both nuclear CHOP and cytoplasmic insulin immunopositivity was determined. The ratio of CHOP-positive β-cells was calculated by adjusting the number of CHOP-positive β-cells by the insulin-positive area[20]. The effect of Ex-4 treatment on β-cell replication and apoptosis was evaluated histologically by proliferating cell nuclear antigen (PCNA) staining (Abcam, Cambridge, MA, USA) and TdT-mediated dUTP-biotin nick-end labeling (TUNEL) staining (Takara Bio, Otsu, Japan), respectively. The ratio of TUNEL-positive and PCNA-positive β-cells was also calculated as described earlier.

Statistical Analysis

Data are presented as means ± SEM. Statistical analyses were carried out by unpaired *t*-test. A *P*-value of <0.05 was considered significant.

RESULTS

Effect of Ex-4 on Hyperglycemia and Bodyweight in Akita Mice

Akita mice showed acute and progressive hyperglycemia at 14 days after birth and thereafter. Twice-daily intraperitoneal injection of Ex-4 from 3 to 5 weeks-of-age significantly reduced blood glucose levels compared with those in PBS-treated mice (Figure 1a). Plasma glucose levels in phlorizin-treated Akita mice were similar to those in Ex-4-treated mice. Plasma glycoalbumin levels were significantly lower in the Ex-4- and phlorizin-treated groups than those in the PBS-treated group, but no significant difference was observed between the Ex-4- and phlorizin-treated groups (12.9 ± 1.5 *vs* 8.7 ± 0.7 *vs* 8.2 ± 0.6, respectively, n = 10–12). Ex-4 treatment or phlorizin treatment did not change bodyweight compared with PBS treatment (Figure 1b). Ex-4 or phlorizin treatment did not change the amount of food intake assessed at 4 weeks-of-age (data not shown).

Effect of Ex-4 on Insulin-Positive Area and Number of Islets

Preservation of β-cell morphology was observed by treatment with Ex-4, as shown in Figure 2a. Quantitative histological analyses showed that Ex-4 treatment significantly increased both the insulin-positive area and the number of islets, whereas there was no significant difference between the PBS-treated group and the phlorizin-treated group (Figure 2b,c).

Effect of Ex-4 on Pancreatic Insulin Content

Figure 2d shows the effect of Ex-4 treatment on insulin content in pancreatic islets. Treatment with Ex-4 significantly increased insulin content in isolated islets, but phlorizin treatment did not.

Quantitative Estimation of CHOP and BiP Expression Levels by Real-Time PCR

The expression levels of CHOP mRNA are shown in Figure 3a, and those of BiP mRNA are shown in Figure 3b. Ex-4 significantly lowered the expression levels of CHOP and BiP mRNA, but there was no significant difference in the expression levels of CHOP or BiP mRNA between the phlorizin- or PBS-treated groups.

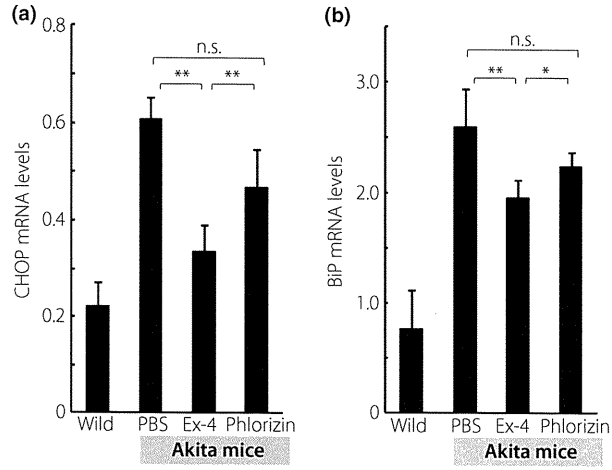

Figure 3 | Ex-4 treatment resulted in a significant decrease in the expression levels of C/EBP-homologous protein (CHOP) mRNA and Bip mRNA in Akita mice. (a) mRNA expression levels of CHOP were evaluated by quantitative real-time polymerase chain reaction (PCR). (b) mRNA expression levels of BiP were evaluated by quantitative real-time PCR. Data are expressed as the ratio to that of glyceraldehyde 3-phosphate dehydrogenase in the same sample (n = 5 for each group). Each column represents mean ± SE. *P < 0.05, **P < 0.01.

Effect of Ex-4 on the Ratio of CHOP-, TUNEL- and PCNA-Positive β-cells

Figure 4a depicts the representative pancreata stained with insulin (red), CHOP (green) and DAPI (blue), respectively. Similarly, Figure 5a shows the representative pancreata stained with insulin (red) and TUNEL (green). Treatment with Ex-4 significantly decreased the ratio of CHOP-positive β-cells and TUNEL-positive β-cells (Figures 4b and 5b), but there was no significant difference in the ratio of CHOP-positive or TUNEL-positive β-cells between the PBS- and phlorizin-treated groups. Figure 6a shows the representative pancreata stained with insulin (red) and PCNA (green). PCNA staining showed no significant difference in proliferation of β-cells among the three groups of Akita mice (Figure 6b). Interestingly, the ratio of PCNA-positive β-cells was increased in all three groups when compared with wild-type C57BL/6 mice.

DISCUSSION

Akita mice are widely used as an animal model of ER stress-mediated diabetes. Akita mice have a point mutation (C96T) in the insulin 2 gene[21] that disrupts the disulfide bond formation between the A and B chains of proinsulin, resulting in a drastic conformational change of the molecule. The unfolded proinsulin accumulates to the ER, causing severe ER stress leading to β-cell apoptosis. In humans, it has recently been shown that a mutation in the insulin gene, which is identical

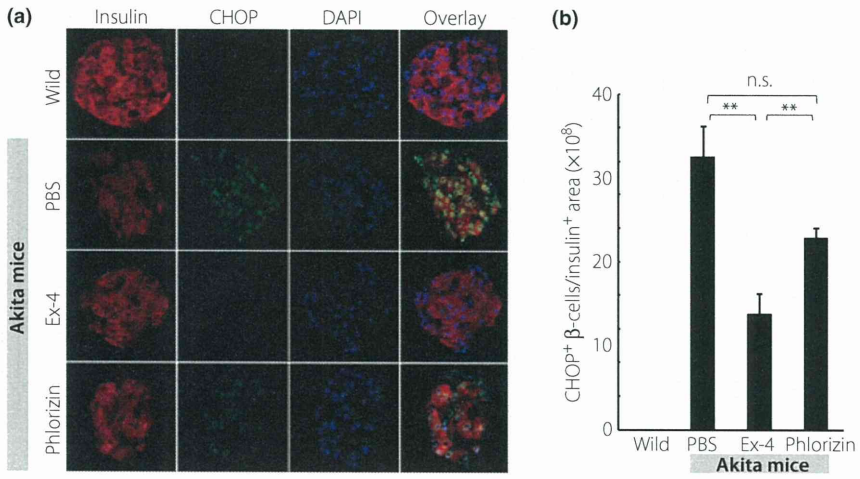

Figure 4 | Ex-4 treatment resulted in a significant decrease in the ratio of C/EBP-homologous protein (CHOP)-positive β-cells in Akita mice. (a) Representative mouse pancreata at 5 weeks-of-age stained with insulin (red), CHOP (green) and DAPI (blue). (b) The number of CHOP-positive β-cells normalized per insulin-positive area was quantified as described in Materials and Methods. Each column represents mean ± SE. $^{**}P < 0.01$.

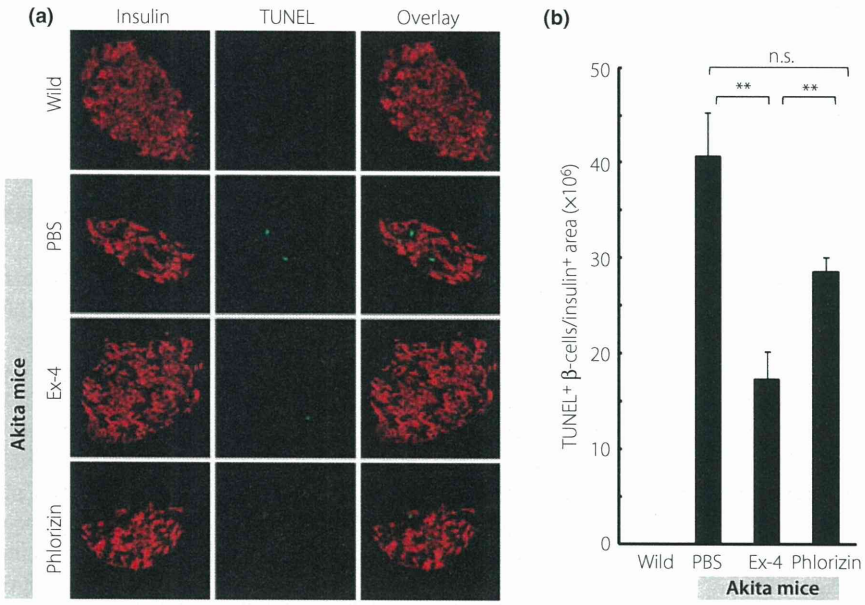

Figure 5 | Ex-4 treatment decreased the ratio of TUNEL-positive β-cells. (a) Representative mouse pancreata at 5 weeks-of-age stained with insulin (red) and TUNEL (green). (b) The number of TUNEL-positive β-cells normalized per insulin-positive area was quantified as described in Materials and Methods. Each column represents mean ± SE. $^{**}P < 0.01$.

to that in the Akita mouse, causes permanent neonatal diabetes within the first month of life that requires lifelong insulin injection[22].

