Evaluation of postprandial hypoglycemia in patients with nonalcoholic fatty liver disease by oral glucose tolerance testing and continuous glucose monitoring

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Objective Nonalcoholic fatty liver disease (NAFLD) is often associated with insulin resistance and glucose intolerance. Postprandial hypoglycemia frequently occurs in NAFLD patients; however, the details remain unclear.

Patients and methods The 75-g oral glucose tolerance test (75gOGTT) in 502 patients with biopsy-proven NAFLD and continuous glucose monitoring (CGM) in 20 patients were performed, and the characteristics and causes of postprandial hypoglycemia were investigated.

Results The proportion of patients in the Hypo subgroup [plasma glucose (PG) at 180 min < fasting-PG (FPG)] among patients with normal glucose tolerance was significantly higher than that with diabetes mellitus and impaired glucose tolerance or impaired fasting glucose. FPG and hemoglobin A1c (HbA1c) were lower, and area under the curve of total insulin secretion within 120 min (< 120 min) was higher in Hypo than Hyper in overall patients. Although FPG and PG at 30 min were higher in Hypo than Hyper, HOMA-IR and the insulinogenic index were not different in normal glucose tolerance and impaired glucose tolerance or impaired fasting glucose. In multivariate logistic regression analysis, low HbA1c, low fasting immunoreactive insulin, and high area under the curve of total insulin secretion (< 120 min) were found to be independent factors associated with hypoglycemia. CGM showed postprandial hypoglycemia until lunch in 70% of NAFLD patients. However, no remarkable relationship in terms of hypoglycemia was identified between the 75gOGTT and CGM.

Conclusion Postprandial hypoglycemia was identified in many NAFLD patients detected by 75gOGTT and CGM. It was clarified that important causes of postprandial hypoglycemia were related to low HbA1c, an early elevation of PG, low fasting and relatively low early insulin secretion, and delayed hyperinsulinemia. Eur J Gastroenterol Hepatol 30:797–805

Introduction

Nonalcoholic fatty liver disease (NAFLD) includes a wide spectrum of liver diseases that range from simple steatosis to nonalcoholic steatohepatitis (NASH) [1,2]. NAFLD is also considered to be a hepatic manifestation of metabolic syndrome, which is associated with insulin resistance (IR) and abnormal glucose metabolism [3]. As the prevalence of metabolic syndrome has increased in the general population worldwide, the numbers of patients with NAFLD have also increased [4].

Type 2 diabetes mellitus (DM) is considered to be an independent risk factor for the development of NAFLD, including NASH [5,6]. Hyperinsulinemia and hyperglycemia are common not only in obese patients but also in nonobese and nondiabetic patients with NASH [7]. Kimura *et al.* [8] reported that the insulin concentration at 120 min in the 75-g oral glucose tolerance test (75gOGTT) was correlated independently with advanced fibrosis in patients with NAFLD.

Postprandial hyperglycemia and glycemic variability involve progression of atherosclerosis through increased oxidative stress and activation of inflammatory cytokines and inflammation. Oxidative stress is one of the most important factors in the development of inflammation and progression of hepatic fibrosis in patients with NAFLD [9]. Continuous glucose monitoring (CGM) systems have been introduced as useful tools with which to detect postprandial hyperglycemia [10] and 24-h glycemic variability in patients with DM. We previously reported that hyperinsulinemia, hyperglycemia, and glycemic variability are important predictive factors for the progression of hepatic fibrosis in patients with NAFLD by CGM [11].

However, details on the background and causes of hypoglycemia remain incompletely understood. Furthermore,

European Journal of Gastroenterology & Hepatology 2018, 30:797–805

Keywords: continuous glucose monitoring, diabetes mellitus, hyperinsulinemia, nonalcoholic fatty liver disease, postprandial hypoglycemia

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Received 12 October 2017 **Accepted** 8 November 2017

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, www.eurojgh.com.

whether hypoglycemia detected by 75gOGTT also occurs during the daily life of patients with NAFLD who have normal eating patterns and whether the characteristics of hypoglycemia detected by 75gOGTT are consistent with those detected by CGM among patients with NAFLD remain unknown. We therefore investigated the characteristics of hypoglycemia in patients with NAFLD by comparing the results of the 75gOGTT and CGM.

Patients and methods

Patients

In total, 502 patients with biopsy-proven NAFLD (197 female and 305 male patients) who underwent the 75gOGTT were enrolled in this study after they had provided informed written consent. Patients with known use of methotrexate, tamoxifen, corticosteroids, or more than 20 g/day of alcohol and patients with other known causes of liver disease including viral hepatitis, hemochromatosis, Wilson's disease, autoimmune hepatitis, and primary biliary cholangitis were excluded from this study. None of the patients had ever received any antidiabetic drugs or insulin. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki [12] and was approved by the Research Committee of Kochi Medical School and JA Hiroshima General Hospital.

Clinical and laboratory evaluation

Venous blood samples were obtained in the morning after a 12-h overnight fast. Laboratory tests in all patients included measurements of the serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), total bilirubin, cholinesterase, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, fasting plasma glucose (FPG), fasting immunoreactive insulin (f-IRI), creatinine, blood urea nitrogen, hemoglobin A1c (HbA1c), and fibrosis markers. These parameters were measured using standard clinical chemistry techniques in the laboratory section of Kochi Medical School Hospital and JA Hiroshima General Hospital.

