



Effect of glycemc control on plasma plant sterol levels and post-heparin diamine oxidase activity in type 1 diabetic patients

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Abstract

We examined the effect of glycemc control on the plasma plant sterol levels (a measure of cholesterol absorption efficacy) and the plasma post-heparin diamine oxidase (DAO) activity (a measure of intestinal mucosal mass) in type 1 diabetes. The plasma plant sterol levels (mmol/mol of cholesterol) and the DAO activities after 30 U/kg of intravenous heparin were determined in age- and sex-matched three groups (12 type 1 diabetic patients undergoing conventional insulin therapy, ten patients undergoing intensive insulin therapy, and ten normal subjects). All patients continued their indicated insulin regimen for 14 days with a weight-maintaining energy restricted diet. The conventional group showed a significant higher ($P < 0.001$) level of the fasting plasma glucose (FPG) or the glycated albumin (GA), a higher ($P < 0.01$) DAO activity (2-fold of the peak level), which was observed 10–30 min after the heparin injection, and a higher ($P < 0.01$) plasma plant sterol levels (1.5-fold) compared with those in the other two groups, respectively. The DAO activity 30 min after the heparin injection significantly correlated with either the glycated albumin (GA) concentration or the plant sterol levels in all subjects. Furthermore, the acute glycemc control by the changes of insulin regimen from conventional to intensive showed a significant reduction of the DAO activity and plant sterols in the same patients. These results suggest that glycemc control in part relates to the intestinal adaptation to cholesterol absorption efficacy in type 1 diabetes. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Abnormalities in lipids and lipoproteins have key roles for the development and the progression of atherosclerotic vascular diseases in type 1 diabetes mellitus [1,2]. Studies on lipoprotein metabolism have clarified the importance of decreased lipoprotein lipase (LPL) activity as a cause of impaired triglyceride metabolism in patients with insulin deficiency [3,4]. However, abnormal cholesterol metabolism is not clearly defined in type 1 diabetic patients.

Plasma cholesterol levels were reported to be increased in type 1 diabetic patients with poor glycemc control in comparison with those in patients with strict

glycemc control [5–7]. Since glycemc control itself depends on the suitable way for insulin supplementation, lack of appropriate insulin action and the resulting hyperglycemia may also largely influence cholesterol metabolism in type 1 diabetic patients. Recently, intensive insulin therapy significantly decreased both plasma cholesterol level and macrovascular events as compared with conventional insulin therapy [8]. However, the mechanism was not clearly demonstrated. In the development of abnormal cholesterol metabolism, there are two possible important causes associated with insulin deficiency. One is the impaired lipoprotein metabolism including cholesterol synthesis, and another is alteration in intestinal cholesterol absorption.

In terms of lipoprotein metabolism in type 1 diabetic patients, increased influx of both plasma fatty acids and glucose to the liver contribute to the increased production of very low density lipoprotein (VLDL) and the decreased insulin action on receptor-mediated cholest-

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terol uptake impairs clearance of low density lipoprotein (LDL) from the plasma [2]. On the other hand, there are a few reports concerning the abnormality of intestinal cholesterol absorption in type 1 diabetic patients. The reduction of dietary cholesterol highly effective in lowering plasma cholesterol levels in type 1 diabetic patients [9], therefore, it is speculated that type 1 diabetic patients are suggested to have higher cholesterol levels in response to the increased its absorption from the small intestine [10]. Cholesterol absorption has been aggressively studied in both normal subjects and the patients with dyslipidemia [11]. Rapid intestinal transit time [12] or decreased intestinal absorptive area [13] may decrease the cholesterol absorption. On the other hand, ϵ 4 allele of apolipoprotein E phenotype may positively regulate the absorption [14]. However, there are very few studies in type 1 diabetic subjects with insulin deficiency. Recently, a study on two brothers with poor glycemic type 1 diabetes had been reported [10]. Interestingly, those two brothers had chronic diarrhea and increased both cholesterol and plant sterol absorption together with the increased plant sterol levels in the plasma. Since the plasma plant sterol level was reported to be a marker of cholesterol absorption efficacy in both non-diabetic subjects [15], and was also reported in type 2 diabetic subjects [16], it is worthwhile to clarify the relation between plasma plant sterol and either hyperglycemia or insulin deficiency in type 1 diabetes mellitus.

