Impact of Insulin Resistance and Nephropathy on Homocysteine in Type 2 Diabetes

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OBJECTIVE — To assess the impacts of insulin resistance and renal function on plasma total homocysteine (tHcy) levels in patients with type 2 diabetes with a wide range of nephropathy.

RESEARCH DESIGN AND METHODS — Plasma tHcy levels were measured using the enzyme immunoassay method in 75 patients with type 2 diabetes and compared with those in 54 healthy control subjects. Insulin sensitivity indexes were assessed in patients with type 2 diabetes by hyperinsulinemic-euglycemic clamp using artificial pancreas.

RESULTS — Plasma tHcy levels and their log-transformed values (log tHcy) were significantly higher in all patients with diabetes than in control subjects (tHcy, 12.0 ± 0.7 [SE] vs. 8.7 ± 0.3 μmol/l, $P < 0.0001$; log tHcy, 1.040 ± 0.021 vs. 0.920 ± 0.016 μmol/l, $P < 0.0001$). Plasma tHcy levels in patients with diabetes were significantly increased according to degree of nephropathy ($P < 0.0001$). On simple regression analyses, log tHcy correlated with insulin sensitivity indexes ($r = -0.319$, $P = 0.005$) as well as creatinine clearance ($r = 0.634$, $P < 0.0001$) in all patients with diabetes. Multiple regression analyses showed that insulin sensitivity indexes ($\beta = -0.245$) as well as creatinine clearance were independent contributors to log tHcy in all patients with diabetes ($R^2 = 0.750$, $P < 0.0001$). For the 59 patients with diabetes with creatinine clearance >60 ml/min, insulin sensitivity indexes were also shown to be a significant contributor to log tHcy ($\beta = -0.438$, $R^2 = 0.561$, $P < 0.001$).

CONCLUSION — Insulin resistance and renal function are independent determinants of tHcy levels in patients with type 2 diabetes.

Diabetes Care 24:533–538, 2001

Homocysteine is a sulfur-containing amino acid formed during the conversion of methionine to cysteine. Since the first report by Wilcken et al. (1), increased plasma total homocysteine (tHcy) level has been recognized as an independent risk factor for coronary atherosclerotic disease (2,3), which is the most common cause of mortality in patients with type 2 diabetes. There is controversy concerning plasma tHcy levels in type 2 diabetes. Plasma tHcy levels in patients with type 2 diabetes are reported to be similar to or higher than those in healthy subjects (4–6). Plasma tHcy levels are reported to be associated with hypertension, hyperuricemia, impaired renal function, and increased risk for development of coronary atherosclerotic disease (7–10). Insulin resistance has been hypothesized to play an important role in the development of atherosclerotic disease (11). Therefore, the association of insulin resistance with plasma tHcy levels must be clarified as it relates to development of atherosclerotic disease. Only a few studies have investigated the association between insulin resistance and plasma tHcy levels in healthy subjects (12–15), and a few studies in animals have suggested that insulin affects the activities of key enzymes in homocysteine metabolism (16,17). It has not been clarified whether insulin resistance in type 2 diabetes is associated with plasma tHcy levels.

The purpose of the present study was to investigate the impacts of insulin resistance and nephropathy on plasma tHcy levels in patients with type 2 diabetes by the hyperinsulinemic-euglycemic clamp technique. We report here that insulin resistance and impaired renal function are independent determinants of plasma tHcy levels in patients with type 2 diabetes with a wide range of nephropathy.

RESEARCH DESIGN AND METHODS

Subjects

A total of 75 patients with type 2 diabetes (46 men and 29 women) participating in diabetes education programs were selected for the present study from among patients attending the diabetes center at Osaka City University Hospital. The diagnosis of diabetes was based on a previous history of diabetes or on the American Diabetes Association criteria (18). The mean values of age, BMI, and known duration of diabetes were 56.0 ± 9.9 years, 22.4 ± 3.1 kg/m^2, and 11.1 ± 8.8 years, respectively. All patients with diabetes had detectable fasting levels of serum C-peptide (mean 0.76 ± 0.50 nmol/l, range 0.23–2.55 nmol/l) and no history of diabetic ketoacidosis. To measure urinary albumin and creatinine excretions in all patients with diabetes, 24-h urine specimens were

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Received for publication 31 July 2000 and accepted in revised form 15 November 2000.

