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Determination of Urinary *Myo-/Chiro*-Inositol Ratios from Korean Diabetes Patients

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Of two major forms (*myo*- and *chiro*-inositol) of inositols, only *chiro*-inositol enhances the activity of proteins involved in intracellular glucose metabolism. This study aims to determine the urinary *myo-/chiro*-inositol ratio in type 1 and type 2 diabetes patients and compare its ratio with the normal control group. The 24-hour urinary *myo*- and *chiro*-inositols in 71 Korean diabetes patients and 39 control subjects have been quantified using high-performance liquid chromatography, and their ratios have been evaluated as indices of insulin resistance. The level of 24-hour urinary *myo*-inositol was significantly higher in both type 1 and type 2 diabetes than with the control group, whereas the urinary *chiro*-inositol in type 1 or type 2 diabetes was lower than that in normal subjects. The *myo-/chiro*-inositol ratio in diabetes patients was higher than that in the control group. Twenty four-hour urinary *myo-/chiro*-inositol ratios were significantly elevated in type 1 and type 2 diabetes patients compared to the control group, suggesting that a high ratio of urinary *myo-/chiro*- inositol in type 2 diabetes patients might be used for an index of insulin resistance.

Key Words: *Myo*-inositol, *chiro*-inositol, inositol, insulin resistance, diabetes mellitus

INTRODUCTION

The main pathophysiologic mechanism of type 2 diabetes mellitus is the insulin resistance, resulting from defects in receptor binding or the post-receptor signaling pathway during the insulin action. Therefore, a major strategy for treatment of type 2 diabetes focuses on how to overcome this insulin resistance. Currently, the prevalence of type 2 diabetes is rapidly increasing, and reaches approximately 10% of the world's population. Many studies assessed the severity of insulin resistance and investigated its mechanism. When insulin binds to its receptor, it activates phospholipase C to hydrolyze phosphatidylinositols, attached to the cell membranes, to inositols and diacylglycerols that act as secondary messengers or mediators in the insulin action.[1-3]

Inositols could be directly obtained from inositol-rich foods or synthesized in the body. Moreover, they exist as two major forms: chiro-inositols and *myo*-inositols. *Chiro*- and *myo*-inositols have the same schematic structure except for the configuration of one hydroxyl group at the carbon 1 site (Fig. 1).[4-6] Most inositols exist as *myo*-inositols in the body, and *myo*-inositols are then converted to *chiro*-inositols by the insulin action in insulin-sensitive tissues (muscle, liver, adipose tissues etc). Therefore, the level of the *chiro*-inositols in those tissues can be used for the biomarker of insulin action.

Chiro-inositol activates pyruvate dehydrogenase phosphatase and glycogen synthase, which are rate-limiting enzymes during the insulin-mediated glucose metabolism. When *chiro*-inositols increase in the body, the glucose metabolism is then elevated by the activated pyruvate dehydrogenase phosphatase and glycogen synthase. As a result, the overall insulin reaction is accelerated in

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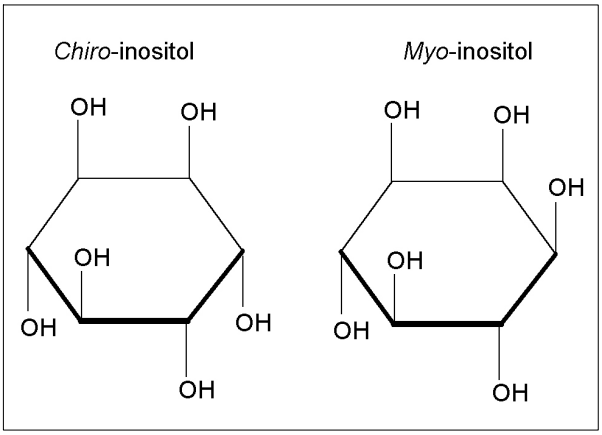

Fig. 1. Chemical structures of *chiro*-inositol and *myo*-inositol. The *myo*-inositol is epimerized at the carbon 1 position to form *chiro*-inositol.

insulin-sensitive tissues.[4-8] Kennington et al.[5] demonstrated that levels of *myo*-inositols and *chiro*-inositols in the muscles and liver are correlated with those in urine and serum. Thus, the urinary levels of *myo*-inositols and *chiro*-inositols can be found in other tissues. According to recent studies, plasma, urinary and muscular *chiro*-inositol levels in type 2 diabetes patients were lower than those in non-diabetic patients.[5] It has also been reported that *chiro*-inositol levels were greatly decreased in the first relatives of patients with type 2 diabetes or in the non-diabetes insulin resistant group.[9] In this study we determined the level of *myo*-inositols and *chiro*-inositols in the 24-hour urine sample of type 1 and type 2 diabetes or the control group to examine that the *myo*-/*chiro*-inositol ratio is elevated in patients with type 1 and type 2 diabetes mellitus more than the control.