In the present study, we have shown that Ex-4 treatment has a protective effect on β-cells in Akita mice. The insulin-positive area and the number of islets were maintained along with a decreased ratio of CHOP- and TUNEL-positive cells in the islets, showing that the major effect of Ex-4 treatment in the maintenance of β-cell mass is through decreasing β-cell apoptosis in response to ER stress. Because phlorizin decreases blood glucose levels without increasing insulin secretion, it might well reduce ER stress by decreasing the insulin demand. However, in contrast to the Ex-4 treatment, phlorizin treatment failed to show a reduction of ER stress or β-cell protective effects against

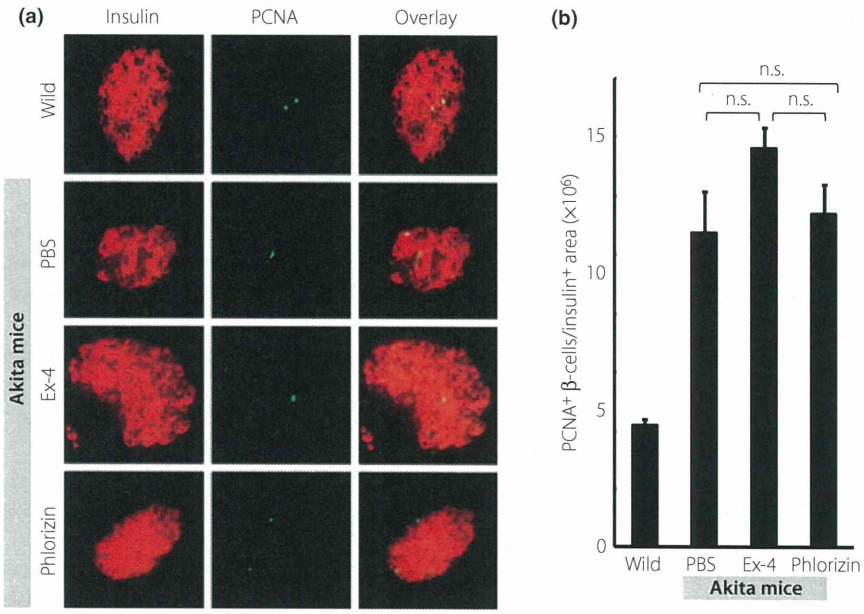

Figure 6 | Ex-4 treatment did not significantly increase the ratio of PCNA-positive β-cells. (a) Representative mouse pancreata at 5 weeks-of-age stained with insulin (red) and PCNA (green). (b) The number of PCNA-positive β-cells normalized per insulin-positive area was quantified as described in Materials and Methods. Each column represents mean ± SE.

apoptosis in our conditions. These findings show that Ex-4 has a direct effect on ER stress-mediated β-cell apoptosis that is independent of decreased insulin demand.

There are several *in vitro* and *in vivo* studies showing that GLP-1R agonists inhibit β-cell apoptosis[9–16], and several molecular mechanisms have been suggested. For example, GLP-1 treatment decreases the expression levels of proapoptotic protein caspase-3 and increases those of anti-apoptotic protein bcl-2 in isolated human islets[10]. It also has been shown that the anti-apoptotic effect of Ex-4 is associated with the activation of protein kinase B/Akt through PKA-dependent phosphorylation of CREB[11]. There are some reports that GLP-1 ameliorates ER stress. Yusta *et al.* found that treatment by Ex-4 reduces blood glucose levels in obese *db/db* mice along with a decrease in the number of CHOP-positive β-cells[20]. Tsunekawa *et al.*[23] reported a beneficial effect of Ex-4 on β-cell damage in calmodulin-over-expressing transgenic (CaMTg) mice that develop diabetes through ER stress-mediated β-cell apoptosis. They found that Ex-4 treatment reduced blood glucose levels while retaining the insulin-positive areas and decreasing the expression levels of CHOP mRNA in CaMTg mice. *In vitro* studies have found that rapid recovery from translational attenuation[19] or upregulation of BiP and JunB[24] accounts for the attenuation of ER stress-mediated β-cell damage by Ex-4 treatment. However, results of chronic Ex-4 treatment in animal models of type 2 diabetes should be carefully interpreted, because enhancement of GLP-1R signaling reduces the blood glucose level by its insulinotropic action. Therefore, the possibility remains that reduced hyperglycemia attenuates persistent ER stress and ameliorates β-cell apoptosis. Our present findings clearly show that Ex-4 treatment attenuates ER stress-mediated β-cell damage in Akita mice through a reduction of apoptotic cell death that is independent of decreased blood glucose levels.

Although several studies have found that the cytoprotective effect of GLP-1R signaling is not only through inhibition of β-cell apoptosis, but also through stimulation of β-cell proliferation[5–9], we did not find any effect of Ex-4 treatment on β-cell proliferation. It is possible that the administration period in the present study was too short to observe β-cell proliferation by Ex-4 or that stimulation of β-cell proliferation does not play a significant role in the cytoprotective effect of GLP-1R signaling in Akita mice. The ratio of PCNA-positive β-cells was increased not only in the Ex-4-treated group of Akita mice, but also in the phlorizin-treated group and the untreated group compared with that in wild-type C57BL/6 mice. Whether or not this result can be attributed to the phenotype of Akita mice requires further study.

Islet mass is reported to be decreased in patients with type 2 diabetes at the time of diagnosis[25]. Although Ex-4 is in clinical use for treatment of type 2 diabetes[26], superiority of Ex-4 over the other antidiabetic drugs has not been shown. Our data confirm the previous findings of a beneficial effect of Ex-4 on glycemic control, but also suggest that Ex-4 has a direct β-cell-protective effect independently of improved glycemic control. Thus, Ex-4 and other GLP-1R agonists might well be more effective than other antidiabetic drugs in clinical use in terms of alleviating β-cell damage and maintaining β-cell mass for diabetic patients.

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Plasma gastric inhibitory polypeptide and glucagon-like peptide-1 levels after glucose loading are associated with different factors in Japanese subjects

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ABSTRACT

Aims/Introduction: Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are major incretins that potentiate insulin secretion from pancreatic β-cells. The factors responsible for incretin secretion have been reported in Caucasian subjects, but have not been thoroughly evaluated in Japanese subjects. We evaluated the factors associated with incretin secretion during oral glucose tolerance test (OGTT) in Japanese subjects with normal glucose tolerance (NGT).

Materials and Methods: We measured plasma GIP and GLP-1 levels during OGTT in 17 Japanese NGT subjects and evaluated the factors associated with GIP and GLP-1 secretion using simple and multiple regression analyses.

Results: GIP secretion (AUC-GIP) was positively associated with body mass index ($P < 0.05$), and area under the curve (AUC) of C-peptide ($P < 0.05$) and glucagon ($P < 0.01$), whereas GLP-1 secretion (AUC-GLP-1) was negatively associated with AUC of plasma glucose ($P < 0.05$). The insulinogenic index was most strongly associated with GIP secretion ($P < 0.05$); homeostasis model assessment β-cell was the most the strongly associated factor in GLP-1 secretion ($P < 0.05$) among the four indices of insulin secretion and insulin sensitivity.

Conclusions: Several distinct factors might be associated with GIP and GLP-1 secretion during OGTT in Japanese subjects.
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KEY WORDS: Gastric inhibitory polypeptide, Glucagon-like peptide-1, Incretin

INTRODUCTION

Oral glucose administration leads to greater insulin release from pancreatic islets than intravenous glucose loading that yields equivalent glucose levels. Gut hormonal substances released in response to glucose include the incretins, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), which are responsible for 50–60% of postprandial insulin secretion[1]. GIP is secreted on meal ingestion from K-cells in the proximal small intestine, whereas GLP-1 is secreted from L-cells in the distal small intestine and colon, and binds to their respective receptors (GIP receptor [GIPR] and GLP-1 receptor) on the surface of pancreatic β-cells to stimulate insulin secretion by increasing the intracellular adenosine 3′,5′-monophosphate (cAMP) concentration[2–4].

Type 2 diabetes is characterized by both decreased insulin secretion and reduced insulin sensitivity[5–7]. The incretin effect has been shown to be reduced in type 2 diabetic subjects compared with those with normal glucose tolerance (NGT) in previous studies[8,9], suggesting that a reduced incretin effect might be associated with hyperglycemia after food intake and glucose loading in type 2 diabetes. When intravenous infusion of GIP or GLP-1 was carried out in type 2 diabetic subjects, GLP-1 potentiated insulin secretion from pancreatic β-cells, but GIP did not, showing that the GIPR signal is downregulated in β-cells in type 2 diabetes[10]. In studies using rodent models, it was reported that GIPR mRNA and protein expression levels in islets are decreased in the diabetic state[11]. In contrast, in the non-diabetic obese state, GIP plays an important role in maintaining blood glucose levels[12]. The GIP signal might be enhanced as a result of increased GIPR sensitivity of β-cells to GIP or increased GIP secretion from K-cells in the non-diabetic obese state. Indeed, GIP concentrations are reported to be increased in obese rodent models and human subjects compared with those in lean rodents and human subjects, respectively[13–15]. Furthermore, we have previously shown the hypersensitivity of GIPR to GIP in β-cells of high fat-induced obese mice[16]. Plasma GLP-1 concentrations in type 2 diabetic patients are reported to be reduced after meal ingestion and glucose loading[9,17]. However, in other studies it was reported that GLP-1 concentrations did not differ

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in NGT and type 2 diabetic subjects[18–20]. Thus, the measurement of GIP and GLP-1 concentrations in various metabolic states is important to evaluate the effects of incretin on insulin secretion.