In the 75gOGTT, the plasma glucose (PG) and insulin concentrations were measured at 0, 30, 60, 120, and 180 min. DM, impaired glucose tolerance/impaired fasting glucose (IGT/IFG), and normal glucose tolerance (NGT) were defined in accordance with the WHO criteria [13]. NGT was defined as an FPG of less than 110 mg/dl and a PG at 120 min after oral glucose loading of less than 140 mg/l. DM was defined as an FPG of greater than 126 mg/dl or PG at 120 min after oral glucose loading of greater than 200 mg/dl. IGT/IFG was considered to be present in patients with neither. IR was calculated by the homeostatic model assessment (HOMA) of IR (HOMA-IR) and Quantitative Insulin-sensitivity Check Index (QUICKI) using the following formulas: HOMA-IR = f-IRI (μU/ml) × FPG (mg/dl)/405 and QUICKI = 1/[log f-IRI (μU/ml) + FPG (mg/dl)]. Insulin secretion was measured with the insulinogenic index and HOMA of β-cell function (HOMA-β) using the following formulas: insulinogenic index = (Δplasma insulin 0–30 min)/(ΔPG 0–30 min) and HOMA-β = (IRI × 360)/FPG (mg/dl) – 63. The fibrosis (FIB)-4 score was calculated using the following formula: age × AST (IU/l)/platelet count ($\times 10^9$/l) × $\sqrt{}$ALT (IU/l).

Histological evaluation

Liver biopsy specimens were obtained from all patients percutaneously under ultrasonographic guidance. The specimens were obtained from the liver parenchyma of the upper region of the right lobe using a 15-G biopsy needle (Surecut; TSK Laboratory, Tochigi, Japan). All specimens were routinely fixed in 10% phosphate-buffered formalin (pH 7.4), embedded in paraffin, and sectioned for hematoxylin and eosin staining. Hepatic fibrosis was assessed by Brunt's classification as follows [14]: 0 = no fibrosis, 1 = zone 3 fibrosis only, 2 = zone 3 and portal/periportal fibrosis, 3 = bridging fibrosis, and 4 = cirrhosis. Histological evaluation was performed by two pathologists blinded to the patients' clinical data.

Continuous glucose monitoring

Continuous glucose concentrations in 20 patients with biopsy-proven NAFLD who underwent the 75gOGTT were monitored using a Gold CGM system (Medtronic MiniMed, Northridge, California, USA). None of the patients had received any antidiabetic drugs, including insulin injections. According to the operating guidelines, the CGM system was installed in the patients to monitor the glucose concentration in the interstitial fluid [15]. The glucose sensor was inserted into the subcutaneous tissue of the abdomen at 3:00–4:00 PM and monitored for 30 h. Finger-stick blood glucose concentrations were checked to calibrate the first glucose concentration read by the CGM system after 1 h of initialization. Glucose concentrations were determined at least four times per day using an automatic blood glucose meter (Glutest; Sanwa Kagaku Kenkyusho Co. Ltd, Nagoya, Japan). Meals were strictly standardized (1800 kcal/day of a standard diet at Kochi Medical School Hospital, Kochi, Japan) during the examination.

Statistical analyses

Results are presented as mean ± SD for quantitative data and as number or percentage for categorical or qualitative data. Statistical differences in quantitative data were determined using Student's *t*-test and Welch's *t*-test. Qualitative data were compared using the χ^2-test or Fisher's exact test. These statistical analyses were carried out using EZR (version 1.27; Saitama Medical Center, Jichi Medical University, Saitama, Japan) [16], which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).

To identify variables to predict the Hypo subgroup, multivariate logistic regression analyses were carried out. All variables with significant differences between the two subgroups ($P < 0.05$) were included in a multivariate logistic regression analysis. Fasting insulin, albumin, ALT, and histological findings including hepatic fibrosis and steatosis were also included in the same model because

they are known to be related closely to glucose metabolic disorder in NAFLD. Statistical analyses for multivariate logistic regression analysis were carried out using Stata 14.0 (StataCorp, College Station, Texas, USA). Results were considered significant when the *P* value was less than 0.05.

Results

Clinical and physiological characteristics of patients with nonalcoholic fatty liver disease according to the grade of glucose intolerance

The 502 patients with NAFLD were classified into three groups (DM, IGT/IFG, and NGT) on the basis of the grade of glucose intolerance indicated by the 75gOGTT, and the clinical and physiological characteristics of these patients were investigated as shown in Table 1 and Supplementary Table 1 (Supplemental digital content 1, *http://links.lww.com/EJGH/A274*). AST, ALT, and GGT concentrations in patients with NGT were lower than those in patients with DM and IGT/IFG. Hepatic fibrosis markers (type IV collagen 7 s and type III procollagen N peptide) in patients with NGT were significantly lower than those in patients of the other groups.

Table 1. Clinical and physiological characteristics of patients with nonalcoholic fatty liver disease

	Total (*N* = 502)
Sex (female/male)	197/305
Age (years)	53.38 ± 15.13
BW (kg)	75.95 ± 15.26
BMI (kg/m^2)	28.43 ± 4.64
AST (IU/l)	53.36 ± 30.37
ALT (IU/l)	89.59 ± 53.51
ALP (IU/l)	247.20 ± 117.48
GGT (IU/l)	82.68 ± 72.20
T-Bil (mg/dl)	0.91 ± 0.37
ChE (IU/l)	384.78 ± 84.64
Albumin (g/dl)	4.62 ± 0.40
BUN (mg/dl)	13.33 ± 3.64
Crn (mg/dl)	0.75 ± 0.36
TC (mg/dl)	211.26 ± 40.62
LDL (mg/dl)	132.99 ± 38.08
HDL (mg/dl)	50.74 ± 13.27
TG (mg/dl)	164.27 ± 89.88
Hb (g/dl)	14.89 ± 1.54
Plt (×10^4/μl)	22.38 ± 5.80
WBC (×10^3/μl)	6.24 ± 1.64
Fe (mg/dl)	117.46 ± 36.97
Ferritin (ng/ml)	258.28 ± 243.13
FIB-4 index	1.61 ± 1.29
Type IV collagen 7s (ng/ml)	4.14 ± 1.41
Type III procollagen N peptide (U/ml)	0.66 ± 0.33
FPG (mg/dl)	106.12 ± 20.82
f-IRI (μU/ml)	15.17 ± 10.07
HbA1c (%)	6.00 ± 0.82
HOMA-IR	4.03 ± 3.19
Insulinogenic index	1.07 ± 1.01
HOMA-β	141.00 ± 95.74
QUICKI	0.32 ± 0.03
AUC-IRI (≤ 120 min)	11 390.19 ± 7503.41
AUC-IRI (≤ 180 min)	16 858.73 ± 10 822.28
Histological findings	
Fibrosis (F0/F1/F2/F3/F4)	21/150/197/124/10
Steatosis (G1/G2/G3)	249/141/112