MATERIALS AND METHODS

Subjects

Participants in this study included 77 diabetes patients (12 type 1 diabetes patients, 65 type 2 diabetes patients) and 39 control subjects. They visited Gyeongsang National University Hospital as outpatients or inpatients between April and September of 2002. We explained our study to them and received written consent from all participants. Patients were excluded if any of the following circumstances were present: age less than 20 or over 70 years; severe vascular complications such as coronary artery disease, cerebrovascular disease, or peripheral vascular disease; creatinine clearance was less than 60 mL/kg/min; malignancy or severe illness such as liver cirrhosis, congestive heart failure, chronic obstructive lung disease; drug use within 4 weeks that would influence inositol metabolism such as vitamin B complex, anticonvulsants, or herbal medications; severe mental retardation or psychiatric disorders; infection within the last two weeks; malnutrition; pregnancy and abnormal hemoglobin, white blood cell count, or platelet count; serum aspartate transferase, alanine transferase or cholesterol level. Among the 77 subjects, six patients were excluded, because they either failed to collect the 24-hour urinary samples adequately or had a decreased creatinine clearance below 60 mL/min. Therefore, a total of 71 subjects (type 1 diabetes in 11 patients, type 2 diabetes in 60 patients) participated in this study. We defined type 1 diabetes patients as those whose fasting C-peptide is less than 0.01 ng/dL (0.003 nmol/L) and their serum responses to anti-glutamic acid decarboxylase antibodies are positive. The control subjects do not have hypertension or a history of hyperglycemia, and their fasting glucose levels should be below 110 mg/dL (6.1 mmol/L). They have less than 5.8% glycated HbA1c and no first relative with diabetes mellitus. They were selected based on visits to the health examination promotion center at Gyeongsang National University Hospital between April and September 2002. Protocols were approved by the National University of Gyeongsang Institutional Review Board and informed consent was obtained from every subject. The trial was conducted in accordance with the Helsinki Declaration.[10] We analyzed the clinical features between the type 2 diabetes (or type 1) and control group (Table 1). There was no statistical difference for age, sex ratio, body weight, height, body mass index, alcohol drinking history, smoking history or 24-hour urinary creatinine amounts. Only the creatinine clearance was significantly decreased in the type 2 diabetes group compared to the control group. The statistical difference of age, body weight, height, alcohol-drinking history, smoking history and 24-hour uri-

Table 1. Comparison of Clinical Parameters of Subjects

Parameters	Type 1 diabetes (N=11)	Type 2 diabetes (N=60)	Control group (N=39)	Statistical analysis		
				A	B	C
Age (yrs)	32.0 ± 10.5	53.0 ± 11.2	50.3 ± 5.5	‡	‡	NS
Sex (M/F)	6/5	29/31	19/20	NS	NS	NS
Weight (kg)	56.3 ± 15.1	64.1 ± 10.6	64.2 ± 10.6	†	NS	NS
Height (cm)	164.5 ± 7.9	162.7 ± 7.8	163.4 ± 7.6	NS	NS	NS
BMI (kg/m^2)	20.6 ± 4.0	24.2 ± 3.2	23.9 ± 2.4	‡	†	NS
Alcohol	0% (N=0)	8% (N=5)	10% (N=4)	NS	NS	NS
Smoking	18% (N=2)	28% (N=17)	21% (N=8)	NS	NS	NS
Urine Cr (mg/day)	906.4 ± 280.9	932.6 ± 226.6	1041 ± 343.1	NS	NS	NS
CCr (mL/kg/min)	77.1 ± 11.0	72.1 ± 10.2	83.7 ± 20.5	NS	†	†
Urine protein (mg/day)	119.4 ± 179.1	683.7 ± 1717.9	-	NS		
HbA1c (%)	10.7 ± 3.3	7.9 ± 2.1	5.0 ± 2.1	NS		
DM duration (yrs)	2.8 ± 3.2	7.5 ± 6.6	0	NS		
Family history of DM	18% (N=2)	20% (N=12)	0% (N=0)	NS		
DM neuropathy	9% (N=1)	33% (N=20)	0% (N=0)	NS		
DM retinopathy	18% (N=2)	32% (N=19)	0% (N=0)	NS		