Hyperglycemia and insulin deficiency are suggested to affect the small intestinal adaptation and to change the mucosal morphology in experimental insulin-deficient animals [17,18]. Prior to our human study, we have evaluated the usefulness of the determination of plasma post-heparin diamine oxidase (DAO) activities in control and insulin-deficient diabetic rats. The activity was increased in the insulin-deficient state and was well correlated with intestinal mucosal mass [19], even though those rats were pair-fed with controls [20]. DAO is synthesized in most of the tissues in the body but its activity in post-heparin plasma almost depends on the release from the small intestine, and is highly related to the intestinal mucosal mass [21]. Moreover, clinical application of the DAO determination has already been demonstrated in the field of gastroenterology to evaluate mature enterocytic mass in patients with chronic inflammatory bowel diseases [22–24]. These evidences in human and animals indicate that the two parameters (plasma post-heparin DAO activity and plant sterol levels) may be good indicators to evaluate the intestine and sterol responsiveness in type 1 diabetic patients.

In this study, we hypothesized that poor glycemic control might induce intestinal mucosal changes, and that the normal intestinal sterol responsiveness might be affected in type 1 diabetic subjects. To evaluate the

relation of the intestinal absorptive mass and the sterol absorption efficacy in response to insulin deficiency and hyperglycemia, we determined plasma DAO activity and plasma plant sterol levels in patients with type 1 diabetic patients, who were subjected to conventional or intensive insulin treatment.

2. Methods

2.1. Study protocol and subjects

We studied in 22 in-patients with IDDM based on the clinical criteria (age < 40 years at the onset of the disease, the presence of ketonuria, and insulin-dependence within 1 month of diagnosis), and in ten non-diabetic normal subjects based on the WHO criteria [25] after 75 g oral glucose tolerance test. To be eligible, patients with age 17 to 60 years old, and under conventional insulin treatment without advanced major diabetic complications were recruited in this study. The patients with advanced complications (overt nephropathy, proliferative retinopathy, gastroenteropathy, autonomic nerve abnormalities, cardiovascular diseases, or cerebrovascular diseases) were excluded. None had major gastrointestinal and hematological abnormalities, or had received any medication except insulin injection.

After admission to our metabolic ward, all patients received dietary information from a clinical dietician. Then, they received a standard hospital diet for diabetic patients containing 28 kcal·kg of ideal body weight⁻¹ day⁻¹ of energy with low cholesterol content, which contained 58% calories as carbohydrate, 24% as fat, and 18% as protein based on energy%. Phytosterol content of the diet in the stabilization periods was very similar to experimental conditions since the diet was provided the subjects in hospital for at least 5–7 days prior to the day when the program was initiated. To quantify the dietary sterols in all of the meals (breakfast, lunch and dinner; a standard diet with 1600 kcal), sterols were extracted from a blended mixture of foods. Sterol concentrations were quantified by high performance liquid chromatography (HPLC). The mean \pm S.D. sterol composition of the three major meals over the 14-day study program was 257 \pm 22 mg of cholesterol, 69.4 \pm 5.2 mg of campesterol, and 106.5 \pm 10.9 mg of β -sitosterol per day. Prior to this study, we have tested the effect of continuous oral plant sterol load (1.0 g/day of moristerol, Morisita Pharmaceutical Co., Osaka, Japan), in which the dose of plant sterol was about a 5.7-fold of the present study diet. The mean ratio of plasma campesterol/cholesterol and β -sitosterol/cholesterol ratios were significantly increased by about 1.7-fold on day 14 compared with those on the start day. Further increment of those ratios were not observed at day 28. Therefore, we have set the 14-day study program in this study.

Normal subjects in the same age range as the diabetic patients (from 18 to 52 years old) were chosen. They were in good health and were not given any regular medication. Before the beginning of the study, all normal subjects went on the same dietary programs as the diabetic patients. These programs lasted for 14 days, when the DAO activities were determined. Thus, all subjects ate the diet containing similar proportion of the contents of the nutrients and the total energy was the same per kg ideal body weight.