Data are shown as mean ± SD for quantitative data and as numbers for qualitative data.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC-IRI, area under the curve of total insulin secretion; BUN, blood urea nitrogen; BW, body weight; ChE, cholinesterase; Crn, creatinine; Fe, plasma iron; f-IRI, fasting immunoreactive insulin; FPG, fasting plasma glucose; GGT, γ-glutamyl transpeptidase; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-β, homeostatic model assessment beta cell function; LDL, low-density lipoprotein cholesterol; Plt, platelet; QUICKI, Quantitative Insulin-sensitivity Check Index; T-Bil, total bilirubin; TC, total cholesterol; TG, triglycerides; WBC, white blood cell.

According to the patterns of the PG concentration investigated by 75gOGTT, the PG concentration at 180 min after oral glucose loading [PG (180 min)] (89.2 ± 22.3 mg/dl) was markedly lower than the FPG concentration (94.5 ± 8.1 mg/dl) in patients with NGT ($P < 0.01$, Fig. 1). In contrast, the PG (180 min) concentration was significantly higher than the FPG concentration in patients with IGT/IFG and DM (112.8 ± 30.3 and 102.1 ± 10.3 mg/dl in IGT/IFG, and 182 ± 77.5 and 126 ± 27.5 mg/dl in DM, respectively, $P < 0.001$).

We further divided the patients into the Hypo subgroup [PG (180 min) < FPG] and the Hyper subgroup [PG (180 min) ≥ FPG]. The number of patients in the Hypo subgroup was markedly higher than that in the Hyper subgroup (58%) in the NGT group. In contrast, for both the DM and the IGT/IFG groups, the number of patients in the Hypo subgroup (16 and 36%, respectively) was lower than that in the Hyper subgroup (84 and 64%, respectively) (Fig. 2a). From the viewpoint of the Hypo and Hyper subgroups, the proportion of patients with NGT in the Hypo subgroup was markedly higher than that in the Hyper subgroup (Fig. 2b).

Table 2 compares the laboratory characteristics associated with glucose metabolism between the Hyper and Hypo subgroups. Notably, the insulinogenic index and area under the curve of total insulin secretion (AUC-IRI) at less than or equal to 120 min after oral glucose loading [AUC-IRI (≤ 120 min)] in the Hypo subgroup were higher than those in the Hyper subgroup. Conversely, the FPG concentration was lower in the Hypo than Hyper subgroup.

We further divided the patients into Hyper, Hypo, and less than or equal to 70 [PG (180 min) of ≤ 70 mg/dl]

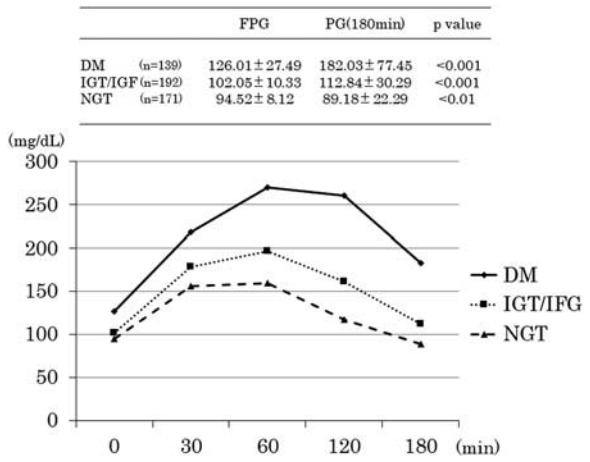

Fig. 1. Patterns of glucose concentration changes in the 75-g oral glucose tolerance test (75gOGTT) in each group. The plasma glucose (PG) concentration at 180 min [PG (180 min)] was markedly lower than the fasting-PG (FPG) concentration in patients with normal glucose tolerance (NGT). PG (180 min) was significantly higher than the FPG concentration in patients with impaired glucose tolerance or impaired fasting glucose (IGT/IFG) and diabetes mellitus (DM). Data are presented as mean ± SD.

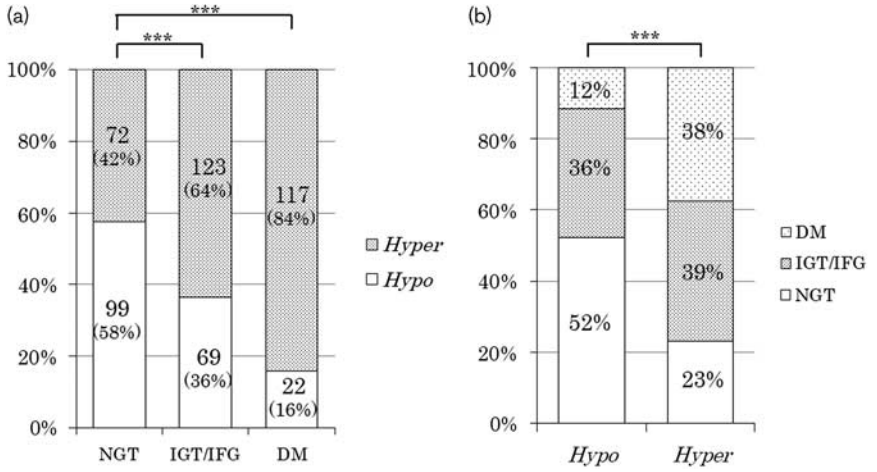

Fig. 2. Proportion of Hyper and Hypo subgroups in each grade of glucose intolerance as determined by the 75-g oral glucose tolerance test (75gOGTT). The patients were divided into two subgroups: Hyper [plasma glucose (PG) (180 min) ≥ fasting-PG (FPG)] and Hypo [PG (180 min) < FPG]. (a) The number of patients in the Hypo subgroup was lower than that in the Hyper subgroup among patients with impaired glucose tolerance or impaired fasting glucose (IGT/IFG) and diabetes mellitus (DM) (36 and 16%, respectively). Among patients with normal glucose tolerance (NGT), the number of patients was higher in the Hypo than the Hyper subgroup (58%). (b) From the viewpoint of Hypo and Hyper subgroups, the proportion of patients with NGT in the Hypo subgroup was markedly higher than that in the Hyper subgroup. ***$P < 0.001$.