Insulin sensitivity in Asian subjects has been shown to be higher than in Mexican Americans and Caucasians in previous reports[21,22], which is partly as a result of the fact that Asians, including Japanese, are generally less obese. Furthermore, insulin secretion rather than insulin sensitivity is the more important factor in progression from NGT to diabetes in Japanese subjects[23]. We have reported that early-phase insulin secretion is considerably decreased, even in Japanese NGT subjects with 1-h plasma glucose (PG) levels during oral glucose tolerance test (OGTT) of more than 180 mg/dL[24]. Thus, it is especially important to evaluate incretin secretion and determine the factors associated with incretin secretion in Japanese NGT subjects, because GIP and incretin is responsible for more than 50% of postprandial insulin secretion after glucose ingestion. The factors responsible for incretin secretion have been reported in Caucasian subjects, but have not been thoroughly elucidated in Japanese subjects.

In the present study, we evaluated GIP and GLP-1 levels during OGTT and determined the factors involved in GIP and GLP-1 secretion (area under the curve [AUC] of GIP and GLP-1 during OGTT) in Japanese NGT subjects.

MATERIALS AND METHODS

Subjects

We recruited 17 Japanese healthy volunteers. The subjects had no history of hypertension, hyperlipidemia or kidney and liver diseases, and did not take any drugs 2 weeks before the study. The study was designed in compliance with the ethics regulations of the Helsinki Declaration and Kyoto University. Informed consent was obtained from all subjects.

Study Procedure

The subjects' age, height and bodyweight were determined. Blood samples for the measurement of liver and kidney function, HbA_{1c}, serum triglyceride (TG), total cholesterol and high-density lipoprotein (HDL)-cholesterol levels were drawn after an overnight fast. All subjects received OGTT. After the subjects fasted overnight for 10–16 h, standard OGTT with 75 g glucose was given according to the National Diabetes Data Group recommendations[25]. NGT was diagnosed according to World Health Organization (WHO) criteria[26].

Blood samples were collected at −15, 0, 10, 20, 30, 60, 90, 120, 150 and 180 min after glucose loading and were centrifuged at 1800 *g* at 4°C for 10 min. After collecting supernatant of the samples, plasma and serum were stocked at −80°C. Plasma GIP, GLP-1 levels and the various parameters (PG, serum immunoreactive insulin [IRI], serum C-peptide reactivity [CPR], TG, serum free fatty acid [FFA] and plasma glucagon) were measured at the indicated times (plasma GIP and GLP-1 levels were measured at −15, 0, 10, 30, 60, 90, 120 and 180 min after glucose loading, and plasma glucagon levels were measured at −15, 0, 30, 60, 90, 120 and 180 min after glucose loading). The PG levels were measured by glucose oxidase method. Serum IRI levels were measured by two-site radioimmunoassay. Total GIP and total GLP-1 levels were measured using human GIP ELISA kit (Linco Research, St Charles, MO, USA; range of detection from 8.2 pg/mL to 2000 pg/dL) and human GLP-1 ELISA kit (Meso Scale Discovery, Gaithersburg, MD, USA; range of detection from 2.4 pg/mL to 1,000,000 pg/dL), respectively, as previously described[27,28]. The AUC of PG, IRI, CPR, TG, FFA, glucagon, total GIP (AUC-GIP) and total GLP-1 (AUC-GLP-1) were calculated. We then analyzed the relationship between the AUC of GIP (GIP secretion) and GLP-1 (GLP-1 secretion) and age, body mass index (BMI) and the parameters during OGTT.

Statistical Analysis

Basal insulin secretion and sensitivity were evaluated by homeostasis model assessment (HOMA) β-cell function and homeostasis model assessment of insulin resistance (HOMA-IR)[29,30], respectively. Early-phase insulin secretion and systemic insulin sensitivity during OGTT were evaluated by insulinogenic index[31] and insulin sensitivity index (ISI) composite[32]. The calculations of the four indices were as follows:

$$\text{HOMO } \beta\text{-cell} = 20 \times \text{fasting IRI level (FIRI) (pmol/L)} / (\text{fasting PG level [FPG] [mmol/L]} - 3.5)$$

$$\text{HOMO-IR} = \text{FIRI (pmol/L)} \times \text{FPG (mmol/L)} / 22.5$$

$$\text{Insulinogenic index} = (30 \text{ min IRI} - \text{FIRI [pmol/L]}) / (30 \text{ min PG} - \text{FPG [mmol/L]})$$

$$\text{IRI composite} = 10{,}000 / (\text{FGP [mg/dL]} \times \text{FIRI [}\mu\text{U/mL]} \times \text{mean OGTT PG [mg/dL]} \times \text{mean OGTT IRI [}\mu\text{U/mL]})^{0.5}$$

All analyses were carried out using statistical analysis software (SPSS version 17.0, IBM, Somers, NY, USA) system. Statistical analysis was carried out by ANOVA with Fisher's PLSD test for changing levels of GIP, GLP-1, and the parameters during OGTT and differences between the two groups were assessed by unpaired *t*-test. We used simple regression analysis to determine the relationship between AUC-GIP or AUC-GLP-1 and the age, BMI and the parameters during OGTT, and we carried out multiple regression analysis to determine the factors most strongly associated with AUC-GIP and AUC-GLP-1, and the indices of insulin secretion and sensitivity. Probability (*P*) values <0.05 were considered statistically significant. Data are presented as mean ± standard error (SE).

RESULTS

Table 1 shows clinical characteristics of the subjects. Mean age was 31.7 ± 1.3 years and mean BMI was 23.1 ± 0.9 kg/m^2. No subjects had liver or kidney dysfunction. HbA_{1c}, FPG, TG, total

Table 1 | Clinical characteristics of the subjects

n (male/female)	17 (14/3)
Age (years)	31.7 ± 1.3
Body mass index (kg/m^2)	23.1 ± 0.9
Fasting plasma glucose (mmol/L)	6.1 ± 0.2
Fasting insulin (pmol/L)	25.2 ± 3.7
HbA_{1c} (%)	4.7 ± 0.0
Triglycerides (mmol/L)	2.00 ± 0.31
Total cholesterol (mmol/L)	4.56 ± 0.16
HDL-cholesterol (mmol/L)	1.51 ± 0.10
Insulinogenic index	66.22 ± 8.54
HOMA β-cell	60.85 ± 8.89
HOMA-IR	0.94 ± 0.15
ISI composite	11.45 ± 1.67

Means ± SE. HDL, high-density lipoprotein; HOMA, homeostasis model assessment; HOMA-IR, homeostasis model assessment of insulin resistance; ISI, insulin sensitivity index.

cholesterol and HDL-cholesterol levels were within normal limits in the fasting state.

The levels of GIP, GLP-1, PG, IRI, CPR, TG, FFA and glucagon after glucose loading were measured (Figure 1). The subjects were diagnosed NGT according to WHO criteria with fasting plasma glucose and 2-h glucose levels below 6.1 and 7.8 mmol/L, respectively. Levels of PG, IRI and CPR were significantly increased from 10 min after glucose loading compared with fasting level (Figure 1a–c). FFA levels were significantly decreased from 10 min after glucose loading (Figure 1d). TG levels were not significantly changed during OGTT (Figure 1e). Glucagon levels were significantly decreased from 30 min after glucose loading (Figure 1f). Total GIP levels were significantly increased from 10 min during OGTT (Figure 1g). Total GLP-1 levels were significantly increased from 10 min during OGTT with peaks at 30 and 120 min (Figure 1h).

We analyzed the relationship between AUC-GIP or AUC-GLP-1 and age, BMI and the several parameters (AUC of PG, IRI, CPR, TG, FFA and glucagon). AUC-GIP were positively related to BMI and AUC of CPR, IRI and glucagon, but AUC-GLP-1 was not related to these factors (Figure 2a–c; AUC data of IRI during OGTT are not shown; $P < 0.05$). In contrast, AUC-GLP-1 was inversely related to AUC of PG (Figure 2d), but AUC-GIP was not.

We then analyzed the relationship between AUC-GIP or AUC-GLP-1 and indices of insulin secretion and insulin sensitivity. AUC-GIP was positively related to insulinogenic index and HOMA-IR, whereas AUC-GLP-1 was positively related to HOMA β-cell function (Figure 3a–c). ISI composite was not related to either AUC-GIP or AUC-GLP-1 (Figure 3d). In addition, multiple regression analysis was carried out to determine the factors strongly associated with AUC-GIP and AUC-GLP. The insulinogenic index was the most strongly associated factor in AUC-GIP (correlation coefficients 0.56, standardized β 0.56, $P < 0.05$) of the four indices; HOMA β-cell function was the strongest factor in AUC-GLP-1 (HOMA β-cell function: correlation coefficients 0.524, standardized β 0.870, $P < 0.01$, ISI composite: correlation coefficients 0.063, standardized β 0.581, $P < 0.05$).

DISCUSSION

In the present study, we estimated the incretin level after glucose loading in Japanese NGT subjects and found that plasma GIP and GLP-1 levels during OGTT are related to different factors.

