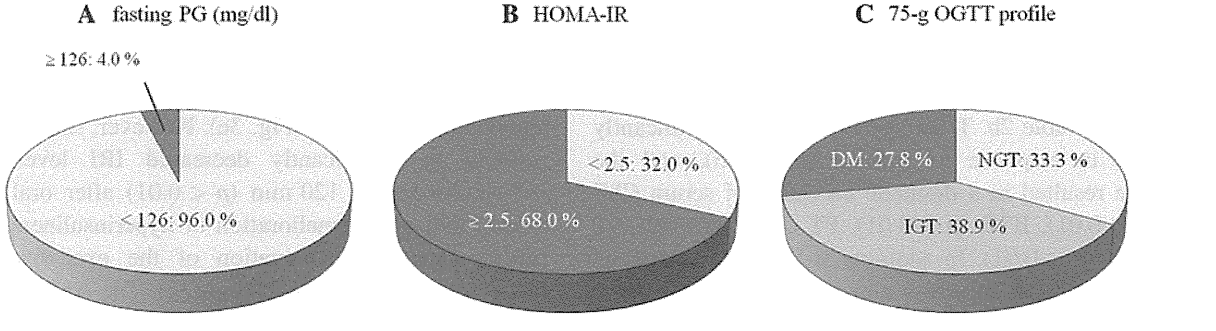

Fig. 1 Preoperative glucose intolerance and insulin resistance. Before the procedure, only one of 25 patients (4.0 %) was diagnosed with diabetes mellitus (DM) according to fasting plasma glucose levels (**a**). However, 68.0 % of the cirrhotic patients enrolled in this study had insulin resistance (IR), defined as homeostasis model assessment of IR ≥2.5 (**b**). The 75-g oral glucose tolerance test revealed that 38.9 % and 27.8 % of the 18 patients who underwent this test had impaired glucose tolerance and DM, respectively, according to the criteria of the American Diabetes Association (**c**). *PG* plasma glucose, *HOMA-IR* homeostasis model assessment of insulin resistance, *OGTT* oral glucose tolerance test, *NGT* normal glucose tolerance, *IGT* impaired glucose tolerance, *DM* diabetes mellitus

Table 2 Changes in hemodynamics and liver function

	Before	After (1 M)	*p* value
(a) Hemodynamic changes			
wHVP (mmH_2O)	251.8 ± 10.9 (255.0)	314.4 ± 11.3 (300.0)	<0.01
HVPG (mmH_2O)	143.2 ± 9.7 (125.0)	177.6 ± 11.4 (160.0)	<0.01
PFVe (cm/s)	13.1 ± 0.9 (14.5)	14.1 ± 0.7 (14.3)	0.38
PFVo (ml/min)	917.4 ± 98.7.7 (886.3)	1163.5 ± 78.7 (1089.9)	0.08
(b) Changes in hepatic function parameters			
Child-Pugh's score (points)	6.8 ± 0.3 (7.0)	6.2 ± 0.2 (6.0)	<0.01
T-Bil (mg/dl)	1.4 ± 0.1 (1.2)	1.2 ± 0.1 (1.0)	<0.05
D-Bil (mg/dl)	0.4 ± 0.1 (0.4)	0.4 ± 0.0 (0.3)	<0.05
Albumin (g/dl)	3.4 ± 0.1 (3.4)	3.5 ± 0.1 (3.5)	0.10
ChE (IU/l)	163.8 ± 13.1 (152.0)	207.6 ± 11.9 (210.0)	<0.01
PT (%)	68.5 ± 3.2 (68.6)	73.5 ± 4.1 (70.0)	<0.05
PT-INR	1.3 ± 0.0 (1.3)	1.2 ± 0.0 (1.2)	<0.05
AT III (%)	64.7 ± 3.0 (67.0)	70.6 ± 3.1 (69.2)	<0.01
BTR	3.5 ± 0.2 (3.6)	4.1 ± 0.3 (4.2)	<0.01
Ammonia (μmol/l)	59.4 ± 6.1 (52.0)	49.3 ± 5.4 (41.0)	0.11
ICG-15 (%)	36.8 ± 3.4 (39.6)	32.5 ± 4.7 (23.0)	0.05