Table 2. Comparison of laboratory characteristics associated with glucose metabolism between the Hyper and Hypo subgroups in patients with nonalcoholic fatty liver disease

Overall	Hyper ($N = 312$)	Hypo ($N = 190$)	P value
FPG (mg/dl)	108.68 ± 24.64	101.92 ± 11.01	< 0.001
f-IRI (μU/ml)	15.41 ± 10.27	14.77 ± 9.75	NS
HbA1c (%)	6.14 ± 0.94	5.76 ± 0.50	< 0.001
HOMA-IR	4.22 ± 3.43	3.72 ± 2.72	NS
Insulinogenic index	0.93 ± 0.86	1.28 ± 1.17	< 0.001
HOMA-β	141.03 ± 100.35	140.95 ± 87.86	NS
QUICKI	0.32 ± 0.03	0.33 ± 0.03	NS
AUC-IRI (≤ 120 min)	10 665.20 ± 6990.48	12 580.71 ± 8157.19	< 0.01
AUC-IRI (≤ 180 min)	16 648.78 ± 10421.61	17 203.50 ± 11 469.76	NS

AUC-IRI, area under the curve of total insulin secretion; f-IRI, fasting immunoreactive insulin; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-β, homeostatic model assessment β-cell function; QUICKI, Quantitative Insulin-sensitivity Check Index.

subgroups for each level of glucose intolerance and compared the data among the three groups (Table 3). Among patients with IGT/IFG and NGT, the FPG concentration in the Hyper subgroup was significantly lower than that in the Hypo subgroup ($P < 0.001$ and <0.01, respectively). In contrast, among patients with DM, the FPG concentration in the Hyper subgroup was significantly higher than that in the Hypo subgroup ($P < 0.01$). For all patients, no differences in f-IRI or HOMA-IR were found between the Hyper and Hypo subgroups. In patients with NGT, however, HOMA-β in the Hypo subgroup was lower than that in the Hyper subgroup, although the FPG concentration in the Hypo subgroup was significantly higher than that in the Hyper subgroup. No difference in the insulinogenic index was found between the Hypo and Hyper subgroups in any groups, although PG concentrations at 30 min in the Hypo subgroup seemed to be higher than those in the Hyper subgroup in all NAFLD patients (Table 2). Among the three groups, the AUC-IRI in the Hypo subgroup tended to be higher than that in the Hyper subgroup (Table 3). Next, we compared the less than or equal to 70 and Hyper subgroups in each group. In patients with NGT, the average AUC-IRI (≤ 120 min) in the less than or equal to 70 subgroup was significantly higher than that in the Hyper subgroup (Table 3). In addition, in patients with DM and IGT/IFG, AUC-IRI (≤ 120 min) tended to be higher in the less than or equal to 70 than the Hyper subgroup.

On the basis of the patterns of the PG concentration and insulin secretion, the early PG concentration at 30 min was significantly higher in the less than or equal to 70 than the Hyper subgroup among patients with NGT and IGT/IFG (Fig. 3). However, the early insulin secretion at 30 min was not different between the less than or equal to 70 and Hyper subgroups.

We also examined the quantity of the elevations in PG and secretion of plasma insulin early after oral glucose loading in the Hyper, Hypo, and less than or equal to 70 subgroups in each group (Table 4). The following definitions were used:

$$\Delta\text{Glu30} = \text{PG30 (PG at 30 min)} - \text{FPG},$$

$$\Delta\text{Glu60} = \text{PG60 (PG at 60 min)} - \text{FPG},$$

$$\Delta\text{ins30} = \text{ins30 (plasma insulin at 30 min)} - \text{f-IRI},$$

$$\Delta\text{ins60} = \text{ins60 (plasma insulin at 60 min)} - \text{f-IRI}.$$

In patients with NGT and IGT/IFG, ΔGlu30 was significantly higher in the Hypo and less than or equal to 70 subgroups than in the Hyper subgroup. In contrast, Δins30 was not different among the subgroups. In patients with DM, no particular differences in these parameters were observed among the subgroups.

Multivariate logistic regression analysis to predict the Hypo subgroups in nonalcoholic fatty liver disease patients

Table 5 shows the findings in the multivariate logistic regression analysis for the prediction of Hypo subgroups.

Table 3. Comparison of laboratory characteristics associated with glucose metabolism among the Hyper, Hypo, and less than or equal to 70 subgroups in each grade of glucose intolerance