Incretin action of GIP is reduced in the diabetic state as a result of decreased GIP receptor expression on pancreatic β-cells[11], whereas GIP signaling is enhanced and maintains

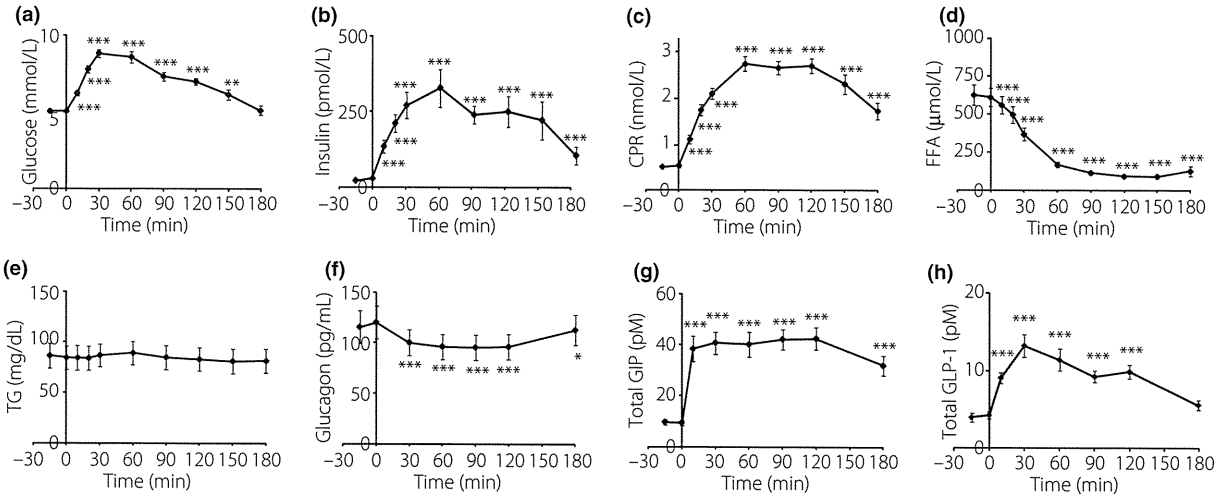

Figure 1 | Concentrations of (a) plasma glucose, (b) serum immunoreactive insulin, (c) serum C-peptide reactivity (CPR), (d) serum free fatty acid (FFA), (e) serum triglyceride (TG), (f) glucagon, (g) total gastric inhibitory polypeptide (GIP) and (h) total glucagon-like peptide-1 (GLP-1) during oral glucose tolerance test in 17 Japanese subjects. Mean ± SE, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* the levels at fasting.

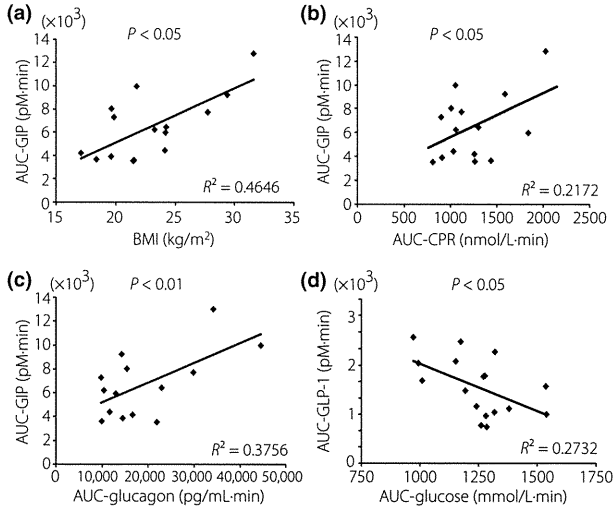

Figure 2 | Simple regression analysis of gastric inhibitory polypeptide secretion (AUC-GIP) and (a) body mass index (BMI), (b) AUC of serum C-peptide reactivity (CPR) and (c) glucagon. (d) Simple regression analysis of glucagon-like peptide-1 secretion (AUC-GLP-1) and AUC of plasma glucose (PG).

glucose homeostasis by compensatory increased insulin secretion in the obese state[15,16]. In some human studies in Caucasians, plasma GIP levels are increased in obese subjects[14,15] and there is a positive relationship between AUC-GIP and AUC of FFA during OGTT[18]. In the present study, AUC-GIP after glucose loading was not associated with AUC of FFA, but was positively associated with BMI, HOMA-IR, and AUC of IRI and CPR after glucose loading. In fact, obese subjects are known to have hyperinsulinemia and insulin resistance[33,34], and BMI was strongly associated with AUC of IRI and CPR. Thus, GIP secretion from K-cells may well be associated with insulin resistance to maintain postprandial hyperinsulinemia in Japanese NGT subjects. It is unknown why there was no correlation between AUC-GIP and AUC-glucose. It might be explained by the fact that GIP secretion is associated with the amount of glucose loading[1], whereas blood glucose levels are maintained within normal levels by GIP-induced compensatory insulin secretion in NGT subjects.

GLP-1 secretions of type 2 diabetes subjects after glucose or meal ingestion are diverse in human studies[9,17–19]. Some studies report that GLP-1 secretion is decreased in Caucasian type 2 diabetes[9,17]. Recently, it is reported that GLP-1 levels after ingestion of glucose and mix meal in Japanese type 2 diabetic subjects were not decreased compared with those in NGT subjects, suggesting that GLP-1 secretion is not decreased in Japanese type 2 diabetes[20,35,36]. Two studies of Caucasian subjects found that AUC-GLP-1 during OGTT is positively associated with age and AUC of glucagon, whereas AUC of GLP-1 is negatively associated with BMI or bodyweight and AUC of FFA[9,18]. In the

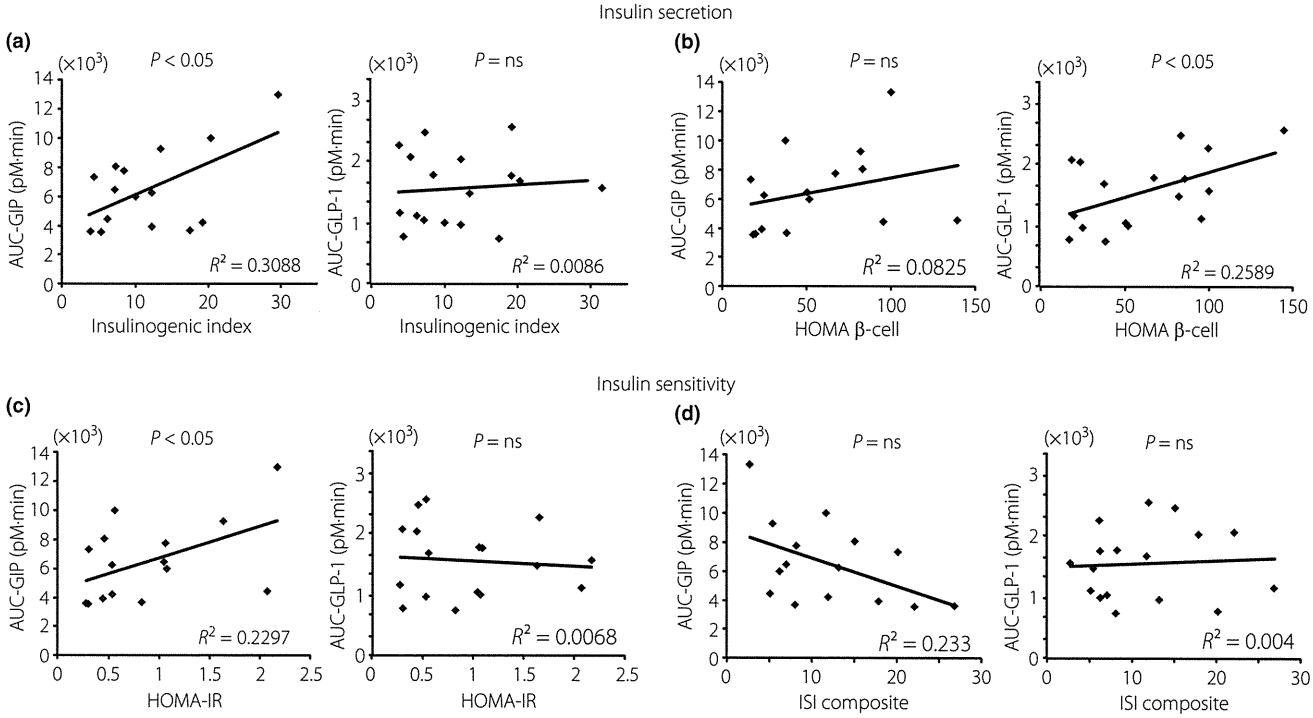

Figure 3 | Relationship between gastric inhibitory polypeptide secretion (AUC-GIP) and glucagon-like peptide-1 secretion (AUC-GLP-1) and the indices of insulin secretion and insulin sensitivity. (a) Insulinogenic index, (b) homeostasis model assessment (HOMA) β-cell function, (c) homeostasis model assessment of insulin resistance (HOMA-IR) and (d) insulin sensitivity index (ISI) composite. Ns, not significant.

present study, AUC-GLP-1 was negatively related to AUC of PG during OGTT, showing that the increase in GLP-1 secretion after glucose loading is associated with a decrease in postprandial glucose levels in Japanese NGT subjects. It has been reported that GLP-1 levels after glucose loading are positively related to gastric empting in Caucasian subjects[37]. Although we did not measure gastric empting of the subjects in the present study, increasing GLP-1 secretion after glucose loading might decrease postprandial glucose levels through gastric emptying. In the present study, BMI and AUC of FFA were not associated with AUC-GLP-1 during OGTT. Obese subjects have higher FFA levels than lean subjects[38]. However, because Japanese subjects are less obese than Caucasian subjects[21], the difference observed in the relationship between AUC-GIP and GLP-1, and AUC of FFA might reflect this ethnic difference in Caucasians and Japanese.