Abbreviations: CBS, cystathione β-synthase; MTHFR, 5,10-methylenetetrahydrofolate reductase; tHcy, total homocysteine; SSPI, plasma insulin level during the steady state; UAE, urinary albumin excretion.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

collected for 3 consecutive days; mean values of these parameters were adopted for data analyses. The 75 patients with type 2 diabetes were divided into four subgroups of diabetic nephropathy, determined according to urinary albumin excretion (UAE) and serum creatinine level for data analyses: stage 1, 39 patients with normal UAE <30 mg per 24 h; stage 2, 17 patients with microalbuminuria (30 < UAE < 300 mg per 24 h); stage 3, 9 patients with overt proteinuria (UAE >300 mg per 24 h); stage 4, 10 subjects with serum creatinine levels >176 μmol/l. Creatinine clearance was estimated in all patients with diabetes from serum creatinine level and Cockcroft-Gault equations (19). A total of 59 patients with diabetes (39 in stage 1, 17 in stage 2, and 3 in stage 3) had creatinine clearances >60 ml/min, and 16 subjects (6 in stage 3 and 10 in stage 4) had creatinine clearances <60 ml/min. Of the patients with diabetes, 37 were treated with sulfonylureas, 2 were treated with α-glucosidase inhibitors, 4 were treated with the combination of sulfonylureas and α-glucosidase inhibitors, 20 were treated with insulin, and 12 were treated with diet alone.

The control subjects (54 healthy individuals: 28 men and 26 women) were selected from among apparently healthy subjects participating in a health check program at Osaka Municipal Health Promotion Center, as previously reported (20). The mean age of healthy subjects was 53.8 ± 10.5 years. Informed consent was obtained from all participants in the present study.

Homocysteine assay

Blood sampling for assay of plasma tHcy level was performed overnight in the fasting state, immediately before hyperinsulinemic-euglycemic clamp, as described below. The blood was collected into a tube containing EDTA-2Na and immediately centrifuged, and the plasma was stored at –20°C until assay. Plasma level of tHcy was measured by an enzyme conversion immunoassay kit (Homocysteine; Axis, Norway) according to the method of Frantzen et al. (21).

Hyperinsulinemic-euglycemic clamp

The hyperinsulinemic-euglycemic clamp protocol was performed according to the method of DeFronzo et al. (22) using an STG 22 artificial pancreas model (Nikkiso, Tokyo). After overnight fasting, venous blood sampling and measurement of blood pressure were performed with the patient in the supine position, and the hyperinsulinemic-euglycemic clamp protocol was begun as previously described (20,23). In brief, insulin (Humulin; Eli Lilly, Indianapolis, IN) was infused in a continuous fashion at a rate of 1.25 mU · kg^{-1} · min^{-1} after the priming insulin infusion during the first 10 min of the clamp at the same doses as reported previously. Blood glucose levels were determined every 5 min during the 120-min clamp study, and euglycemia (5.0 mmol/l) was maintained by infusion of variable amounts of 20% glucose solution. The mean coefficient of variance of blood glucose in maintaining euglycemia was 1.29% and ranged from 0.4 to 2.9%. The total body glucose disposal rate was evaluated as the mean of the glucose infusion rate during the last 30 min of the clamp. The mean plasma insulin level during the steady state (SSPI) was 647 ± 191 pmol/l in all patients with diabetes. The insulin sensitivity indexes from the clamp study were calculated by dividing the mean glucose infusion rate by SSPI levels during the last 30 min of the clamp and multiplying by 100.

Biochemical assay

Plasma glucose levels were measured by the glucose oxidase method, HbA_{1c} levels were measured by high-performance liquid chromatography (reference range 4.0–5.5%), plasma insulin levels were measured by immunoradiometric assay (Insulin RIA Bead II kit; Dainabot, Tokyo), and urinary albumin levels were measured by immunoturbidometry (TIA MicoAlb kit; Nittobo, Tokyo). Serum levels of vitamin B_{12} and folate were measured by an automated chemiluminescence system (ACS:180 VB12 and Folate kit; Bayer Medical, Tokyo); reference ranges were 172–674 pmol/l for vitamin B_{12} and 5.4–22.2 nmol/l for folate. Serum and urinary creatinine, serum total cholesterol, triglyceride, and HDL cholesterol levels were measured by enzymatic methods adapted to an autoanalyzer (Hitachi 7450; Hitachi, Tokyo).