NGT	Hyper ($N=72$)	Hypo ($N=99$)	≤70 ($N=39$)
FPG (mg/dl)	92.18±8.45	96.22±7.47**	95.67±8.15*
f-IRI (μU/ml)	14.61±9.21	13.98±9.79	13.81±7.06
HbA1c (%)	5.49±0.31	5.60±0.32*	5.60±0.34
HOMA-IR	3.36±2.13	3.30±2.53	3.33±1.83
Insulinogenic index	1.54±1.07	1.54±1.16	1.46±0.95
HOMA-β	189.95±127.66	150.80±90.14*	153.08±74.24
QUICKI	0.33±0.03	0.33±0.03	0.33±0.03
AUC-IRI (≤120 min)	11 120.79±6374.52	12 496.22±7591.52	13 811.74±7217.49*
AUC-IRI (≤180 min)	16 054.21±9619.98	16 171.41±9723.04	17 590.73±9405.16
IGT/IFG	**Hyper ($N=123$)**	**Hypo ($N=69$)**	**≤70 ($N=17$)**
FPG (mg/dl)	100.03±10.58	105.64±8.86***	106.47±9.78*
f-IRI (μU/ml)	15.17±10.23	14.86±8.72	14.90±9.77
HbA1c (%)	5.83±0.49	5.90±0.53	5.97±0.47
HOMA-IR	3.75±2.80	3.99±2.43	4.03±2.89
Insulinogenic index	1.03±0.70	1.13±1.23	0.83±0.64
HOMA-β	151.69±95.15	129.80±84.80	120.91±70.99
QUICKI	0.33±0.03	0.32±0.03	0.32±0.03
AUC-IRI (≤120 min)	11 574.26±6378.85	13 173.47±9454.18	12 019.01±8030.75
AUC-IRI (≤180 min)	18 317.58±10351.49	18 773.00±14106.57	16 079.18±10467.07
DM	**Hyper ($N=117$)**	**Hypo ($N=22$)**	**≤70 ($N=6$)**
FPG (mg/dl)	127.92±29.05	115.86±13.18**	118.50±15.40
f-IRI (μU/ml)	16.16±10.96	18.21±12.22	18.68±16.21
HbA1c (%)	6.84±1.09	6.07±0.75**	5.91±0.52*
HOMA-IR	5.25±4.33	5.12±3.88	5.58±5.39
Insulinogenic index	0.46±0.58	0.60±0.55	0.62±0.40
HOMA-β	99.71±64.57	125.22±87.92	128.94±101.75
QUICKI	0.31±0.03	0.31±0.03	0.31±0.03
AUC-IRI (≤120 min)	9429.15±7797.57	11 101.81±6083.30	11 853.75±7087.88
AUC-IRI (≤180 min)	15 260.28±10804.21	16 925.34±9233.66	17 368.25±11430.91

AUC-IRI, area under the curve of total insulin secretion; DM, diabetes mellitus; f-IRI, fasting immunoreactive insulin; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-β, homeostatic model assessment β-cell function; IGT/IFG, impaired glucose tolerance or impaired fasting glucose; NGT, normal glucose tolerance; QUICKI, Quantitative Insulin-sensitivity Check Index.
Versus Hyper: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Nineteen independent factors including age, sex (female = 1 and male = 2), BMI, hemoglobin, AST, ALT, AST/ALT ratio, albumin, FPG, HbA1c, f-IRI, insulinogenic index, AUC-IRI (≤120 min), ferritin, type IV collagen 7s, procollagen-III-peptide, FIB-4 index, and hepatic steatosis grade and fibrosis stage in biopsy were selected as variables. As determined by multivariate logistic regression analysis, HbA1c [$P=0.004$; Z: −2.86; odds ratio (OR): 0.425; 95% confidence interval (CI): 0.237–0.763], f-IRI ($P=0.038$; Z: −2.08; OR: 0.959; 95% CI: 0.921–0.998), AUC-IRI (≤120 min) ($P=0.028$; Z: 2.20; OR: 1.000; 95% CI: 1.000–1.000), and ferritin ($P=0.046$; Z: −2.00; OR: 0.999; 95% CI: 0.997–1.000) were selected as independent factors that were associated significantly with the Hypo subgroup among all 19 variables. The pseudo R^2 in the built model consisting of these 19 variables was 0.1207.

Use of continuous glucose monitoring to clarify the association between hypoglycemia and nonalcoholic fatty liver disease

As described above, the 75gOGTT showed more patients with Hypo in the IGT/IFG and NGT groups than in the DM group. However, whether this hypoglycemia was similar to that occurring in everyday life in patients with normal eating habits remained unclear. We therefore analyzed these patients by CGM.

We investigated the variability in the glucose concentration by CGM in six patients with DM, five patients with IGT/IFG, and nine patients with NGT as defined by the 75gOGTT in the patients with NAFLD. Table 6 compares the CGM parameters between the patients with Hyper (CGM) (PG 180 min after breakfast ≥ FPG by CGM) and Hypo (CGM) (PG 180 min after breakfast <FPG by CGM), and among the patients with DM, IGT/IFG, and NGT. Supplementary Fig. 1 (Supplemental digital content 2, *http://links.lww.com/EJGH/A275*) shows the time course of the typical pattern CGM in the Hypo (CGM) subgroup of patients.

There were no significant differences in the average PG, average SD, maximum PG, minimum PG, peak PG (after breakfast), time to the peak PG, and Δpeak – FPG between Hyper (CGM) and Hypo (CGM) subgroups (Table 6). In addition, no differences were found in the distribution of glucose intolerance (DM, IGT/IFG, and NGT) and in the stage of hepatic fibrosis between Hyper (CGM) and Hypo (CGM) subgroups. According to the 75gOGTT, the number of patients in the Hyper and Hypo subgroups were nine and four in Hyper (CGM) and six and one in Hypo (CGM) respectively, with no significant difference. Conversely, the 75gOGTT showed that among the DM, IGT/IFG, and NGT groups, the distribution of Hyper (CGM)/Hypo (CGM) patients was not correlated with that of Hyper (OGTT)/Hypo (OGTT) patients. The time to the peak PG in patients with DM was markedly longer than that in patients with IGT/IFG and NGT (DM, 75±23 min; IGT/IFG, 44±9.6 min; and NGT, 48±18.7 min; DM vs.