Conventional insulin treatment was continued for 12 patients as the same dose as they received at the outpatient clinic. Two patients received single subcutaneous insulin (Penfil N, Novo Nordisk Pharmaceuticals, Inc.) injection before breakfast, and ten patients received two subcutaneous insulin (Penfil N) injections; one before breakfast and one before dinner. The insulin dosages for these individuals were kept constant except during severe hypoglycemia or hyperglycemia as determined by capillary glucose monitoring ($\frac{1}{2}$ h before and 2 h after each meal, and at 11 o'clock p.m.). Intensive insulin therapy was started for other ten patients after admission. Two patients had four subcutaneous insulin (Penfil R; Novo Nordisk Pharmaceuticals, Inc., before each meal and Penfil N before sleep) injections daily as given at outpatient clinic, three patients changed from two to four injections daily, and five patients changed from two injections (Penfil N) to continuous subcutaneous insulin (Novorin R, Novo Nordisk Pharmaceuticals, Inc.) injection (CSII) by an automatic microinfusion pump (SP3HQ, Nipro) with three extra-injections before each meal. The insulin dosages for these intensive therapy individuals were changed to maintain a strict glycemic control according to the level of capillary blood glucose as measured 7 times per day in the conventional insulin treated group. To avoid the acute effects of the changes of dietary therapy, the 14-day study program was started on the prior stabilization periods (5–7 days from the day of admission). We evaluated glycemic control state by measuring the fasting plasma glucose (FPG) and the glycated albumin (GA) levels [26] of all diabetic patients and the normal subjects.

To further examine the effect of acute glycemic control on intestinal sterol absorption, eight patients with type 1 diabetic subjects undergoing conventional therapy (five female and three male) in the cross sectional study were switched to the intensive insulin treatment protocol (five for CSII and three for four times subcutaneous injection) after the prior 14-day conventional program. Then, they received intensive insulin therapies for the next following 14 days and the second DAO measurements. The other four patients among the conventional therapy in the cross-sectional study were excluded from this protocol since they wanted to keep conventional insulin therapy.

The study protocols were approved by the ethical committee of Shiga University of Medical Science. Informed consent was obtained from all patients and normal subjects.

2.2. Measurements of serum lipids, plasma glucose, glycated albumin, IRI, DAO activity and LPL mass

The subjects were asked not to smoke on the day of the study and to avoid any food or drink before the heparin injection after an overnight fast. The patients usually treated with single or multiple insulin injections, did not receive a morning injection, and the patients treated with insulin by the microinfusion pump continued to receive basal subcutaneous insulin injections without the extra morning injection.

Blood was taken before the intravenous heparin injection for the determination of their plasma glucose (glucose oxidase method), GA (HPLC method), cholesterol (enzymatic method), high density lipoprotein (HDL) cholesterol (heparin-Mn method), plant sterol (HPLC method described afterward), triglycerides (enzymatic method), immunoreactive antibody-free insulin (IRI, RIA method) level and basal DAO activities (described afterward). The LDL-cholesterol was calculated according to the Friedewald formula. Thirty U/kg body weight of heparin (Novo Nordisk Pharmaceuticals, Inc.; 1000 U/ml) was injected in the right cubital vein. Then blood was drawn from the left cubital vein at 10, 30, 60 and 120 min after the heparin injection. The subjects were in supine position throughout the study. Plasma was stored at -70°C until their DAO activities were determined. Intravenous heparin injection at doses of 10, 20, 30, 40 and 50 U/kg body weight revealed a linear dose-dependent increase in DAO activities at 10 min after the injection, which were determined in two normal subjects (DAO activity in one normal subject is 0.21, 0.46, 1.28, 1.60, and 1.85 mU/l, and that in another subject is 0.43, 1.27, 1.51, 1.88 and 2.69 mU/l, respectively). Thus, we injected 30 U/kg body weight of heparin in this experiment.

DAO activity was measured using Tryding's modification [27] of the method described by Okuyama and Kobayashi [28]. In brief, under the influence of DAO, [^{14}C]-putrescine is converted to γ -aminobutyraldehyde that then forms Δ^{-1} -pyrroline, provided its conversion to γ -aminobutyric acid is blocked by the addition of acetaldehyde. One international unit of DAO equals to 1 μmol Δ^{-1} -pyrroline/min at 37°C . For the plasma DAO assay, duplicate measurements were performed using a 200 μl aliquot of plasma. The coefficient of variation of the measurement was 3.4% when five DAO readings were taken from the same sample. Plasma LPL masses were measured 10 min after heparin injection using an enzyme immunoassay [29].