Data represent mean ± standard error (median)

wHVP wedged hepatic venous pressure, *HVPG* hepatic venous pressure gradient, *PFVe* portal flow velocity, *PFVo* portal flow volume, *1 M* 1 month, *T-Bil* total bilirubin, *D-Bil* direct bilirubin, *ChE* cholinesterase, *PT* prothrombin time, *PT-INR* prothrombin time-international normalized ratio, *AT* anti-thrombin, *BTR* branched chain amino acids/tyrosine molar ratio, *ICG-15* indocyanine green retention rate at 15 min

this study had IR, defined as HOMA-IR ≥2.5 (Fig. 1b). In addition, the 75-OGTT revealed that 38.9 and 27.8 % of the 18 patients who underwent this test had IGT and DM, respectively, according to the criteria of the American Diabetes Association (Fig. 1c) [23].

Hemodynamic changes

Hemodynamic indices, including wHVP, HVPG, PFVe, and PFVo, before and after B-RTO, are shown in Table 2a. Acute portal compression was achieved through the procedure, as evidenced by a significant elevation of both wHVP ($p < 0.01$) and HVPG ($p < 0.01$). Furthermore, an increase in PFVo ($p = 0.08$) was observed post-B-RTO without significant change in PFVe ($p = 0.38$).

Changes in hepatic function parameters

Changes in markers of hepatic function after B-RTO are presented in Table 2b. T-Bil and D-Bil levels significantly decreased in response to B-RTO ($p < 0.05$), and the procedure resulted in a dramatic elevation of serum ChE level ($p < 0.01$), BTR ($p < 0.01$), PT ($p < 0.05$) and AT III activity ($p < 0.01$). In addition, B-RTO led to a trend towards an increase in serum albumin level ($p = 0.10$), a decrease in plasma ammonia level ($p = 0.11$), and a decrease in ICG-15 ($p = 0.05$). As a result, shunt occlusion improved hepatic function in terms of the Child-Pugh's score, from 6.8 ± 0.3 to 6.2 ± 0.2 points ($p < 0.01$).

Changes in glucose and insulin metabolism

While B-RTO had little effect on the fasting PG values, a statistically significant decrease in fasting IRI levels was seen after the procedure ($p < 0.01$) (Fig. 2a, b). Neither HbA1c nor GA concentration varied by shunt occlusion via B-RTO (data not shown). In addition, although no significant change was observed in HOMA-β, B-RTO significantly decreased HOMA-IR ($p < 0.01$), suggesting a pronounced recovery from IR in response to the procedure (Fig. 2c, d). In five of the 17 patients (29.4 %) who had IR, defined as HOMA-IR ≥2.5, before the procedure, B-RTO resulted in a reduction in HOMA-IR to <2.5.

Changes in glucose tolerance

There was no significant decline in PG levels at any time point on the 75-OGTT after B-RTO, and the AUC for PG on the 75-OGTT was similar when comparing pre-B-RTO and post-B-RTO (Fig. 3a, c). Furthermore, B-RTO had no significant effect on II (Fig. 3e). However, shunt occlusion resulted in significantly decreased IRI levels before ($p < 0.05$) and at 120 min ($p < 0.01$) after oral glucose intake, indicating amelioration of hyperinsulinemia in the fasting state and attenuation of the excessive insulin response to a glucose load (Fig. 3b). The AUC for IRI on the 75-OGTT was significantly lower after B-RTO than before the operation ($p < 0.01$), suggesting an improvement of postprandial hyperinsulinemia by occlusion of PSS (Fig. 3d). Before B-RTO, seven and five patients had IGT and DM, respectively, in accordance with the criteria of the