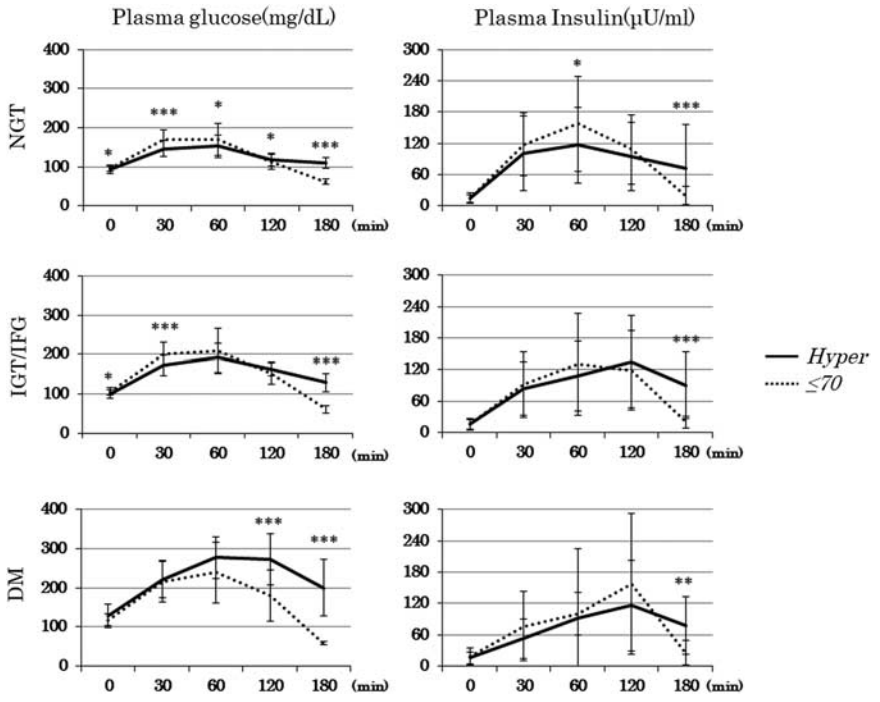

Fig. 3. Patterns of glucose level and insulin secretion in the Hyper and less than or equal to 70 subgroups as determined by the 75gOGTT in each grade of glucose intolerance. The glucose concentration in the less than or equal to 70 subgroup in the early stage (at 30 and 60 min) after oral glucose loading was significantly higher than that in the Hyper subgroup among patients with normal glucose tolerance (NGT) and impaired glucose tolerance or impaired fasting glucose (IGT/IFG). Insulin secretion in the less than or equal to 70 subgroup at 60 min was also higher than that in the Hyper subgroup among patients with NGT. Hyper versus less than or equal to 70: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Table 4. Comparison of the quantity of the elevations in plasma glucose and secretion of plasma insulin early after oral glucose loading in the Hyper, Hypo, and less than or equal to 70 subgroups in each group

NGT	Hyper ($N=72$)	Hypo ($N=99$)	≤70 ($N=39$)
ΔGlu30	54.36±21.26	67.85±22.73***	73.00±22.67***
Δins30	83.56±65.36	97.53±68.59	101.77±56.14
ΔGlu60	59.75±27.51	67.97±38.05	71.49±41.45
Δins60	100.25±65.65	118.83±82.98	140.38±86.15*
IGT/IFG	**Hyper ($N=123$)**	**Hypo ($N=69$)**	**≤70 ($N=17$)**
ΔGlu30	73.24±21.91	83.38±24.71**	94.77±26.57***
Δins30	68.19±44.32	86.55±87.85	75.32±58.78
ΔGlu60	92.84±34.39	99.48±45.82	102.18±54.85
Δins60	92.15±59.09	108.60±79.78	109.00±93.78
DM	**Hyper ($N=117$)**	**Hypo ($N=22$)**	**≤70 ($N=6$)**
ΔGlu30	93.69±28.75	83.19±30.97	96.33±54.63
Δins30	36.03±31.56	46.82±43.31	58.12±64.58
ΔGlu60	149.14±34.82	116.67±51.47**	121.00±77.33
Δins60	75.90±128.74	79.47±51.58	81.82±50.57

ΔGlu30 = PG30 (PG at 30 min) − FPG, ΔGlu60 = PG60 (PG at 60 min) − FPG. Δins30 = ins30 (plasma insulin at 30 min) − f-IRI, Δins60 = ins60 (plasma insulin at 60 min) − f-IRI.
DM, diabetes mellitus; f-IRI, fasting immunoreactive insulin; FPG, fasting plasma glucose; IGT/IFG, impaired glucose tolerance or impaired fasting glucose; NGT, normal glucose tolerance; PG, plasma glucose.
Versus Hyper: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

IGT/IFG, $P=0.02$; DM vs. NGT, $P=0.028$). Taken together, these findings indicate that no remarkable relationships were present between the 75gOGTT and the CGM system with respect to hypoglycemia. However, although only seven of 20 patients were Hypo (CGM), 14 of 20 patients had a PG concentration after breakfast that became lower than the FPG concentration until lunch. In other words, 70% of patients with NAFLD had postprandial hypoglycemia until lunch. Moreover, the average time until the PG concentration became lower than the FPG concentration after breakfast was 178.9±48.8 min in 14 patients.

Discussion

Patients with NAFLD and NASH often have metabolic disorders including IR and type 2 DM. In particular, IR is considered to be one of most important background factors for the development of NAFLD and NASH [7,17]. In the present study, we have clarified the mechanisms of and relationship between postprandial hypoglycemia and hyperinsulinemia in patients with NAFLD by performing the 75gOGTT and CGM.

Figure 2 shows that the proportion of postprandial hypoglycemia among patients with NGT was particularly higher than that among patients with IGT/IFG and DM. Among patients with NGT, the secretion of f-IRI was relatively low in the Hypo subgroup because HOMA-β was significantly lower in the Hypo than the Hyper subgroup (although the FPG concentration was higher in the Hypo than the Hyper subgroup) (Table 3). Moreover, although ΔGlu30 (Table 4) and PG concentration at 30 min (Fig. 3) were higher in the Hypo than Hyper subgroups, there were no significant differences in Δins30 (Table 4) or the insulinogenic index (Table 3) between the Hypo and Hyper subgroups. These results indicate that not only fasting but also early secretion of insulin after a meal were relatively low in the Hypo subgroup of NAFLD

Table 5. Univariate analysis and multivariate logistic regression analysis to predict the Hypo subgroups in nonalcoholic fatty liver disease patients