Insulin secretion, rather than insulin sensitivity, is the more important factor in the progression from NGT to type 2 diabetes in Japanese patients[23,39]. Because incretin is an intestinal hormone that induces postprandial insulin secretion[1], we hypothesize that GIP and GLP-1 secretion is more crucial in Japanese subjects than in Caucasian subjects. Indeed, GLP-1 mimetics and DPP-4 inhibitors improve glycemic control better in Japanese type 2 diabetic patients than in Caucasian type 2 diabetic patients in clinical trials[40–43]. We therefore evaluated the correlation between GIP secretion (AUC-GIP) and GLP-1 secretion (AUC-GLP-1), and the indices of insulin secretion and insulin sensitivity in Japanese NGT subjects during OGTT. The values of HOMA β-cell, insulinogenic index, HOMA-IR and ISI composite were similar to those in previous studies of Japanese subjects[24,30,39]. AUC-GIP was positively associated with the insulinogenic index and HOMA-IR, and the insulinogenic index was strongly associated with AUC-GIP, whereas AUC-GLP-1 was associated only with HOMA β-cell among the four indices. It has been reported that early-phase insulin secretion is an important factor in the progression from NGT through impaired glucose tolerance (IGT) to type 2 diabetes[39], and that basal insulin secretion (HOMA β-cell) and insulin resistance are important factors in the progression from NGT through impaired fasting glucose (IFG) to type 2 diabetes in Japanese patients[44]. Thus, enhancing the GIP and GLP-1 signals might be particularly useful in inhibiting the progression of type 2 diabetes in Japanese patients. Recently, variants at the GIP receptor gene locus associated with 2-h glucose levels during OGTT were identified by meta-analysis of genome-wide association studies[45]. In subjects who carry this GIP receptor risk allele, early-phase insulin secretion is decreased. These data seem to support our results that GIP secretion is associated with insulinogenic index in Japanese NGT subjects.

In conclusion, we evaluated plasma GIP and GLP-1 levels during OGTT in Japanese NGT subjects. GLP-1 secretion was associated with PG during OGTT, and basal insulin secretion (HOMA β-cell) and GIP secretion was associated with BMI and early-phase insulin secretion (insulinogenic index). Thus, there might be different factors associated with GIP and GLP-1 secretion during OGTT in Japanese subjects.

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Utility of indices using C-peptide levels for indication of insulin therapy to achieve good glycemic control in Japanese patients with type 2 diabetes

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ABSTRACT

Aims/Introduction: Type 2 diabetes is progressive in that therapy must be altered over time, which is partly as a result of the progressive loss of pancreatic β-cell function. To elucidate the relationship between residual endogenous insulin secretion and the necessity of insulin therapy to achieve good glycemic control, indices using serum C-peptide immunoreactivity (CPR) were analyzed in patients with type 2 diabetes.

Materials and Methods: The data of 201 Japanese patients with type 2 diabetes who achieved the target of glycemic control during admission were analyzed retrospectively. Indices using CPR including fasting CPR (FCPR), CPR 6 min after intravenous injection of glucagon (CPR-6 min), increment of CPR (ΔCPR), secretory unit of islet in transplantation index (SUIT) and C-peptide index (CPI) were compared between the group requiring insulin (insulin group) and the group not requiring insulin (non-insulin group). A receiver–operator characteristic (ROC) curve was made, and optimal cut-off point and likelihood ratio were determined for each index.

Results: All indices of CPR were lower in the insulin group compared with those in the non-insulin group. Likelihood ratios at the optimal point of FCPR, CPR-6 min, ΔCPR, SUIT, and CPI were 2.0, 2.1, 1.6, 2.3 and 2.8, respectively. Optimal cut-off point of CPI was 1.1 ng/mg. Sensitivity and specificity at optimal point of CPI were 61 and 78%, respectively.

Conclusions: The advantage of CPI of the indices of CPR to select insulin therapy to achieve good glycemic control was shown, but limitations of the predictive abilities of the indices using CPR should be taken into account. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2010.00096.x, 2011)

KEY WORDS: C-peptide, Insulin therapy, Glycemic control

INTRODUCTION

Type 2 diabetes is a heterogeneous disease characterized by insulin resistance and defective insulin secretion[1], and is progressive in that therapy must be altered over time. Initially on diagnosis, diet and exercise are generally adequate to achieve good glycemic control; oral hypoglycemic agents (OHA) are required later, when patients cannot achieve control with diet and exercise alone. Daily insulin injection is indicated when patients are unable to achieve control with a combination of oral agents, diet and exercise[2,3]. Insulin therapy is required in these patients not for survival, as is found in type 1 diabetes, but for good glycemic control[4]. This requirement is, at least in part, as a result of the progressive loss of pancreatic β-cell function. The results of the United Kingdom Progressive Diabetes Study (UKPDS) shows that pancreatic β-cell function (%β), assessed by Homeostasis Model Assessment (HOMA) in patients allocated to diet or OHA, decreased approximately 25% in 5 years[5]. A decline in endogenous insulin secretion over more than several decades of diabetes was observed in a cross-sectional study[6].

Determination of fasting serum C-peptide level and stimulated serum C-peptide level by intravenous glucagon is used widely to assess endogenous insulin secretory reserves[7–10]. There are several reports regarding the correlation between levels of residual endogenous insulin secretion and the choice of insulin therapy to achieve glycemic control[11–14]. However, in these studies, because the glycemic goal was not described clearly or was inappropriate, patients with insufficient glycemic control by the selected mode of therapy were sometimes included.

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In the present study, to evaluate the clinical significance of measures of serum C-peptide in achieving good glycemic control, we retrospectively analyzed the use of indices of endogenous insulin secretion in type 2 diabetes patients admitted to our hospital. Using data of patients who achieved the target of glycemic control during the period of admission, the patients were divided into two groups: one that achieved good control without the use of insulin (non-insulin group) and the other that required the use of insulin (insulin group), and the indices using serum C-peptide were compared between them. Optimal values and the utility of indices using serum C-peptide to select insulin therapy to achieve good glycemic control were analyzed.

MATERIALS AND METHODS

Subjects

A total of 746 Japanese patients with type 2 diabetes admitted between 2003 and 2009 to Kyoto University Hospital for poor glycemic control were enrolled in the present study. Type 2 diabetes mellitus was diagnosed based on the criteria of the American Diabetes Association (ADA)[15]. As indicated in Figure S1, 76 patients including those with pancreatic disease and liver disease, those taking diabetogenic medication and pregnant women were excluded. A total of 40 patients with incomplete clinical examinations also were excluded, and 66 patients with serum creatinine ≥1.3 mg/dL were excluded, as serum C-peptide immunoreactivity (CPR) is elevated by decreased renal function[16]. The data of 90 patients taking oral hypoglycemic agents (OHA) plus insulin at discharge were excluded. Good control was defined as mean preprandial capillary plasma glucose level <130 mg/dL, according to the glycemic control recommendation of ADA[17]. The 474 patients were divided into two groups: 201 patients who achieved good glycemic control (achieved group) and 273 patients who did not (non-achieved group). As shown in Figure S2, of the 201 patients in the achieved group, 47, 107, 38 and nine patients were treated with diet alone, OHA, insulin and insulin plus OHA at admission, respectively. At discharge, 24, 95 and 82 patients were treated with diet alone, OHA and insulin, respectively. Patients treated with diet alone and OHA at discharge comprised the non-insulin group; patients treated with insulin at discharge comprised the insulin group. A total of 166 patients of the 474 patients in the achieved or non-achieved group at discharge who could be confirmed within 6 months after discharge to achieve <7.4% in HbA_{1c}, which excludes 'not good' and 'poor' for assessment of glycemic control in the treatment guide for diabetes of the Japan Diabetes Society (JDS guide)[18], were re-analyzed to determine the cut-off point for C-peptide index (CPI) for longer duration of glycemic control. Of the 201 patients in the achieved group at discharge, 85 were excluded as a result of readmission or alteration to the mode of therapy, or were not followed as outpatients due to a change of hospital. Of the remaining 116 patients, 90 showed <7.4% HbA_{1c} within 6 months after discharge. Of the 273 patients in the non-achieved group at discharge, 137 were excluded as a result of readmission or alteration to the mode of therapy, or were not followed as outpatients due to a change of hospital. In the remaining 136 patients, 76 achieved <7.4% HbA_{1c} within 6 months after discharge. In these 166 patients, analysis of optimal values and the utility of CPI during admission was carried out.