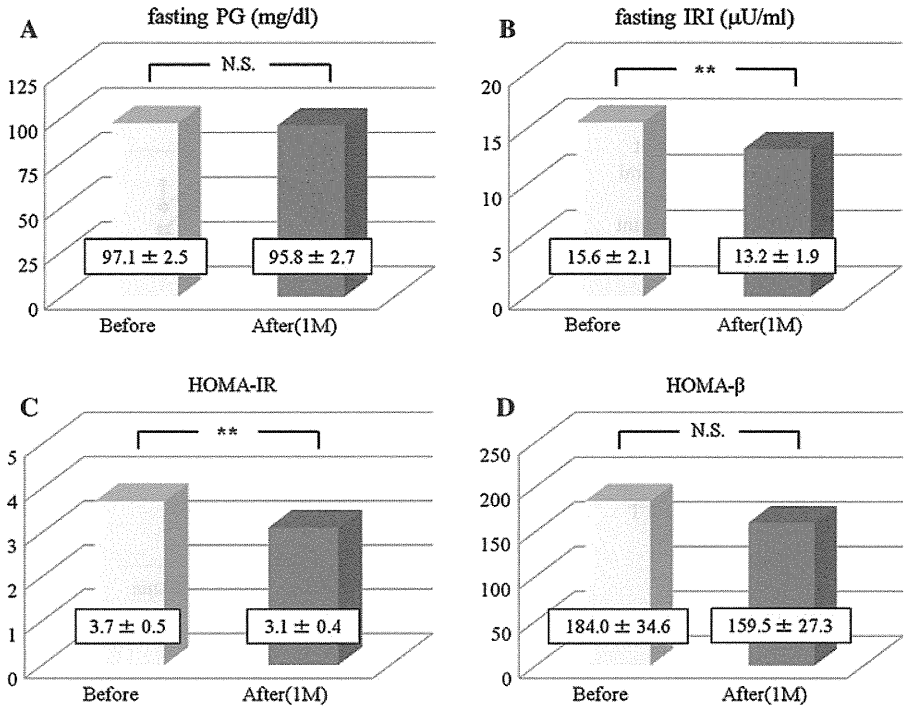

Fig. 2 Changes in markers of glucose and insulin metabolism. Balloon-occluded retrograde transvenous obliteration (B-RTO) had little effect on fasting plasma glucose values (**a**), but produced a significant decrease in fasting immunoreactive insulin levels ($^{**}p < 0.01$) (**b**). Although no significant change was observed in homeostasis model assessment (HOMA) of β-cell function (**d**), B-RTO resulted in a significant decrease in HOMA of insulin resistance ($^{**}p < 0.01$) (**c**). *PG* plasma glucose, *IRI* immunoreactive insulin, *HOMA-IR* homeostasis model assessment of insulin resistance, *HOMA-β* homeostasis model assessment of β-cell function, *1 M* 1 month, *NS* not significant. Data represent mean ± standard error

American Diabetes Association (Fig. 4) [23]. Shunt occlusion resulted in an improved 75-OGTT profile in seven of 12 patients (58.3 %) with glucose intolerance before the procedure, from IGT to NGT in four cases, from DM to IGT in two cases, and from DM to NGT in one case (Fig. 4). Finally, correlation between change in PFVo and change in 75-OGTT profile is shown in Table 3. In all of the cases with an ameliorated 75-OGTT profile, B-RTO resulted in an increase in portal venous flow. In addition, a decreased PFVo following the procedure could not lead to an improvement of 75-OGTT in this investigation.

Discussion

Hepatogenous diabetes differs from type 2 DM [25] and is characterized by IR [5] and hyperinsulinemia not only in the fasting state [2, 3] but also in response to a glucose load [1, 4]. The prevalence of IGT and overt diabetes in patients with LC is reported to be approximately 60 and 20 %, respectively [26]. Several lines of evidence suggest that hyperinsulinemia in cirrhotic patients results from various abnormalities, including impaired hepatic insulin clearance, portosystemic shunting, and enhanced insulin secretion related to IR [8, 9]. Previous studies have demonstrated that the OGTT in patients with LC reveals a prolonged hyperglycemia in spite of high serum insulin levels, suggesting that hepatic and extrahepatic IR is a hallmark of glycometabolism abnormalities in cirrhosis [5, 8, 27].

The TIPS procedure is a useful strategy for management of refractory PH, but may be associated with exacerbation of IR in LC with a significant increase in plasma insulin without any change in glucose levels [13]. Su et al. [12] showed that statistically significant hyperinsulinemia persisted after TIPS and the procedure was followed by worsened IR. Deschênes and Somberg [10] also found marked deterioration in glycemic control after TIPS in cirrhotic patients with overt diabetes.