			Univariate	Multivariate			
	Hyper ($N=312$)	Hypo ($N=190$)	P value	P value	Z value	Odds ratio	95% CI
Sex (female/male)	133/179	64/126	<0.05	–	–	–	–
Age (years)	55.04 ± 16.03	50.66 ± 13.12	<0.001	–	–	–	–
BMI (kg/m^2)	28.11 ± 4.45	28.95 ± 4.91	<0.05	–	–	–	–
AST (IU/l)	56.05 ± 31.01	48.97 ± 28.84	<0.05	–	–	–	–
ALT (IU/l)	91.67 ± 54.78	86.19 ± 51.32	NS	–	–	–	–
AST/ALT ratio	0.68 ± 0.27	0.61 ± 0.21	<0.005	–	–	–	–
Albumin (g/dl)	4.59 ± 0.39	4.65 ± 0.4	NS	–	–	–	–
FPG (mg/dl)	108.68 ± 24.64	101.92 ± 11.00	<0.001	–	–	–	–
f-IRI (mg/dl)	15.41 ± 10.27	14.77 ± 9.75	NS	0.038	−2.08	0.959	0.921–0.998
HbA1c (%)	6.14 ± 0.94	5.76 ± 0.50	<0.001	0.004	−2.86	0.425	0.237–0.763
Insulinogenic index	0.93 ± 0.86	1.28 ± 1.17	<0.001	–	–	–	–
AUC-IRI(≤ 120 min)	10 665.20 ± 6990.48	12 580.71 ± 8157.19	<0.01	0.028	2.2	1.000	1.000–1.000
Hb (g/dl)	14.77 ± 1.58	15.1 ± 1.46	<0.05	–	–	–	–
Plt ($\times 10^4$/μl)	22.17 ± 5.95	22.73 ± 5.55	NS	–	–	–	–
Ferritin (ng/ml)	280.61 ± 251.57	221.71 ± 224.51	<0.01	0.046	−2.00	0.999	0.997–1.000
Type IV collagen 7s (ng/ml)	4.28 ± 1.55	3.92 ± 1.12	<0.01	–	–	–	–
Type III procollagen N peptide (U/ml)	0.68 ± 0.36	0.61 ± 0.27	<0.05	–	–	–	–
FIB-4 index	1.75 ± 1.39	1.37 ± 1.05	<0.001	–	–	–	–
Histological findings							
Fibrosis (F0/F1/F2/F3/F4)	14/93/116/81/8	7/57/81/43/2	NS	–	–	–	–
Steatosis (G1/G2/G3)	149/93/70	100/48/42	NS	–	–	–	–

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC-IRI, area under the curve of total insulin secretion; f-IRI, fasting immunoreactive insulin; FPG, fasting plasma glucose; Hb, hemoglobin; Plt, platelet; HbA1c, hemoglobin A1c.

Table 6. Comparison of the parameters of continuous glucose monitoring between the patients with Hyper (continuous glucose monitoring) and Hypo (continuous glucose monitoring), and among the patients with diabetes mellitus, impaired glucose tolerance or impaired fasting glucose, and normal glucose tolerance

	Hyper (CGM) ($N=13$)	Hypo (CGM) ($N=7$)	P	NGT(on OGTT) ($N=9$)	IGT/IFG (on OGTT) ($N=5$)	DM (on OGTT) ($N=6$)
Average PG (mg/dl)	123.71 ± 25.67	127.24 ± 36.86	NS	110.53 ± 11.73	111.76 ± 13.21	157.55 ± 32.25**,##
Average SD (mg/dl)	27.91 ± 11.64	28.13 ± 21.32	NS	20.45 ± 9.56	26.81 ± 11.81	40.27 ± 18.10*
Maximum PG (mg/dl)	195.15 ± 43.59	212.57 ± 95.81	NS	174.56 ± 52.38	188.00 ± 46.16	252.33 ± 71.89*
Minimum PG (mg/dl)	74.77 ± 19.43	82.86 ± 10.95	NS	75.89 ± 9.92	66.20 ± 16.05	89.67 ± 20.88
ΔMaximum–minimum PG (mg/dl)	120.38 ± 45.27	129.71 ± 91.18	NS	98.67 ± 50.08	121.80 ± 56.37	162.67 ± 73.93
Peak PG (mg/dl)	182.23 ± 43.48	209.86 ± 97.79	NS	170.33 ± 54.22	160.40 ± 25.30	250.50 ± 74.45*,#
Time to the peak PG (min)	56.54 ± 25.44	52.86 ± 14.68	NS	48.33 ± 18.71	44.00 ± 9.62	75.00 ± 23.02*,#
ΔPeak−FPG (mg/dl)	74.23 ± 28.61	83.86 ± 76.84	NS	65.33 ± 46.28	56.80 ± 21.39	108.67 ± 62.21
ΔPeak−FPG/time (mg/dl × min)	1.42 ± 0.53	1.41 ± 0.98	NS	1.33 ± 0.65	1.37 ± 0.64	1.49 ± 0.97
DM/IGTorIFG/NGT	4/3/6	2/2/3	NS	–	–	–
Hyper/Hypo (on OGTT)	9/4	6/1	NS	5/4	5/0	5/1
Hyper/Hypo (on CGM)	–	–	NS	6/3	3/2	4/2
Histological findings						
Fibrosis (F0/F1/F2/F3/F4)	0/5/2/4/2	1/3/1/2/0	NS	1/5/1/1/1	0/2/9/0/0	0/1/2/2/1
Steatosis (G1/G2/G3)	5/3/5	2/2/3	NS	3/2/4	2/2/1	2/1/3

Data are expressed as average ± SD.
Average PG: average of the average PG of the patients during a 24-h monitoring period.
Average SD: average SD of PG of the patients during a 24-h monitoring period.
Maximum and Minimum PG: highest and lowest PG during a 24-h monitoring period, respectively.
ΔMaximum–minimum PG: difference between maximum and minimum PG.
Hyper (CGM): the patient whose PG (180 min) after breakfast ≥ FPG on CGM.
Hypo (CGM): the patient whose PG (180 min) after breakfast < FPG on CGM.
Peak PG : the first peak PG after breakfast on CGM.
Time to the peak PG: the time to the peak PG from breakfast on CGM.
ΔPeak−FPG: difference between peak PG and FPG.
P values were calculated using Student's t-test and Welch's t-test and Fisher's exact test.
CGM, continuous glucose monitoring; DM, diabetes mellitus; FPG, fasting plasma glucose; IGT/IFG, impaired glucose tolerance or impaired fasting glucose; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PG, plasma glucose.
Versus NGT: *$P<0.05$, **$P<0.01$.
Versus IGT/IFG: #$P<0.05$, ##$P<0.01$.