Statistical analyses

Statistical analyses were performed with the Stat View 5 system (Abacus Concepts, Berkeley, CA) for the Apple Macintosh computer. All values were expressed as means ± SE, unless otherwise indicated. Student's *t* tests, one-way analyses of variance (Scheffe type), and χ^2 tests were appropriately performed for comparison of groups. Univariate linear regression analyses and multiple regression analyses were performed to evaluate the relationships among tHcy levels and various clinical factors. *P* values less than 0.05 were considered significant.

RESULTS

Clinical characteristics of subjects

Clinical characteristics of all patients with diabetes and control subjects are shown in Table 1. There were no significant differences in age, sex, or BMI between the two groups. Smoking indexes, systolic blood pressure, and fasting plasma glucose levels were significantly higher in all patients with diabetes than in control subjects. There were no significant differences in either total cholesterol or triglyceride levels between the patients with type 2 diabetes and the healthy subjects (total cholesterol 4.95 ± 0.15 vs. 5.08 ± 0.09 mmol/l, triglyceride 1.28 ± 0.08 vs. 1.27 ± 0.09 mmol/l, respectively). Serum HDL cholesterol levels were significantly lower in patients with type 2 diabetes than in healthy subjects (1.14 ± 0.04 vs. 1.57 ± 0.07 mmol/l, $P < 0.01$). Systolic blood pressures in patients in stages 3 and 4 were significantly higher than those in patients in stage 1. Serum levels of vitamin B_{12} and folate in patients with diabetes exhibited skewed distributions, and their log-transformed values (log vitamin B_{12} and log folate) were used for analyses. There were no significant differences in either log vitamin B_{12} or log folate among the four groups of patients with diabetic nephropathy. Serum creatinine levels varied from 26.5 to 716.0 μmol/l with the inclusion of patients with diabetes in stage 4. Creatinine clearances in patients in stages 3 and 4 were significantly lower than those in stage-1 patients. There were no significant differences in age, smoking indexes, or fasting plasma insulin levels among the four groups.

Plasma tHcy levels

Plasma tHcy levels in patients with diabetes and control subjects exhibited skewed distributions; therefore, data were analyzed as log-transformed parameters (log tHcy). Plasma tHcy and log tHcy were sig-

Table 1—*Clinical profiles of type 2 diabetic subjects with various stages of nephropathy*

	Control	All patients with diabetes	Stages of diabetic nephropathy			
			Stage 1	Stage 2	Stage 3	Stage 4
n	54 (28/26)	75 (46/29)	39	17	9	10
Age (years)	53.8 ± 1.4	56.0 ± 1.1	54.6 ± 1.7	53.9 ± 2.2	57.0 ± 2.4	64.0 ± 1.3
BMI (kg/m^2)	22.0 ± 0.3	22.4 ± 0.3	22.2 ± 0.6	22.8 ± 0.7	22.4 ± 0.9	22.8 ± 0.6
Duration of diabetes (years)	—	11.1 ± 1.0	8.9 ± 1.1	8.1 ± 1.3	15.9 ± 2.3	20.4 ± 4.2††
Smoking index (cigarette-years)	183 ± 43	417 ± 59*	472 ± 88	466 ± 112	289 ± 192	230 ± 117
Systolic blood pressure (mmHg)	122 ± 3	135 ± 3**	120 ± 2	137 ± 6	159 ± 7†††	165 ± 8†††
Diastolic blood pressure (mmHg)	77 ± 2	73 ± 1	70 ± 1	76 ± 3	81 ± 3	76 ± 6
HbA_{1c} (%)	—	8.7 ± 0.3	9.2 ± 0.4	8.5 ± 0.4	8.6 ± 0.8	7.0 ± 0.4†
Creatinine (μmol/l)	72 ± 2	113 ± 17	59 ± 2	68 ± 4	90 ± 16	416 ± 68†††
Log vitamin B_{12} (pmol/l)	—	2.76 ± 0.02	2.78 ± 0.03	2.65 ± 0.04	2.79 ± 0.05	2.82 ± 0.08
Log folate (nmol/l)	—	1.20 ± 0.02	1.20 ± 0.03	1.16 ± 0.03	1.22 ± 0.06	1.22 ± 0.05
tHcy (μmol/l)	8.7 ± 0.3	12.0 ± 0.7***	9.6 ± 0.4	11.2 ± 1.1	13.3 ± 1.8	21.6 ± 2.0†
Log tHcy (μmol/l)	0.920 ± 0.016	1.040 ± 0.02***	0.966 ± 0.018	1.017 ± 0.044	1.092 ± 0.060*	1.319 ± 0.040***†††