2.3. Measurement of plasma cholesterol and plant sterols

Plasma sterol concentrations and the sterol content of the food were determined by a method described by Kasama et al. [30]. Briefly, for the determination of plasma sterol concentrations, 0.1 ml of plasma with 10 µg of desmosterol (5,24-cholestadien-3β-ol) as an internal standard was treated with 1 mol/l of ethanolic KOH and extracted twice with n-hexane. For the determination of the sterol content of the food, 5β-cholestane 3α-ol was used as the standard. The sterols in the extracts were converted into their benzoate derivatives with a benzoyl chloride reagent that was freshly prepared for each assay. The benzoate derivatives of the sterols were re-extracted with 1,2-dichloroethane, and dissolved again in 250 µl of acetonitrile-dichloroethane 2:1 after evaporation under a stream of nitrogen. Fifty µl of the solution was injected into an HPLC system. The sterols were separated using a reverse-phase column (SBC-ODS 150 × 2.5 mm, Shimadzu, Kyoto, Japan) maintained in an incubator at 50°C and monitored at 228 nm. The instrument was an LC-6A system (Shimadzu), equipped with a column oven and chromatogram data processor (Chromatopac C-R5A, Shimadzu). The solvent used for the elution was acetonitrile-water-acetic acid 97:3:0.2 at a flow rate of 0.5 ml/min. A calibration was done after the derivatization of authentic sterols obtained from Sigma, St. Louis, MO or Nakarai Tesque, Kyoto, Japan. The coefficients of variations for measurement of plasma concentrations of cholesterol, campesterol and β-sitosterol were all within 5% as described before [31].

2.4. Statistical analysis

The multiple comparison test to compare the differences among the three groups were calculated by Scheffe's comparison test except the DAO activities. The time-course of plasma DAO activities were compared by two-factor ANOVA and the differences among the three groups were calculated by Fisher's protected LSD using a software of Macintosh Super ANOVA ver 1.11. Univariate correlation and regression analyses were performed to determine the relation between DAO activities and other clinical variables in all subjects. Effects of acute insulin-treatment were evaluated by the paired t-test. The results are expressed as mean ± S.E. *P*-values of < 0.05 were considered to indicate statistical significance.

3. Results

The fasting plasma glucose concentration was significantly higher in the diabetic patients undergoing

conventional insulin therapy than those in patients undergoing intensive insulin therapy or normal subjects (Table 1). Plasma concentrations of GA in the diabetic patients undergoing intensive therapy were significantly lower than those in the patients undergoing conventional therapy. Body mass indices, fasting plasma IRI levels, serum concentrations of cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol were not significantly different among the three groups. Duration of diabetes and daily insulin dosages were also not different between the diabetic patients undergoing conventional insulin therapy and the intensive insulin therapy.

The plasma DAO activities in type 1 diabetic patients and normal subjects were measured before and after intravenous injection of the heparin. Maximal plasma DAO activity was observed between 10 and 30 min after the heparin injection (Fig. 1). Patients undergoing conventional insulin therapy had increased plasma post-heparin DAO activities when compared with the other two groups. On the other hand, both diabetic groups had lowered plasma LPL mass 10 min after the heparin injection compared with the normal subjects (Fig. 1). As shown in Fig. 2, DAO at 30 min after the heparin was positively correlated with either campesterol ($r = 0.56$, $P < 0.001$) or β-sitosterol ($r = 0.44$, $P < 0.05$), respectively. Furthermore, we also found significant relationship between DAO at 30 min after the heparin and glycated albumin ($r = 0.49$, $P < 0.005$). However, plasma IRI levels were not related to the DAO activities (data not shown).

Plasma plant sterol levels (mmol/mol of cholesterol) were increased in patients undergoing conventional insulin therapy (campesterol; 3.84 ± 0.44 , β-sitosterol; 2.33 ± 0.25) compared with those in normal subjects (campesterol; 2.02 ± 0.20 , $P < 0.001$, β-sitosterol; 1.38 ± 0.06 , $P < 0.001$) or those in patients undergoing intensive therapy (campesterol; 2.63 ± 0.15 , $P < 0.05$, β-sitosterol; 1.44 ± 0.14 , $P < 0.005$) (Table 1). However, there was no statistical difference in plasma cholesterol levels among the three groups of subjects.