However, little is known regarding the effect of B-RTO, which is widely performed in Asian countries for gastric varices and/or hepatic encephalopathy caused by PSS, on glucose and insulin metabolism in patients with LC. The present study demonstrated that occlusion of PSS via the B-RTO procedure resulted in decreased collateral venous flow and increased portal blood flow, leading to augmented insulin uptake by hepatocytes and increased hepatic insulin clearance. As was seen in previous reports [16, 17, 19, 28, 29], our study also demonstrated that B-RTO resulted in an improvement of hepatic function accompanied by increased portal venous flow and enlarged liver volume (data not shown), leading to increased degradation of insulin by the liver due to ameliorated hepatic parenchymal function. On the other hand, Tanabe et al. [30] previously reported that the B-RTO procedure did not affect insulin secretion by the pancreas, as the C-peptide immunoreactivity response to the OGTT was similar before and after B-RTO. This is consistent with observations from the present study, which showed no significant difference in HOMA-β and II when comparing pre-B-RTO and

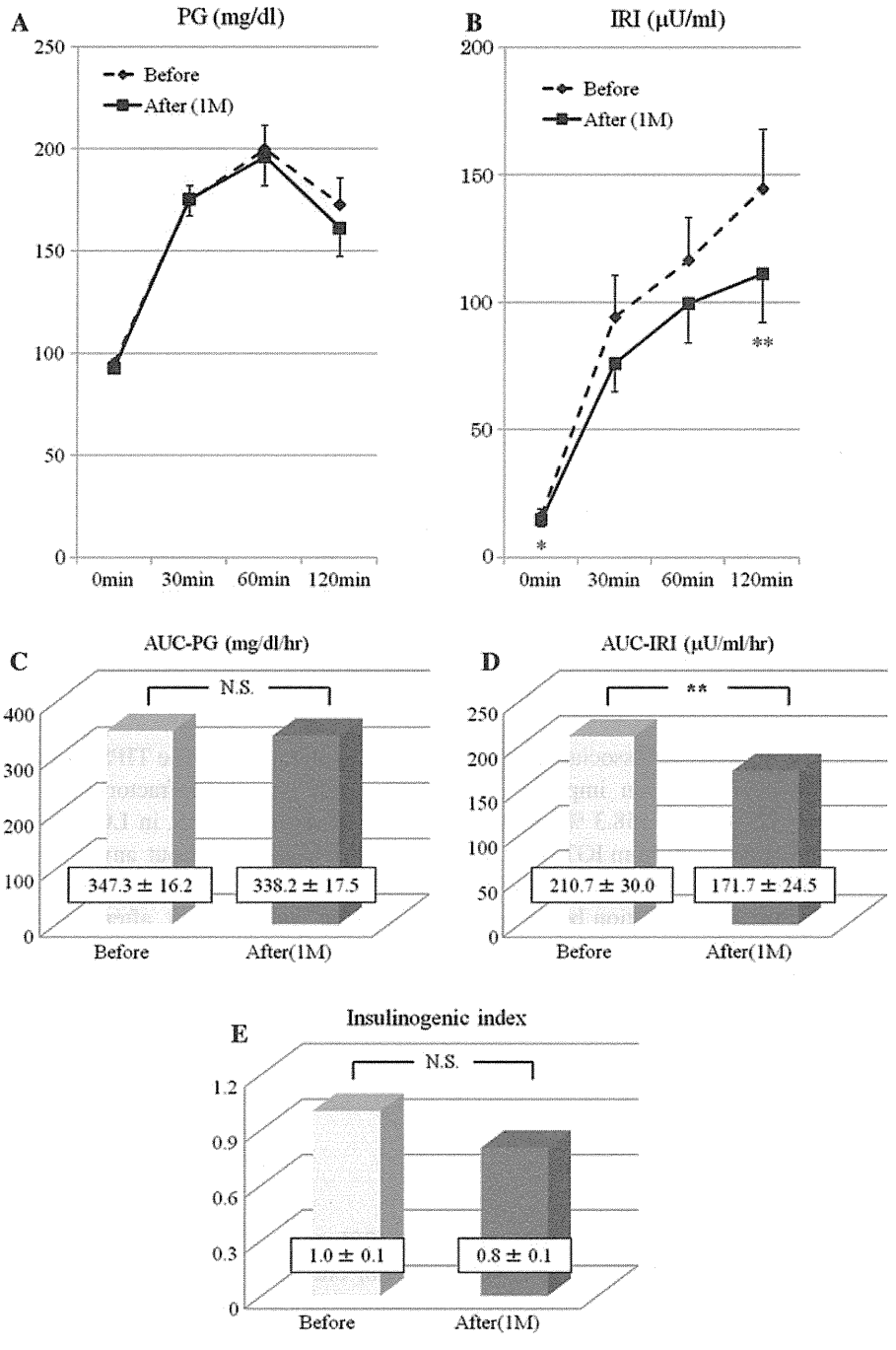

Fig. 3 Changes in 75-g oral glucose tolerance test results. Balloon-occluded retrograde transvenous obliteration (B-RTO) had no effect on plasma glucose (PG) levels at any time point on the 75-g oral glucose tolerance test (75-OGTT) (**a**). However, shunt occlusion via B-RTO resulted in a significant decrease in immunoreactive insulin (IRI) levels before ($*p < 0.05$) and at 120 min ($**p < 0.01$) after oral glucose intake (**b**). While the area under the curve (AUC) for PG on the 75-OGTT was similar between values before B-RTO and values post-B-RTO (**c**), the AUC for IRI on the 75-OGTT was significantly lower after B-RTO than before the procedure ($**p < 0.01$) (**d**). Furthermore, there was no statistical change in insulinogenic index in response to the procedure (**e**). *PG* plasma glucose, *IRI* immunoreactive insulin, *1 M* 1 month, *AUC-PG* area under the curve for plasma glucose, *AUC-IRI* area under the curve for immunoreactive insulin, *NS* not significant. Data represent mean ± standard error