NS, $p>0.05$, † $p<0.05$, ‡ $p<0.01$.
Statistical significance between type 1 and type 2 diabetes (A), between type 1 diabetes and controls (B), between type 2 diabetes and controls (C).

nary creatinine amounts between the type 1 group and the control group was not significantly different. The type 1 diabetes group was younger than the control group. However, the body mass index and creatinine clearance was higher in the control group than in the type 1 diabetes group. Compared to type 1 and type 2 diabetes groups, the statistical values for age, height, alcohol drinking history, smoking history, family history of diabetes, duration of diabetes, 24-hour urinary creatinine amounts and creatinine clearance were acceptable. Also, the severity markers of diabetes including diabetic neuropathy, diabetic retinopathy, HbA1c, and 24-hour urine proteinuria showed no statistical difference. The type 1 diabetes group was younger as well as having a lower body weight than the type 2 diabetes group. The body mass index of the type 1 diabetes group was lower than the type 2 diabetes group.

Collection and preparation of blood and urine samples

Blood samples were collected at 8 a.m. after fasting overnight, and serums were quickly separated. Twenty-four-hour urinary samples were collected from subjects who had regular diets. Diabetes patients taking medications such as sulfonylureas, antihypertensive medicines, antilipidemic agents, and aspirins were not restricted. All serum and urinary samples were immediately stored at -40℃.

Measurement of the concentration of *myo*-inositol and *chiro*-inositol in 24-hour urine samples

Myo-inositols and *chiro*-inositols in 24-hour urinary samples were determined by a Dionex Bio LC GP 50 Gradient Pump system (Dionex Corp. 01020506, Austin, TX, USA). Urine specimens were analyzed with Dionex Carbopak MA-1 columns (Dionex Corp. P/N 053699-01, Austin, TX, USA) maintained at 50℃ by columns-heater. All samples were then quantified by a Dionex ED 50 electrochemical Detector (Dionex Corp. 00120946, Austin, TX, USA). Urine samples were filtered by 0.2μm Costar Spin-X membranes (Corning Incor-

porated Costar, 8161, Corning, NY, USA). Buffered solutions of 60 mmol Sodium Hydroxide and ion-free distilled water were used as catalysts, and urinary components were eluted for one hour at the speed of 0.4 mL/minute. *Myo*-inositols and *chiro*-inositols were mixed at the density of 0.018 mg/mL each and 25μL of them were injected to be used as experimental standards. Chromatograms were recorded by a DS chrom 99 chromatogram Data System (Donam Instrument, DS2000, Seoul, South Korea) and under this condition, *myo*-inositols showed a retention time of about 8 minutes, and *chiro*-inositols one of about 10 minutes. The concentration of *myo*-inositols and *chiro*-inositols in specimens were determined from comparison with standard materials.

Statistical analysis

All data were expressed as means±standard deviation. We performed statistical analyses with the Kruskal-Wallis method, and used independent Student's t-tests for independent samples. *P* values of less than 0.05 were considered to be statistically significant.

RESULTS

The metabolic level of *myo*-inositols or *chiro*-inositols in our body is an important indicator to determine insulin resistance. From the three experimental groups - type 1 diabetes, type 2 diabetes, and the control group - we determined the level of *myo*-inositols and *chiro*-inositols in 24-hour urinary samples (Table 2). The amount of 24-hour urinary *myo*-inositols in both type 1 diabetes and type 2 diabetes groups was about three-fold higher than in the control group. On the contrary, the amount of 24-hour urinary *chiro*-inositols in type 1 diabetes was lower than that in the control group, but the value was not statistically significant. The type 2 diabetes group revealed more severe decreases of 24-hour urinary *chiro*-inositol level than the results from the type 1 diabetes group. From these values, we determined the *myo-/chiro*-inositol ratio among three experimental groups (Table 2). The ratio in type 1 and type 2 diabetes was four- and six-fold higher than that in control group, respectively, suggesting that diabetes causes a decrease of *chiro*-inositol production due to a defect of insulin action.