Methods

On the first day in hospital, medical history, physical examination and laboratory evaluation including glycosylated hemoglobin were carried out. HbA_{1c} was measured using HPLC (HA-8180; Arcray, Kyoto, Japan). The HbA_{1c} (%) value was estimated as an National Glycohemoglobin Standardization Program equivalent (%) calculated by the formula: HbA_{1c} (%) = HbA_{1c} (JDS) (%) + 0.4%, considering the relational expression of HbA_{1c} (JDS) (%) measured by the previous Japanese standard substance and measurement methods and HbA_{1c} (National Glycohemoglobin Standardization Program)[19]. β-cell function was evaluated within 1 week after overnight fast by glucagon test measuring CPR before (fasting CPR [FCPR]) and 6 min after i.v. injection of 1 mg glucagon (CPR-6 min)[7], as this test is valid in patients taking insulin therapy. Increment of CPR (ΔCPR) was obtained by subtracting FCPR from CPR-6 min. SUIT index (SUIT) (%) was calculated by the formula: 1500 × FCPR (ng/mL)/(fasting plasma glucose [FPG; mg/dL] − 61.7)[20]. CPI (ng/mg) was calculated by the formula: 100 × FCPR (ng/mL)/FPG (mg/dL). Serum CPR was measured by immunoenzymometric assay (EIA; ST AIA-PACK C-Peptide, Toso corporation, Tokyo, Japan). In patients taking OHA, medication was stopped for the glucagon test, but was maintained until 1 day before to prevent hyperglycemia during the test[6]. Fasting plasma glucose was measured by the glucose oxidase method when the glucagon test was carried out. Patients were treated according to the JDS guide[18]. Treatment policy including diet therapy, exercise therapy, pharmacotherapy and education for each patient was determined by Japanese Board Certified Diabetologists certified by the Japan Diabetes Society. Patients took medical nutritional therapy (25–30 kcal/kg of standard bodyweight/day consisting of 58% carbohydrate, 18% protein and 24% fat energy intake percentages) with counseling by a registered dietitian. Preprandial capillary plasma glucose levels were monitored three t.i.d. during hospitalization. The study protocol was approved by the ethics committee of Kyoto University.

Statistical analysis

Statistical analysis was carried out with the Stat View 5.0 system (SAS institute, Cary, NC, USA). Data are presented as mean ± SE unless otherwise stated. Clinical parameters among the two groups were compared by Mann–Whitney *U*-test. *P*-values <0.01 were considered statistically significant. Histograms and receiver–operator characteristic (ROC) curve were made for FCPR, CPR-6 min, ΔCPR, SUIT and CPI respectively, and sensitivity, specificity, cut-off values, area under the ROC curve (AUC) and the likelihood ratio were calculated.

RESULTS

Clinical profiles of patients with mean preprandial capillary plasma glucose levels at discharge of <130 mg/dL (achieved group) and ≥130 mg/dL (non-achieved group), respectively, are shown in Table 1. Patients of the non-achieved group were older, had lower body mass index at admission, higher mean preprandial capillary plasma glucose level both at admission and at discharge, longer years from diagnosis and lower endogenous insulin secretion indices than those of the achieved group. The clinical stages of diabetic nephropathy and retinopathy were more progressed in the non-achieved group than those in the achieved group. The relationships between indices using serum C-peptide and selected modes of therapy at discharge were analyzed based on the data of the achieved group.

The clinical profiles of patients not requiring insulin for good glycemic control (non-insulin group) and those requiring insulin (insulin group) are shown in Table 2. The patients of the insulin group were older, has lower body mass index, higher HbA_{1c} at admission, higher mean preprandial capillary plasma glucose level at admission, longer years from diagnosis and lower endogenous insulin secretion indices compared with those of the non-insulin group. As shown in Figure S2, the mode of therapy in 41 patients was altered from diet alone or OHA to insulin during admission. The average number of hospital days before altering the therapeutic mode of these patients was 3.1 ± 3.4 (mean ± SD). The reasons for the change to insulin therapy were the necessity of tight glycemic control before operation in five patients, marked hyperglycemia (a fasting plasma glucose level of 250 mg/dL or above, or a causal plasma glucose of 350 mg/dL or above)[21] or both the presence of hyperglycemia and ketosis in 11 patients, and persistent hyperglycemia with OHA in 25 patients. HbA_{1c} at admission of these patients was 10.2 ± 2.2% (mean ± SD). In five patients, the mode of therapy was altered from insulin to OHA. The average number of hospital days before this change was 7.6 ± 4.3 (mean ± SD); the reason was improved glycemic control despite a decrease in the required dosage of insulin. HbA_{1c} at admission of these patients was 10.1 ± 4.4% (mean ± SD). Another patient treated with OHA plus insulin at admission was changed to OHA alone after nine hospital days because of improved glycemic control. Of the 113 patients with therapy of diet alone or OHA both at admission and at discharge, 19 transiently used insulin during the period of admission.

The category of OHA at discharge is shown in Table S1a. In 95 patients treated with OHA, 60 and 29 patients were prescribed sulfonylurea alone or in combination, and biguanide alone or in combination, respectively. In the insulin group, 50 of 86 patients were given premixed insulin b.i.d. at discharge. As shown in Table S1b, the prescribed daily dosages of gliclazide, glimepiride and metformin required were <80, 4 and 750 mg,

Table 1 | Clinical profiles of patients who achieved good glycemic control

	Achieved	Non-achieved	*P*
No. subjects	201	273	
Duration of hospitalization (days)	22.0 ± 0.7	23.6 ± 0.7	0.1115
Age (years)	60.2 ± 0.9	64.5 ± 0.7*	0.0002
Male/female	127/74	159/114	
Systolic blood pressure (mmHg)	124.5 ± 1.0	126.9 ± 1.1	0.1076
Diastolic blood pressure (mmHg)	74.6 ± 0.7	73.6 ± 0.6	0.2653
BMI (kg/m^2)	25.2 ± 0.3	23.8 ± 0.3*	0.0005
HbA_{1c} at admission (%)	9.5 ± 0.1	9.8 ± 0.1	0.0776
PG at admission (mg/dL)	181.1 ± 4.7	209.5 ± 3.9*	<0.0001
PG at discharge (mg/dL)	112.2 ± 0.9	163.2 ± 1.9*	<0.0001
Years from diagnosis	9.1 ± 0.6	13.5 ± 0.6*	<0.0001
FCPR (ng/mL)	1.87 ± 0.06	1.65 ± 0.05*	0.0054
CPR-6 min (ng/mL)	3.99 ± 0.14	3.41 ± 0.10*	0.0006
ΔCPR (ng/mL)	2.12 ± 0.09	1.76 ± 0.07*	0.0011
SUIT (%)	40.6 ± 1.9	32.4 ± 2.0*	0.0043
CPI (ng/mg)	1.34 ± 0.05	1.09 ± 0.04*	<0.0001
Clinical stage of nephropathy (normal/microalbuminuria/macroalbuminuria)	129/56/16 (64/28/8)	133/80/60 (49/29/22)	
Clinical stage of retinopathy (NDR/mild NPDR/moderate NPDR/severe NPDR/PDR)	141/25/26/4/5 (71/12/13/2/2)	112/53/45/22/41 (41/20/16/8/15)	

Data are presented as mean ± SE. *$P < 0.01$ versus achieved. Achieved group: mean preprandial capillary plasma glucose levels at discharge <130 mg/dL compared with those who did not achieve good glycemic control (non-achieved group ≥130 mg/dL). BMI, body mass index; CPI, C-peptide index; ΔCPR, increment of C-peptide immunoreactivity; CPR-6 min, C-peptide immunoreactivity 6 min after intravenous injection of glucagon; FCPR, fasting C-peptide immunoreactivity; NDR, no diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; PG, mean preprandial capillary plasma glucose level; SUIT, secretory unit of islet in transplantation index. Numbers in parentheses indicate percentages.

Table 2 | Clinical profiles of patients who achieved good glycemic control without requiring the use of insulin and those requiring insulin to achieve good glycemic control

	Non-insulin	Insulin	*P*
No. subjects	119	82	
Male/female	82/37	45/37	
Age (years)	58.4 ± 1.1	62.9 ± 1.3*	0.0099
Systolic blood pressure (mmHg)	124.4 ± 1.4	126.4 ± 1.7	0.3598
Diastolic blood pressure (mmHg)	77.3 ± 1.0	73.3 ± 1.3	0.0135
BMI (kg/m^2)	26.0 ± 0.4	24.0 ± 0.4*	0.0019
HbA_{1c} at admission (%)	9.2 ± 0.2	10.0 ± 0.2*	0.0050
PG at admission (mg/dL)	163.2 ± 5.0	206.9 ± 8.0*	<0.0001
PG at discharge (mg/dL)	110.9 ± 1.2	114.2 ± 1.3	0.0602
Years from diagnosis	7.8 ± 0.6	10.9 ± 1.0*	0.0052
FCPR (ng/mL)	2.06 ± 0.07	1.61 ± 0.09*	0.0001
CPR-6 min (ng/mL)	4.48 ± 0.18	3.29 ± 0.19*	<0.0001
ΔCPR (ng/mL)	2.43 ± 0.12	1.68 ± 0.12*	<0.0001
SUIT (%)	47.2 ± 2.5	31.1 ± 2.7*	<0.0001
CPI (ng/mg)	1.57 ± 0.07	1.06 ± 0.06*	<0.0001

Data are presented as mean ± SE. *P < 0.01 versus non-insulin. Good glycemic control: mean preprandial capillary plasma glucose levels at discharge <130 mg/dL.
BMI, body mass index; CPI, C-peptide index; ΔCPR, increment of C-peptide immunoreactivity; CPR-6 min, C-peptide immunoreactivity 6 min after intravenous injection of glucagon; FCPR, fasting C-peptide immunoreactivity; PG, mean preprandial capillary plasma glucose level; SUIT, secretory unit of islet in transplantation index.