post-B-RTO results, suggesting that the procedure did not influence β-cell secretion of insulin either in the fasting state or in response to a glucose load. Taken together, these data suggest that shunt occlusion attenuates hyperinsulinemia by reducing IR in cirrhotic patients with PH. The mechanism by which B-RTO improved insulin metabolism is likely related to increased portal venous flow into the liver and promoted hepatic function. Moreover, it is theoretically possible that insulin antagonists, such as glucagon and fatty acids, may be also metabolized adequately by the liver after occlusion of collateral vessels, thereby leading to improved glycemic control. Further study would be of benefit to characterize changes in hormonal and non-hormonal antagonists in response to B-RTO.

On the other hand, although patients who were receiving oral antidiabetic drugs or subcutaneous insulin before the operation were not enrolled in the present clinical study, the B-RTO procedures were actually performed for them as the treatment for ruptured gastric varices. In one patient who depended on a daily dose of 54 U of rapid-acting

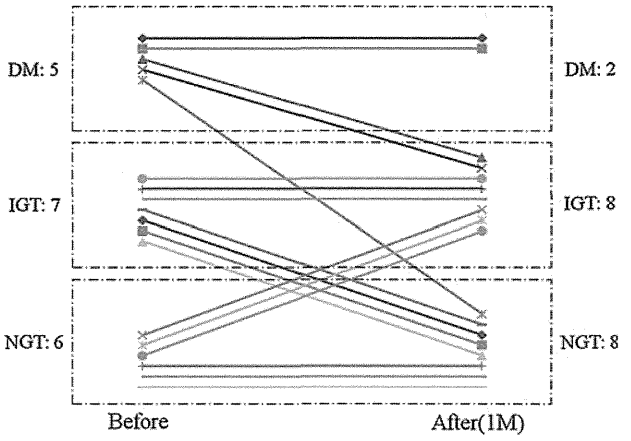

Fig. 4 Changes in 75-g oral glucose tolerance test profile. Before balloon-occluded retrograde transvenous obliteration, seven and five patients had impaired glucose tolerance (IGT) and diabetes mellitus (DM), respectively, according to the criteria of the American Diabetes Association. Shunt occlusion resulted in an improvement in the 75-g oral glucose tolerance test profile in seven of 12 patients who had glucose intolerance before the procedure, from IGT to normal glucose tolerance (NGT) in four cases, from DM to IGT in two cases, and from DM to NGT in one case. *NGT* normal glucose tolerance, *IGT* impaired glucose tolerance, *DM* diabetes mellitus, *1 M* 1 month. *Number* represents no. of cases