Data are mean ±SE or *n*. *, **, or *** $P < 0.05$, 0.01, or 0.001 vs. healthy subjects, respectively. †, ††, or ††† $P < 0.05$, 0.01, or 0.001 vs. patients with diabetes with stage 1 nephropathy, respectively.

nificantly higher in all patients with diabetes than in control subjects (tHcy 12.0 ± 0.7 vs. 8.7 ± 0.3 μmol/l [$P < 0.0001$], log tHcy 1.040 ± 0.021 vs. 0.920 ± 0.016 [$P < 0.0001$], respectively). Plasma tHcy levels in patients with diabetes were significantly increased according to degree of nephropathy ($P < 0.0001$) (Table 1). Log tHcy in neither patients in stage 1 nor patients in stage 2 differed significantly from that in control subjects ($P = 0.615$ or $P = 0.150$ for patients in stages 1 and 2, respectively) (Table 1). Log tHcy values in patients in both stage 3 and stage 4 were significantly higher than those in control subjects ($P = 0.0147$ and $P < 0.0001$ for patients in stages 3 and 4, respectively) (Table 1). The percentages of subjects with hyperhomocysteinemia (i.e., level higher than 13.8 μmol/l, equal to mean + 2 SD in control subjects) were 2.6% in stage 1, 11.8% in stage 2, 44.4% in stage 3, and 90.0% in stage 4. The frequencies of hyperhomocysteinemia in patients in stages 3 and 4 were also significantly higher than the 7.4% of control subjects ($P = 0.002$ and $P < 0.0001$ for patients in stages 3 and 4, respectively).

Impacts of insulin resistance and nephropathy on plasma tHcy levels

In all patients with diabetes, log tHcy levels were significantly correlated with the insulin sensitivity index ($r = -0.319$, $P = 0.005$) on simple regression analysis (Table 2). Simple regression analyses showed that log tHcy was significantly correlated with various clinical factors. Among these factors, serum creatinine levels and creatinine clearance exhibited the strongest associations with log tHcy in all patients with diabetes. Neither log vitamin B_{12} nor log folate exhibited significant correlations with log tHcy on simple regression analyses. To assess the impacts of the insulin sensitivity index and nephropathy on plasma tHcy levels in all patients with diabetes, multiple regression analyses were performed. In model 1 (all patients with diabetes), the log tHcy level was entered as a dependent variable, and age,

Table 2—*Correlation coefficients determined by simple regression analyses between log-transformed plasma tHcy levels and clinical variables for all 75 diabetic subjects and those of 59 type 2 diabetic subjects with creatinine clearance >60 ml/min*

	All diabetic subjects		Diabetic subjects with creatinine clearance >60 ml/min	
	r	*P*	*r*	*P*
Age	0.397	0.0004‡	0.223	0.089
BMI	0.221	0.057	0.205	0.120
Duration of diabetes	0.218	0.060	−0.100	0.450
Smoking index	0.075	0.524	0.261	0.046*
Systolic blood pressure	0.495	<0.0001‡	0.167	0.207
Diastolic blood pressure	0.153	0.188	0.120	0.365
HbA_{1c}	−0.377	0.0009‡	−0.229	0.081
Fasting plasma insulin	0.182	0.118	0.125	0.344
Uric acid	0.539	<0.0001‡	0.255	0.051
Serum creatinine	0.636	<0.0001‡	0.388	0.002†
Total cholesterol	−0.036	0.761	−0.005	0.970
HDL cholesterol	−0.294	0.010*	−0.171	0.195
Triglyceride	0.172	0.140	0.165	0.213
Log vitamin B_{12}	−0.142	0.248	−0.258	0.060
Log folate	−0.118	0.338	−0.267	0.051
Creatinine clearance	−0.634	<0.0001‡	−0.206	0.117
Insulin sensitivity index	−0.319	0.0054†	0.237	0.070

The serum levels of vitamin B_{12} and folate were log-transformed (log vitamin B_{12} and log folate, respectively). Creatinine clearance was estimated by Cockcroft-Gault derived equation. Simple correlation coefficients are shown with level of significance. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.0001$.