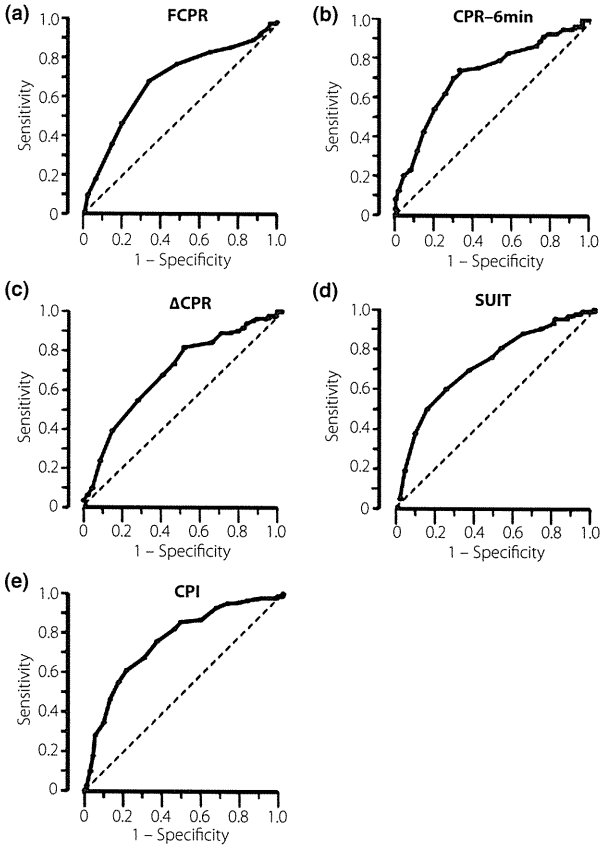

Figure 1 | Receiver–operator characteristic curves of (a) fasting C-peptide immunoreactivity (FCPR), (b) CPR 6 min after intravenous injection of glucagon (CPR-6 min), (c) increment of CPR (ΔCPR), (d) secretory unit of islet in transplantation index (SUIT) and (e) C-peptide index (CPI) of patients with mean preprandial capillary plasma glucose levels of <130 mg/dL at discharge.

respectively in almost all (more than 95%) patients. Daily insulin dosage was 22.0 ± 11.1 U (mean ± SD) in the insulin group.

In Figure S3, peak relative frequency of indices using CPR of patients with mean preprandial capillary plasma glucose levels of <130 mg/dL at discharge in the insulin group and the non-insulin group, respectively, is shown (FCPR: 1.50–1.75, 2.00–2.25 ng/mL; CPR-6 min: 2.75–3.00, 4.00–4.25 ng/mL; ΔCPR: 1.25–1.50, 1.25–1.50 plus 2.25–2.50 ng/mL; SUIT: 15–20, 25–30 plus 35–40 plus 45–50%; and CPI: 0.8–0.9, 1.5–1.6 ng/mg). According to ROC curves of indices using CPR shown in Figure 1, AUC, cut-off values and values at optimal cut-off points including sensitivity, specificity and the likelihood ratio were determined and shown in Table 3. CPI is the most relevant of these indices for selecting insulin therapy to achieve good glycemic control, because the likelihood ratio and AUC of CPI is greatest.

The ROC curve of CPI of patients who achieved <7.4% HbA_{1c} within 6 months after discharge is shown in Figure 2. According to ROC curves of CPI in Figure 2, the AUC (0.75), cut-off values (optimal: 1.2; 90% specificity 0.8; 90% sensitivity 1.7 ng/mg), and values at optimal cut-off points including sensitivity (73%), specificity (71%) and the likelihood ratio (2.5) were determined.

DISCUSSION

Medical nutritional therapy (MNT) improves glycemic control in patients with type 2 diabetes regardless of their modes of therapy including diet alone, OHA and insulin[22–24]. Diet therapy is the basis and starting point of treatment of all patients with diabetes[25], and failure of diet therapy alone might predict the inability to attain optimal glycemic control by any of these modes of therapy. To precisely analyze the relationship between endogenous insulin secretion and the appropriate mode of therapy for achieving good glycemic control, we used data of hospitalized patients under optimal therapy including proper MNT. Thus, our results are more likely to be valid in patients with appropriate care behaviors. Although inappropriate care behavior is an obstacle to achieving good glycemic control over a longer duration, our results suggest a basis for beginning insulin therapy in patients who do not achieve good glycemic control with diet alone or OHA despite the practice of appropriate care behavior.

Table 3 | Analysis of indices using serum C-peptide of patients with mean preprandial capillary plasma glucose levels of <130 mg/dL at discharge

	FCPR	CPR-6 min	ΔCPR	SUIT	CPI
AUC	0.69	0.71	0.69	0.72	0.75
Cut-off values	(ng/mL)	(ng/mL)	(ng/mL)	(%)	(ng/mg)
Optimal	1.75	3.75	2.25	30	1.1
90% Specificity	1.00	2.25	1.00	20	0.7
90% Sensitivity	2.75	5.25	3.25	55	1.7
Values at optimal cut-off points					
Sensitivity (%)	70	74	82	61	61
Specificity (%)	66	65	49	73	78
Likelihood ratio	2.0	2.1	1.6	2.3	2.8

AUC, area under receiver–operator characteristics curve; CPI, C-peptide index; ΔCPR, increment of C-peptide immunoreactivity; CPR-6 min, C-peptide immunoreactivity 6 min after intravenous injection of glucagon; FCPR, fasting C-peptide immunoreactivity; SUIT, secretory unit of islet in transplantation index

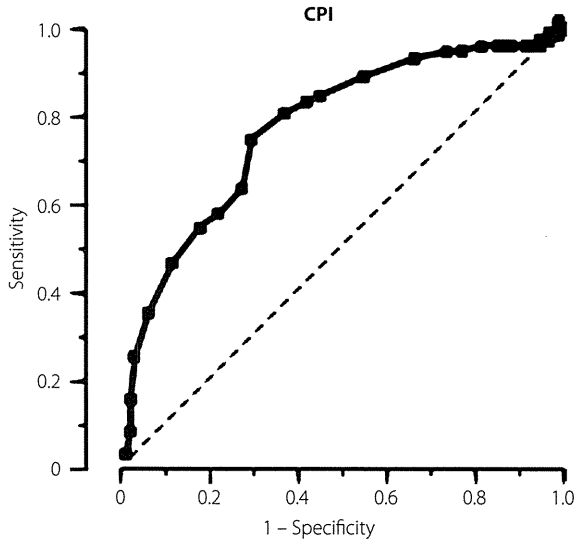

Figure 2 | Receiver–operator characteristic curve of C-peptide index (CPI) of patients who achieved <7.4% HbA_{1c} within 6 months after discharge.

In the present study, just 42% of patients achieved good control during hospital admission, partly because the aim of admission was not necessarily to achieve good control during the period of admission, but to establish a treatment policy for the achievement of good control after discharge. The percentage of patients treated with insulin at discharge was higher in the non-achieved group than in the achieved group (non-achieved group: 67%; achieved group: 41%). Of the patients treated with OHA at admission in the achieved group, 39% had therapy changed to insulin, whereas 73% of the patients treated with OHA at admission in the non-achieved group had therapy changed to insulin. These results might indicate more intensive therapy in the case of the non-achieved group. Of the 136 patients in the non-achieved group at discharge, 76 showed <7.4% HbA_{1c} within 6 months after discharge, showing fair glycemic control in some of the patients of this group over the longer term. As shown in Table 1, the non-achieved group had more progressive diabetic complications and more years from diagnosis compared with the achieved group. These factors might prompt therapy that aims at a more gradual improvement of glycemic control to prevent hypoglycemia. In addition, the non-achieved group showed higher glycemic levels at admission than that of the achieved group, whereas the duration of hospitalization was similar.

Although there have been several reports regarding the utility of indices of endogenous insulin secretion to indicate initiation of insulin therapy to improve glycemic control[11–14], none has compared the utility of the various indices. In the present study, as shown by the likelihood ratio and by AUC, CPI is shown to be the most useful among the five indices.

CPI was used as an index of endogenous insulin secretion in several reports[26–28], but its advantage over other indices and the scientific basis was unclear. The SUIT index (SUIT) was developed using FCPR and plasma glucose level after islet transplantation[19]. The linear relationship between FCPR and FPG in individual subjects shows a plasma glucose level (61.7 mg/dL) assumed to suppress C-peptide to zero. Transplantation of islets from non-diabetic donors increases the slope (FCPR/[FPG − 61.7]), suggesting an index of transplanted β-cell mass. Although a correlation between SUIT and CPR 6 min after intravenous injection of 1 mg glucagon (CPR-6 min) is observed in type 2 diabetes ($r = 0.58$), it is weaker than that in patients after islet transplantation ($r = 0.82$)[19].

Autopsy reveals that β-cell mass is decreased in patients with type 2 diabetes compared with that in healthy subjects[29–31]. Recently, in 33 subjects at various stages of glucose tolerance, a correlation between β-cell areas of a sample obtained during pancreatectomy, and serum levels of CPR and insulin before the operation was analyzed[32]. Interestingly, β-cell areas are positively correlated with fasting insulin/FPG ($r = 0.51$, $P = 0.0024$) and FCPR/FPG ($r = 0.63$, $P < 0.0001$), but are not significantly

correlated with homeostasis model assessment β-cell function (HOMA-β). Because SUIT resembles HOMA-β in that insulin secretion is assumed to be suppressed to zero at approximately 60 mg/dL glucose in the formula, CPI might be a better index of residual β-cell mass than SUIT in subjects with glucose intolerance. Furthermore, CPI is not affected by exogenous insulin[27], which might favor reproducibility of the results in patients with insulin therapy. Determination of the index using a one-point blood sample without the use of loading agents also favors CPI.