patients. Conversely, AUC-IRI (≤ 120 min) was significantly higher in the Hypo than the Hyper subgroup (Table 3). Similar tendencies were found in patients with IGT/IFG, but not in those with DM. According to the multivariate logistic regression analysis, low HbA1c, low f-IRI, and high AUC-IRI (< 120 min) were shown to be independent factors associated with the hypoglycemia (Table 5). However, no association was found between the development of hepatic fibrosis and hypoglycemia because the hepatic fibrosis markers, FIB-4 index, type IV collagen 7s, type III procollagen N peptide, or histological findings were not selected as the independent factors for hypoglycemia in multivariate logistic regression analysis. Manchanayaka *et al.* [18] reported that all nondiabetic patients with NAFLD

in their study had postprandial hyperinsulinemia. Taken together with these results, the cause of postprandial hypoglycemia in patients with NGT and IGT/IFG might be related closely to low HbA1c, early elevation in the PG concentration, low fasting and relatively low early insulin secretion, and delayed hyperinsulinemia.

Late dumping syndrome is a known cause of reactive hypoglycemia after a meal. This type of postprandial hypoglycemia occurs after gastrectomy or bariatric surgery [Roux-en-Y gastric bypass (RYGB) surgery or laparoscopic sleeve gastrectomy] [19,20]. Up to 30% of patients reportedly develop postprandial hypoglycemia after RYGB or laparoscopic sleeve gastrectomy [21–23]. After bariatric surgery, massive meals are more rapidly delivered from the stomach to the small intestine. This results in exposure of the distal intestine to higher volumes of carbohydrates, and absorption of glucose into the bloodstream is thus encouraged. Postprandial hyperglycemia then develops, which stimulates rapid and excessive secretion of insulin, and late hypoglycemia follows [19,20]. According to the abovementioned report [20], the time to peak PG early after oral glucose loading was around 45 min in patients who developed hypoglycemia after RYGB and insulin secretion was more excessive than before RYGB. In the present study, the time to the peak PGs from breakfast in patients with NGT (48.33 ± 18.71 min) and IGT/IFG (44.00 ± 9.62 min) were markedly shorter than that in patients with DM (75.00 ± 23.02 min, Table 6). Although no patients with NAFLD in the present study underwent gastrectomy or bariatric surgery, it was clarified that the PG concentration after oral glucose administration increased more quickly (Tables 4 and 6), and insulin secretion seemed to be excessive in the NGT and IGT/IFG groups than in the DM group (Tables 3 and 4). In addition, the average AUC-IRI (≤ 120 min) of the Hypo subgroups were higher in the NGT and IGT/IFG groups than in the DM group (Table 3). Considering the findings of previous studies as well as our results, common mechanisms between postprandial hypoglycemia in patients with NAFLD and postoperative symptoms of late dumping syndrome may exist from the point of view that early hyperglycemia leads to excessive secretion of insulin and delayed hyperinsulinemia, particularly in patients with NGT and IGT/IFG.

In 20 patients with NAFLD who underwent CGM, the Hyper and Hypo distributions according to the 75gOGTT were nine and four of 13 patients in the Hyper (CGM) group and six and one of seven patients in the Hypo (CGM) group, respectively (Table 6). The distributions of glucose intolerance according to the 75gOGTT were six, five, and nine patients with DM, IGT/IFG, and NGT, respectively, and the distributions of Hyper and Hypo according to the OGTT were five and one, five and zero, and five and four, respectively. The proportion of Hypo according to the 75gOGTT among patients with NGT was higher than that in the other groups. However, the distributions of Hyper and Hypo according to CGM for each level of glucose intolerance were four and two (DM), three and two (IGT/IFG), and six and three (NGT). In other words, the number of patients with hypoglycemia determined by CGM did not always match that determined by 75gOGTT. Overall, the distributions of Hyper and Hypo were not always correlated between CGM and the OGTT.

Although seven of 20 patients had Hypo (CGM) (PG 180 min after breakfast < FPG by CGM), in 14 of 20 patients, PG concentration decreased more than the FPG concentration until lunch. In other words, 70% of patients with NAFLD had postprandial hypoglycemia until lunch. Moreover, the average time until postprandial hypoglycemia after breakfast was 178.9 ± 48.8 min in 14 patients (data not shown). This result indicates that the hypoglycemia time was mostly concentrated around 180 min after breakfast.

Our study has several limitations. First, energy intake, body fat mass, and skeletal muscle mass were not evaluated. Second, the evaluation of the type and amount of meal in the daily life were not performed.

Conclusion

We have clarified that the proportion of patients who develop postprandial hypoglycemia is higher among those with NGT than among those with IGT/IFG and DM. The development of postprandial hypoglycemia in patients with NAFLD depends on low HbA1c, early elevation in the PG concentration, low fasting and relatively low early insulin secretion, and delayed hyperinsulinemia. In addition, the development and characteristics of Hyper and Hypo are not always correlated between CGM and the OGTT. Therefore, the 75gOGTT and/or CGM should be performed because they provide data that are very important and useful for the evaluation and understanding of postprandial hyperglycemia and hypoglycemia in patients with NAFLD.

Acknowledgements

This study was funded by Grants-in-Aid for Scientific Research (C) 2011 (#23590979) and 2014 (#26461010) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and the Program for Basic and Clinical Research on Hepatitis from the Japan Agency for Medical Research and Development (AMED), Japan.

Conflicts of interest

There are no conflicts of interest.

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