Table 3—*Standard correlation coefficients (β) on multiple regression analyses for clinical factors affecting log-transformed plasma total homocysteine levels in all 75 patients with diabetes and those of 59 patients with type 2 diabetes with creatinine clearance >60 ml/min*

	All patients with diabetes		Patients with diabetes with creatinine clearance >60 ml/min	
Independent variables	Model 1	Model 2	Model 3	Model 4
Age	0.123	0.101	0.297	0.241
Gender	0.133	0.179*	0.213*	0.296*
Systolic blood pressure	0.151	0.078	0.122	0.003
HbA_{1c}	–0.008	–0.070	0.014	0.165
Uric acid	0.298†	0.321†	0.308*	0.368†
Log vitamin B_{12}	–0.331†	–0.329‡	–0.386†	–0.375†
Log folate	–0.106	–0.177*	–0.187	–0.336*
Creatinine clearance	–0.521‡	–0.529‡	–0.217	–0.252
Insulin sensitivity index		–0.245†		–0.438†
R^2	0.710‡	0.750‡	0.434†	0.561†

Models 1 and 2 were used for all 75 patients with diabetes, and models 3 and 4 were used for 59 patients with diabetes with creatinine clearance >60 ml/min. In all models, log-transformed plasma tHcy level (log tHcy) was entered as a dependent variable. For sex, female was entered as 0 and male was entered as 1. Creatinine clearance was estimated by Cockcroft-Gault derived equation. The serum levels of vitamin B_{12} and folate were log-transformed (log vitamin B_{12} and log folate). Standard correlation coefficients (β) and multiple coefficients of determination (R^2) are shown with level of significance. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.0001$.

sex, systolic blood pressure, HbA_{1c}, uric acid, creatinine clearance, log vitamin B_{12}, and log folate were entered as independent variables (Table 3). The creatinine clearance, log vitamin B_{12}, and uric acid were significant contributors to log tHcy in this model($R^2 = 0.710$, $P < 0.0001$). The insulin sensitivity index was also a significant contributor to log tHcy in model 2, in which insulin sensitivity index was added as an independent variable in addition to the variables of model 1. The addition of the insulin sensitivity index increased the multiple coefficient of determination (R^2) by 0.040 from 0.710 in model 1 to 0.750 in model 2.

Because the decrease in creatinine clearance was a strong contributor to the increase in tHcy in both simple and multiple regression analyses, the impact of insulin resistance on log tHcy was further analyzed for the 59 patients with diabetes with creatinine clearance >60 ml/min. For these patients with diabetes, simple regression analyses failed to reveal a significant correlation between the insulin sensitivity index and log tHcy (Table 2). Multiple regression analyses (models 3 and 4) for such patients with diabetes were also performed using the same dependent and independent variables as in models 1 and 2 for all patients with diabetes (Table 3). In these analyses, the insulin sensitivity index was a significant determinant of log tHcy, as were sex, uric acid, log vitamin B_{12}, and log folate. Furthermore, R^2 increased by 0.127 from 0.434 in model 3 to 0.561 in model 4.

CONCLUSION — The present study demonstrated the impacts of insulin resistance and diabetic nephropathy on plasma tHcy levels in patients with type 2 diabetes with nephropathy. Plasma tHcy levels in patients with type 2 diabetes were, on the whole, higher than those in healthy subjects. Plasma tHcy levels in patients with type 2 diabetes with advanced nephropathy were higher than those in control subjects or patients with diabetes without nephropathy. Renal function, represented by creatinine clearance and serum creatinine level, was the strongest contributor to plasma tHcy in patients with diabetes. Insulin resistance in patients with type 2 diabetes contributed independently to plasma tHcy levels, even with adjustment for renal function and vitamin status.