Effects of acute glycemic control on both DAO activities and the plant sterol levels were also examined in the same eight IDDM patients who were switched conventional therapy to intensive therapy (Fig. 3). The FPG levels were significantly decreased from 9.8 ± 0.8 mmol/l to 5.9 ± 0.8 mmol/l ($P < 0.05$). The GA level was also decreased from 31.2 to 24.2% ($P < 0.05$). The DAO activity at 30 min after the heparin was significantly decreased from 1.78 ± 0.37 mU/l to 0.81 ± 0.22 mU/l ($P < 0.01$) by the intensive insulin therapy. The plasma cholesterol concentrations decreased significantly from 5.07 ± 0.37 mmol/l to

Table 1
Characteristics of the normal subjects and the diabetic patients^a

Variable	Normal subjects	Diabetic patients	
		Conventional therapy	Intensive therapy
No. (F/M)	10 (5/5)	12 (6/6)	10 (5/5)
Age (years)	32 ± 3	32 ± 4	32 ± 4
Height (m)	1.64 ± 0.02	1.62 ± 0.02	1.63 ± 0.04
BMI (kg/m ²)	22.9 ± 1.0	22.5 ± 1.2	21.2 ± 1.0
Diabetic duration (years)	–	6 ± 2	4 ± 1
Insulin dose (U·kg ⁻¹ ·day ⁻¹)	–	0.70 ± 0.08	0.89 ± 0.10
FPG (mmol/l)	4.6 ± 0.2 ^b	11.2 ± 0.8	6.3 ± 0.6 ^b
Glycated albumin (%)	14.8 ± 0.5 ^b	32.3 ± 1.6	25.5 ± 1.4 ^{b,c}
Fasting plasma IRI (pmol/l)	43 ± 5	45 ± 14	47 ± 14
Serum cholesterol (mmol/l)	4.89 ± 0.26	5.02 ± 0.32	4.75 ± 0.20
Serum triglyceride (mmol/l)	1.23 ± 0.16	1.06 ± 0.11	0.99 ± 0.11
Serum HDL-cholesterol (mmol/l)	1.35 ± 0.06	1.37 ± 0.11	1.46 ± 0.10
Serum LDL-cholesterol (mmol/l)	2.99 ± 0.24	3.16 ± 0.32	2.84 ± 0.23
Serum campesterol (mmol/mol of cholesterol)	2.02 ± 0.20 ^b	3.84 ± 0.44	2.63 ± 0.15 ^d
Serum β -sitosterol (mmol/mol of cholesterol)	1.38 ± 0.06 ^b	2.33 ± 0.25	1.44 ± 0.14 ^c

^a Values are determined on the day of heparin infusion test except the value of insulin dose, and are expressed as mean ± S.E.M. The insulin dose in each group was calculated the mean value on one day before the heparin test. The LDL-cholesterol levels were calculated according to the Friedewald formula. The multiple comparison test to compare the differences among the three groups were calculated by Scheffé's comparison test.

^b Indicates $P < 0.001$ vs. diabetic patients undergoing conventional therapy.

^c Indicates $P < 0.001$ vs. normal subjects.

^d Indicates $P < 0.05$ vs. diabetic patients undergoing conventional therapy.

^e Indicates $P < 0.005$ vs. diabetic patients undergoing conventional therapy.

4.48 ± 0.41 mmol/l ($P < 0.01$) after the intensive insulin therapy. Plasma plant sterol levels (mmol/mol of cholesterol) also significantly decreased (campesterol; from 3.93 ± 0.56 to 3.03 ± 0.46, $P < 0.05$, β -sitosterol; from 2.35 ± 0.28 to 1.48 ± 0.25, $P < 0.01$, respectively). On the other hand, the plasma LPL mass at 10 min after intravenous heparin injection was not significantly changed before (192 ± 11 ng/ml) and after (214 ± 24 ng/ml) the intensive insulin therapy ($P = 0.58$).

4. Discussion

We found that plasma post-heparin DAO activity, an index of small intestinal absorptive area, and plasma plant sterol levels, a marker of cholesterol absorption efficacy, were both increased in poor glycemic type 1 diabetic patients with conventional insulin therapy. Moreover, glycemic control after the intensive insulin therapy decreased these two parameters to the normal levels, indicating an importance of intensive insulin therapy and the good glycemic control for the normalization of small intestinal sterol absorption in type 1 diabetic patients.