In results derived from CPI of patients with mean preprandial capillary plasma glucose levels of <130 mg/dL at discharge, AUC was 0.75, optimal cut-off value was 1.1 ng/mg with 61% sensitivity and 78% specificity, and values at 90% sensitivity and at 90% specificity were 1.7 and 0.7 ng/mg, respectively. Interestingly, in results derived from CPI of patients who achieved <7.4% HbA_{1c} within 6 months after discharge, AUC was 0.75, optimal cut-off value was 1.2 ng/mg with 73% sensitivity and 71% specificity, and values at 90% sensitivity and at 90% specificity were 1.7 and 0.8 ng/mg, respectively, similar to the values evaluated by mean preprandial glucose levels at discharge. These values are also similar to those in a previous report in Japanese using the data of 180 subjects from another institution (optimal cut-off value: 1.0 with 62% sensitivity and 81% specificity; values at 90% sensitivity: 1.8; 90% specificity: 0.7 ng/mg), although good glycemic control was defined as 8.4% in HbA_{1c}, which is somewhat inadequate[14]. Thus, CPI might be a predictor of suitable therapy to achieve fair glycemic control not only for the short-term, but also for longer duration.

The main limitation of the present study is that it is a retrospective analysis of inpatients at one hospital, and the protocol for starting insulin therapy was not defined precisely. However, in the achieved group analyzed as subjects, the decisions as to whether to start insulin therapy made by Japanese Board Certified Diabetologists were confirmed retrospectively to have been made according to the treatment guide for diabetes of the Japan Diabetes Society, as discussed in the results section.

In conclusion, we have shown the advantage of CPI of indices using CPR to select insulin therapy to achieve good glycemic control. However, limitations of the predictive abilities of indices using CPR generally and the importance of observation of the clinical therapeutic course must be taken into consideration.

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The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 | Process of selection of subjects for analysis.
Figure S2 | Therapeutic modes of analyzed patients at admission and discharge, and the required alteration of therapy during the period of admission.
Figure S3 | Relative frequency distribution of C-peptide indices of patients with mean preprandial capillary plasma glucose levels of <130 mg/dL at discharge in the non-insulin and insulin group.
Table S1 | Details of medication and daily dosages of oral hypoglycemic agents used at discharge

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Clinical Study

Fat Restriction Is Associated with Impaired Quality of Life in Patients with Ulcerative Colitis and Crohn's Disease

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Inflammatory bowel disease (IBD), ulcerative colitis (UC) and Crohn's disease, is reported to be associated with impaired health-related quality of life (QOL). Although decreased QOL in these subjects has been reported to be associated with various factors, the effect of nutritional therapy, especially nutrients intake on QOL has received less attention. In this study, we evaluated the various factors including nutrients intake on QOL using SF-8 in 64 patients with IBD. Patients with IBD seem to have decreased QOL especially in the mental aspects. The percentage energy intake from fat of total energy fat intake (% energy) of the whole subjects, was lower than those of the annual National Nutrition Survey in Japan. Multiple regression analyses revealed that fat intake (% energy) was a significant predictor for mental component summary. In conclusion, fat restriction contributes to impaired QOL especially in the mental aspects in IBD patients.

1. Introduction

Inflammatory bowel disease (IBD); ulcerative colitis (UC) and Crohn's disease, is reported to be associated with impaired health-related quality of life (HR-QOL). In this paper, HR-QOL will be simply designated as QOL. Decreased QOL in these subjects has been reported to be related to various factors such as age, gender [1, 2], treatment effects [3], disease activity, and social environment [4]. However, the effect of nutritional therapy on the QOL of IBD patients has received less attention, most of which is devoted to the parenteral nutrition therapy, not the nutritional therapy in general [5, 6].

Since excessive fat intake is considered to worsen the inflammation in the intestine, its restriction has traditionally been employed in Japan as the oral nutritional therapy for IBD patients, especially for those with CD, which, however, has its own pros and cons.

Recently, we have studied the possible involvement of hypovitaminosis D and K in the development of osteoporosis in IBD patients [7]. In face of apparently sufficient intake of these vitamins, their plasma levels were quite low in these patients. Paradoxically, plasma concentrations of vitamin D and K were correlated with the fat intake but not with their intake of these vitamins. These results were more prominent in patients with CD than those with UC. Then it was concluded that fat-soluble substances such as vitamin D and K were not effectively absorbed from the intestine without concomitant intake of enough fat.

Through this paper, we were interested in what fat restriction means from the patients' perspectives and studied

the effect of fat restriction on the QOL of IBD subjects in this paper.

2. Subjects and Methods

2.1. Subjects. Study subjects were 64 patients with IBD attending the gastroenterology clinic at the Kyoto University Hospital; 33 with CD (19 men/14 women) and 31 with UC (20 men/11 women). Detailed information was given and written consent was obtained. The study protocol was approved by the ethical committee of the Kyoto Women's University. Almost all patients (27/33 in CD and 28/33 in UC) were receiving 5-aminosalicylic acid. Glucocorticoid therapy was given to four and two patients with CD and UC, respectively. Immunosuppressive drug therapy was performed in 25 and 4 patients with CD and UC, respectively. Eight patients with CD, but none with UC, were on combined therapy of infliximab, synthetic glucocorticoid, and immunosuppressive drug. Fifteen patients with CD and one with UC were on enteral or total parenteral nutrition therapy, respectively.

2.2. Methods

2.2.1. Dietary Information. Dietary information was obtained from food intake records in 2 weekdays by the patients. By calculating these records, their energy and nutrients intakes were obtained by computer software program (Healthy Maker Pro 501, Mushroom soft Corp.).

2.2.2. QOL Measurement. QOL was assessed using the Japanese Short Form Health Survey (SF-8), a widely used generic questionnaire [8]. Eight subscales are obtained; physical function (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social function (SF), role emotional (RE), and mental health (MH). RP and RE refer to the limitations due to physical or emotional reasons, respectively. They are also summarized into two summary scores: physical component summary (PCS) and mental component summary (MCS). Data are transformed to deviation scores based on Japanese norms [8]. Higher scores indicate better QOL, with 50 corresponding to the national norms.

2.2.3. Statistical Analyses. Statistical analyses were performed using SPSS 17.0 J for Windows (SPSS. Japan Inc., Tokyo, Japan). Comparison of data from IBD patients with Japanese norms was done by one-sample t test. The difference between two independent groups was analyzed by unpaired t test or Mann-Whitney test depending on normality. Correlations between two independent variables were analyzed by Pearson's or Spearman's correlations. Multiple regression analysis was performed to determine independent factors for QOL scores in IBD patients.

3. Result

3.1. Background Profiles and Biochemical Indices. The baseline characteristics of the patients are shown in Table 1.

Table 1: Background profiles and results from blood tests in patients with CD and UC.

	CD	UC	P value
Age (y)	35.6 ± 7.3	41.7 ± 17.3	.343[a]
Sex (F/M)	19/14	20/11	—
Disease duration (y)	13.7 ± 7.4	6.8 ± 4.8	<.001[b]
Body mass index (kg/m^2)	19.5 ± 2.3	21.1 ± 3.3	.025[b]
Disease location (involving small bowel/not involving small bowel)	30/2	0/31	—
Glucocorticoid therpy	4	2	—
Immunosuppressive therapy	25	4	—
Immunopotentiating therapy (TNF-α)	8	0	—
Enteral or total parenteral nutrition therapy	15	1	—
C-reactive protein (g/dl)	0.6 ± 1.0	0.3 ± 0.6	.135[b]
Albumin (g/dl)	3.9 ± 0.4	4.3 ± 0.3	<.001[b]
Total cholesterol (mg/dl)	126.9 ± 25.0	177.1 ± 40.3	<.001[b]

Values represent mean ± SD. Comparison of indices between patients with CD and those with UC was done by unpaired t test[a] or Mann-Whitney test[b] depending on normality.

CD patients had significantly longer disease duration and lower BMI than UC patients. While nutritional indices such as serum albumin and total cholesterol were lower in CD subjects, there was no significant difference in C-reactive protein which is an inflammatory parameter between these groups. Most of patients were in remission.

3.2. Energy and Nutrients Intake in CD and UC Patients. Food intake could be evaluated in 62 patients (31 with CD and 31 with UC). Energy and nutrients intake in these patients is shown in Table 2. Fourteen patients with CD were on enteral nutrition, and each one of subjects with CD and UC was on total parental nutrition. Although the energy intake was not significantly different between the two groups, fat intake was significantly lower in CD patients than UC subjects. The annual National Nutrition Survey in Japan (NNS-J) in 2008 showed that in subjects of 30–39 or 40–49, years of age including both genders [9], the daily fat intake (% energy) was 26.5% or 25.6%, respectively. These were significantly higher than those of IBD subjects in this study (P = .001; data not shown). Subjects with enteral or parental nutrition had fat intake only approximately half of that in subjects with oral intake (data not shown). The percentage energy intake from protein, fat, and carbohydrates was significantly different between CD and UC subjects.