Table 3 Correlation between change in portal flow volume and change in 75-g oral glucose tolerance test profile

	Ameliorated OGTT	Stable OGTT	Deteriorated OGTT	Total
Increased PFVo	5	3	1	9
Decreased PFVo	0	1	1	2
Total	5	4	1	11

The total number of cases is 11 in this investigation because portal flow volume could not be calculated due to failure in measurement of portal flow velocity by Doppler US in seven cases

Number represents no. of cases

PFVo portal flow volume, *OGTT* oral glucose tolerance test

insulin before B-RTO, the procedure resulted in decreased insulin requirements (44 units per day). Another patient was undergoing treatment with sulfonylureas and α-glucosidase inhibitors (α-GI) before the procedure and developed hypoglycemia post-B-RTO; therefore, this patient regimen was narrowed to α-GI monotherapy. These observations suggest that B-RTO might reduce the requirement for antidiabetic therapy.

The present study demonstrated that shunt occlusion resulted in an improved 75-OGTT profile in 58.3 % of patients who had IGT or DM before the procedure. However, as shown in Fig. 4, the operation unfortunately led to a deteriorated 75-OGTT profile in three patients (16.7 %), from NGT to IGT. We compared preoperative and postoperative characteristics of these three patients with those of seven patients with an ameliorated 75-OGTT profile, but we could find no significant differences between them. On the other hand, while B-RTO significantly improved hepatic function parameters such as T-Bil, D-Bil, alanine aminotransferase, ChE, and BTR accompanied with an increase in portal venous flow in cases with an improved OGTT, those with an aggravated OGTT did not have obvious changes after shunt occlusion (data not shown). In addition, our investigation as shown in Table 3 revealed that an improvement of the 75-OGTT profile would be closely related to an increase in portal venous flow following the B-RTO procedure. At the present time, the most fundamental problem is small sample size (overall, 18 cases; ameliorated OGTT, seven cases; deteriorated OGTT, three cases), so further examination must be necessary to explain the mechanism by which B-RTO affects the 75-OGTT in detail.

Previous studies have suggested that hyperinsulinemia and IR contribute to deterioration of chronic liver disease by enhancing hepatic fibrogenesis and carcinogenesis [31–34]. IR reportedly accelerates the progression of fibrosis in the liver due to stimulation of hepatic stellate cells by hyperinsulinemia and hyperglycemia [35, 36]. Furthermore, insulin has mitogenic and proliferative effects, and hyperinsulinemia might be involved in the development and recurrence of HCC [37, 38]. In addition, some meta-analyses have shown that DM is closely related to cancer development not only in the liver [39] but also in other organs, including the colon [40] and pancreas [41]. These observations suggest that B-RTO, which significantly ameliorates glucose and insulin metabolism, might have a potential to improve outcomes for patients with LC. In fact, our preliminary data showed that B-RTO resulted in a marked decrease in fibrosis markers (e.g., type IV collagen 7 s domain) and tumor markers of HCC (e.g., des-γ-carboxy prothrombin) (data not shown).

Of note, this study was limited by its small sample size. To obtain well-supported findings, a larger randomized controlled study is clearly necessary. A longer duration of follow-up is also needed to evaluate the incidence of HCC and overall survival after B-RTO.

In conclusion, this study demonstrated that occlusion of PSS could attenuate IR-related hyperinsulinemia in cirrhosis with PH. This effect is likely mediated by increased portal venous flow, improved liver function, and consequent augmented hepatic insulin clearance. B-RTO may be beneficial for therapeutic management of cirrhotic patients with severe hepatic fibrosis and/or HCC.

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