The type 1 diabetic patients with poor glycemic control showed that their plasma cholesterol levels were increased when compared with non-diabetic subjects [5,6]. It has also been reported that cholesterol intake is one of the major determinants for plasma cholesterol

concentration in uncontrolled diabetic subjects [9]. Moreover, following the National Cholesterol Education Programs [32], recommendation of low-cholesterol

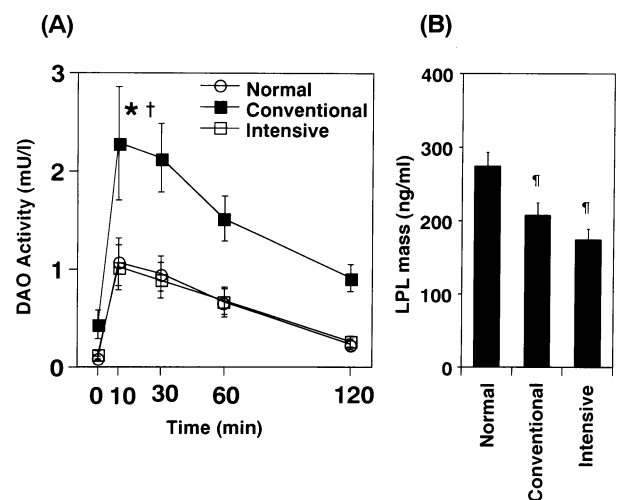


Fig. 1. Plasma DAO activity (A) and LPL mass (B) after 30 U/kg heparin injection. Blood was drawn after an overnight fast and the plasma was stored at -70°C until the DAO activity was measured. The procedure for the measurement of DAO activity was described in Section 2. Plasma LPL mass was determined 10 min after the heparin. The plasma DAO activities were compared by two factor ANOVA and the differences among the three groups were calculated by Fisher's protected LSD using a software of Macintosh Super ANOVA ver 1.11. * $P < 0.05$ vs. normal group; † $P < 0.05$ vs. intensive group, ¶ $P < 0.01$ vs. conventional group.

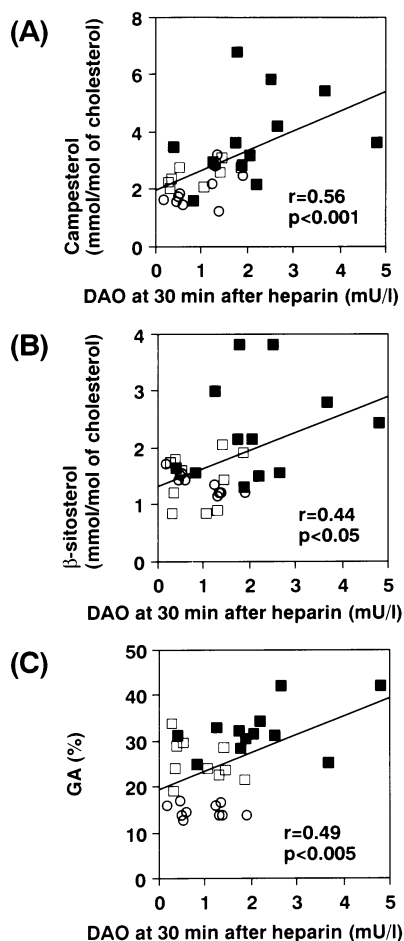


Fig. 2. Relationship between plasma post-heparin DAO activity and plasma level of campesterol (A), β -sitosterol (B), or GA (C). Univariate correlation (r) and regression analyses were performed to determine the relation between DAO activities and other clinical variables in all subjects. Values represent measurements from the patients undergoing conventional insulin therapy (■), the patients undergoing intensive insulin therapy (□), and the normal subjects (○).

diet to type 1 diabetic patients [33,34] results in no abnormal increment of plasma cholesterol levels even if their good glycemic control has not been achieved. These results strongly suggest that plasma cholesterol concentration in type 1 diabetic patients with poor glycemic control may respond more sensitively to the quantity of dietary cholesterol than normal subjects.