Consensus regarding plasma tHcy level in type 2 diabetes has not been achieved. A few studies found no significant differences in fasting plasma tHcy levels between patients with type 2 diabetes and healthy subjects (4,5). Other studies have found increased plasma tHcy levels in patients with type 2 diabetes with atherosclerotic diseases (6,7,9). Microalbuminuria, an early marker of endothelial dysfunction as well as nephropathy, has been reported to be associated with plasma tHcy level in patients with diabetes (4,5,24). However, Smulders et al. (6) recently found no association of microalbuminuria with plasma tHcy levels in patients with type 2 diabetes. In the present study, there were no differences in plasma tHcy levels between patients with type 2 diabetes, either with or without microalbuminuria, and control subjects. Patients with type 2 diabetes with proteinuria more commonly have hyperhomocysteinemia than control subjects, and plasma tHcy levels in subjects with renal failure were markedly increased. The findings of the present study were consistent with those reported by Chico et al. (4). Furthermore, multiple regression analyses in the present study revealed a strong association of hyperhomocysteinemia with decrease in renal function and hypertension. This is supported by the findings of previous studies, in which ~70% of the homocysteine in blood was found to be removed by reuptake and metabolism in the renal tubules, and in which decreased clearance of homocysteine resulted in hyperhomocysteinemia (25–27).

Insulin resistance has been hypothesized to play an important role in the development of atherosclerotic disease via hypertension and dyslipidemia as well as hyperglycemia (11). The causal relationship between insulin resistance and hyperhomocysteinemia is an important issue requiring further clarification. Limited study has been conducted on the direct association between insulin resistance and hyperhomocysteinemia in humans (12–15). Giltay et al. (12) investigated the association between plasma tHcy levels and insulin resistance assessed by hyperinsulinemic-euglycemic clamp in 24 nonobese healthy subjects. They found a significant increase in plasma tHcy level in healthy subjects with insulin resistance among three groups (divided by degree of insulin resistance). Gallistl et al. (15) demonstrated that fasting insulin level was an independent determinant of plasma tHcy level in 84 obese children and adolescents. However, Abbasi et al. (14) failed to find a direct association between plasma tHcy level and insulin resistance assessed by the modified insulin suppression test in 55 healthy subjects with a wide range of BMI.

In this study, vitamin status, an important confounding factor for plasma tHcy level, was not documented. Fonseca et al. (13) demonstrated that acute hyperinsulinemia using hyperinsulinemic-euglycemic clamp decreased plasma tHcy levels in healthy subjects but not in patients with type 2 diabetes. The controversy concerning insulin resistance and plasma tHcy level may be due to the heterogeneity of subjects and the difference in methods used to assess insulin resistance in humans. No studies have previously investigated the impact of insulin resistance on plasma tHcy levels in type 2 diabetes. Vitamin status, renal function, sex, hyperuricemia, and hypertension are known to be major confounding factors for plasma tHcy level. The present study clearly demonstrated that even after adjustment for these confounding factors, insulin resistance was an independent determinant of plasma tHcy level in type 2 diabetes. Furthermore, this independent contribution of insulin resistance to plasma tHcy level was also found when the group of patients with type 2 diabetes was limited to those with normal renal function (Table 3). Although the underlying mechanisms were not demonstrated in the present study, recent studies suggested some possibilities. A few studies have demonstrated that insulin may affect activities of enzymes involved in homocysteine metabolism, cystathione β-synthase (CBS) and 5,10-methylenetetrahydrofolate reductase (MTHFR), which are key enzymes of homocysteine transsulfation and remethylation pathways, respectively (16,17). Jacobs et al. (16) have also reported that plasma homocysteine levels were decreased by the increase in CBS activities in streptozotocin-induced diabetic rats but recovered to initial plasma levels after administration of insulin. Furthermore, Fonseca et al. (17) have recently shown that hyperinsulinemia in the high fat–sucrose rat with insulin resistance induced the decreased CBS activities and compensatorily increased MTHFR activities, followed by hyperhomocysteinemia. Considering our data together with those of these studies, it is suggested that the actions of insulin on the enzyme activities of the homocysteine metabolism contribute to plasma homocysteine levels in humans. Further investigations are needed to clarify the direct causal relationship between insulin resistance and homocysteine metabolism.

In summary, the present cross-sectional study clearly demonstrated the independent impacts of insulin resistance and diabetic nephropathy on plasma homocysteine levels in type 2 diabetes. These clinical factors are considered to contribute to the development and progression of atherosclerotic diseases in patients with type 2 diabetes, especially those with nephropathy. Intervention trials for both insulin resistance and hyperhomocysteinemia are needed to clarify the impacts of modification of these factors on the prognosis of patients with diabetes as well as the mutual causal relationships between these two factors.

Acknowledgments— This study was supported by a grant-in-aid for scientific research (no. 11770646) from the Japanese Ministry of Education, Science, and Culture.

A part of this study was presented at the 55th Annual Meeting of the American Diabetes Association, San Diego, California, June 1999.

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