Recently, Gylling and Miettinen [10] have reported an interesting study about two brothers with type 1 diabetes, in which the patients had increased efficacy of cholesterol absorption together with the increased plasma plant sterol levels (mmol/mol of cholesterol) under the poor glycemic control. Plant sterols (campesterol and β -sitosterol) are not synthesized in humans [35] and the plasma plant sterol levels and fractional cholesterol absorption have been shown to be closely correlated [36]. Furthermore, patients with gut resections were reported to show decreased fractional cholesterol absorption along with decreased plasma plant

sterol levels [12]. Since these findings strongly suggest that plasma plant sterol concentrations may in part be regulated by the functioning enterocytic mass, we measured plasma plant sterols as a convenient and reliable clinical parameter for the evaluation of cholesterol absorption efficacy, and found that the values were increased in type 1 diabetic patients with poor glycemic control. Moreover, we have found small but significant relationship between plasma plant sterol levels and DAO activity. In animal study [19], however, the association between sterol absorption efficiency and DAO activity is closely associated ($r^2 = 0.83$). Since the degree of r^2 is not so marked in this human study, the absorption efficacy of sterols is not solely regulated by the intestinal mass size. Therefore, the biological significance should be justified further in the future study. On the other hand, it is also possible that the degree of hyperglycemia might influence plasma plant sterol clearance in type 1 diabetic patients. Prior to this human study, we examined the metabolic effect of hyperglycemia on plant sterol clearance in insulin-deficient diabetic rats. The rates of disappearance of plant sterols were almost identical between non-diabetic and diabetic rats [37]. Although this evidence suggests that plasma plant sterol clearance may be similar between non-diabetic and type 1 diabetic subjects, it is necessary to be further clarified.

Since the aim of this study was to know the sterol absorption efficacy by plant sterol, we used a standard hospital diet with low cholesterol content for diabetic patients, of which plant sterol/cholesterol ratio was relatively high compared with typical western style diet in contrast to the previous high cholesterol diet [5,6]. When we compare the origins of plasma concentrations of cholesterol and plant sterol, there is a marked difference between the two sterols. Plasma cholesterol is supplied from endogenous biosynthesis as well as exogenous origins, whereas plasma plant sterols are only from the diet. In the present study, we could not observe a significant increment of the plasma cholesterol levels in poor controlled type 1 diabetic patients compared with that in good controlled patients or normal subjects in the cross-sectional study, while plasma plant sterol level in the conventional insulin group was significantly increased compared those in other two groups. These differences of the supplies might produce the discrepancy in plasma comparison between cholesterol and plant sterols. Therefore, individual difference of the body cholesterol biosynthesis and the differences of apolipoprotein ϵ allele-linked cholesterol metabolism [38] were also important to determine plasma cholesterol levels in type 1 diabetic patients.

In the present study, plasma post-heparin DAO activity was significantly increased in poor glycemic type 1 diabetic patients. It has already been reported that the determination of the plasma post-heparin DAO activity

is a useful marker for the evaluation of intestinal mucosal surface area. D'agostino et al. [23], and Rokkas et al. [24] have reported that plasma post-heparin DAO activities were decreased in patients with chronic intestinal diseases such as Crohn's disease or celiac diseases, suggesting the co-existence of intestinal mucosal hypoplasia and decreased DAO activity. After the successful treatment of the active disease, those DAO activities were normalized [24], indicating that the determination of the enzyme activity was a useful index for the evaluation of the mucosal mass. Moreover, there was a close inverse correlation between plasma post-heparin DAO activity and the length of intestine removed at the time of resection [24].

Regarding plasma DAO activity in diabetes mellitus, only a few reports have been published. Yuen et al. [39] have reported that the basal activities of serum DAO, which were measured without heparin injection, were significantly increased in diabetic subjects. They suggested that the changes of DAO might be related to collagen abnormalities, but did not mention the involvement of the small intestine in diabetes mellitus. In order to evaluate a significance of small intestine as a determining factor for plasma post-heparin DAO activity, it had been ruled out that our subjects were not suffering from liver dysfunction since the enzyme DAO was reported to be mainly metabolized by the liver [40]. In addition, patients with chronic liver diseases are reported to have decreased plasma post-heparin DAO activities [41,42]. Though it is postulated that diabetes-induced subclinical liver injury may affect the clearance

of the enzyme, the time course of the post-heparin DAO activities in the present study showed no delay in both the peak value and the following activities in the plasma in patients with conventional insulin treatment. These results indicate that it is unlikely that the plasma DAO activity in type 1 diabetes with poor glycemic control is affected by the changes of the enzyme clearance by the liver.

Consistent with our previous rat study [19,20,37], we have recently reported that the intestinal crypt cell growth by a growth factor (IGF-I) is suppressed by insulin through an autocrine/paracrine secretion of TGF- β 1 [43]. Since the plasma heparin-induced DAO activity is a reflection of the DAO enzyme mass produced from matured enterocytes, the increased DAO activity in the plasma in insulin deficiency may in part be produced by the lack of insulin action for cell growth suppression. These results in both the human and animals in vivo or crypt cell growth in vitro support the hypothesis that the increased plasma post-heparin DAO activity in poor controlled type 1 diabetic patients may be associated with the increased intestinal absorptive area, which needs to be further evaluated.

We also determined the LPL mass as one of the co-evaluating enzymes with DAO during the intravenous heparin. Lipoprotein lipase activity was reported to be significantly low in IDDM patients without insulin therapy, and was increased upon introduction of insulin therapy [3,4]. In this study, however, we observed still significant reduction of post-heparin LPL mass in patients with intensive insulin therapy

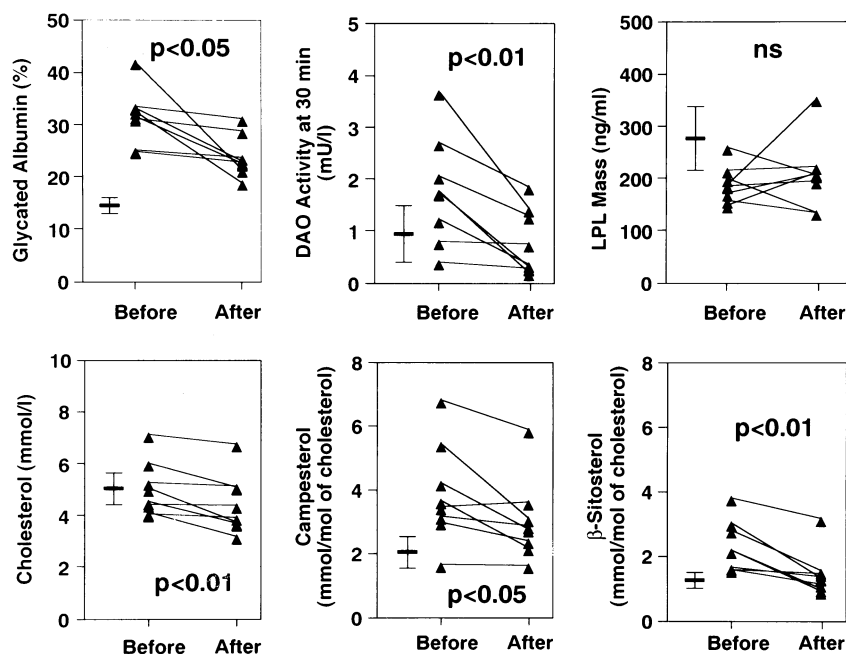


Fig. 3. Plasma glycated albumin, DAO activity, LPL mass, and sterol (cholesterol, campesterol and β -sitosterol) levels in eight diabetic patients before and after 14 days of intensive insulin therapy. Statistical significances were calculated by the paired *t*-test, and the results were shown in the figures. The vertical bar in the left side of the each figures indicate the mean \pm S.D. of the measure in the normal groups.

compared with those in normal subjects, indicating perfect and longer glycemic control was needed for the complete normalization of the enzyme.

In summary, type 1 diabetic patients with conventional insulin therapy showed increased plasma post-heparin DAO activity as well as increased plasma plant sterol levels compared with normal subjects. Moreover, strict glycemic control by intensive insulin therapy normalized both the plasma DAO and sterol levels. These results indicate that the poor glycemic control is associated with the increased small intestinal absorption surface as well as sterol absorption efficacy. Although we have not clearly defined the mechanism by which the DAO activity in post-heparin plasma is increased in poor-controlled insulin-deficient diabetic patients, the relationship between plasma plant sterol levels and DAO activity suggests a close association between intestinal absorption area and sterol absorption efficacy.

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