Furthermore, we compared the absolute amounts of *myo*-inositol and *chiro*-inositol and their ratio between type 1 and type 2 diabetes (Table 2). The amount of *myo*-inositols was not statistically different between the two diabetic groups (665.5 ± 278.6 vs. 601.5 ± 299.1 μmol/day, $p=0.32$). The *chiro*-inositols amount in the 24-hour urine of the type 1 diabetes group was higher than that of type 2 diabetes group (65.5 ± 23.0 vs. 46.1 ± 26.0, $p=0.02$). As a result, the *myo-/chiro*- inositol ratio of the type 1 diabetes group was lower than that of the type 2 diabetes group (10.3 ± 3.2 vs 17.3 ± 14.3) although its statistical significance was not acceptable ($p=0.09$).

DISCUSSION

In this study we investigated the levels of *myo*-inositol and *chiro*-inositol in 24-hour urine of Korean diabetes patients to study their clinical significance. The *myo-/chiro*-inositol ratio in the type 2 diabetes group and the type 1 diabetes

Table 2. Statistical Comparison of 24-hour Urinary *Myo*- or *Chiro*-Inositol in Diabetes

Inositols	Type 1 diabetes (N=11)	Type 2 diabetes (N=60)	Control (N=39)	Statistical analysis		
				A	B	C
Myo-inositols (μM/day)	665.5 ± 278.6	601.5 ± 299.1	193.3 ± 60.7	NS	‡	‡
Chiro-inositols (μM/day)	65.5 ± 23.0	46.1 ± 26.0	78.5 ± 31.7	†	NS	‡
Myo-/chiro-inositol ratio	10.3 ± 3.2	17.3 ± 14.3	2.7 ± 0.9	NS	‡	‡

NS, $p>0.05$; † $p<0.05$; ‡ $p<0.01$.
Statistical significance between type 1 and type 2 diabetes (A), between type 1 diabetes and controls (B), between type 2 diabetes and controls (C).

group was increased more than that in the control group. Therefore, it is likely that the elevated *myo*-/*chiro*-inositol ratio is associated with diabetes in this study.

The total amount of 24-hour urinary *myo*-inositols and *chiro*-inositols of the Korean type 2 diabetes group are higher than those of the American and Japanese type 2 diabetic groups (Table 3).[9,11] However, the *myo*-/*chiro*-inositol ratio (17.3) is similar to those from the American (20.4) and Japanese studies (15.5). Also, the *myo*-/*chiro*-inositol ratios are significantly elevated in the type 2 diabetes group rather than in the control group in all studies, indicating that the high *myo*-/*chiro*-inositol ratio is closely related to type 2 diabetes. The total amount of 24-hour urinary *myo*-inositols in the Korean type 1 diabetes group are higher than that in the American type 1 diabetes group. The 24-hour urinary *chiro*-inositol level of Korean type 1 diabetes group is higher than that of American type 1 diabetes group (Table 3). But the *myo*-/*chiro*-inositol ratios were not statistically different between the Korean type 1 diabetes group and the American type 1 diabetes group (10.3 ± 3.2 vs 13.6 ± 4.1). Compared to each of the normal control groups, the level of 24-hour urinary *myo*-inositols and *chiro*-inositols in the Korean group were similar to the levels in the Japanese group but were much higher than the American control group (Table 3). Nevertheless, the *myo*-/*chiro*-inositol ratio was similar among the Korean, Japanese and American groups (2.7 ± 0.9 vs. 2.5 vs. 2.5).

The low level of *chiro*-inositol in diabetes could be due to defect of conversion from *myo*-inositol to *chiro*-inositol, but it was not affected by race, country, eating habits, etc. The body inositol components might be affected by dietary habits because all studies did not restrict dietary foods, and the Korean and Japanese generally consume more inositol-rich foods such as soybean, brown rice and barley than Americans do. However, it is unlikely that dietary habits have an effect on the body ratio of *myo*-/*chiro*-inositol among races.

Two types of *chiro*-inositols exist in the body. One is *chiro*-inositol metabolically converted from *myo*-inositol, and the other one is a pinitol (methylated D-*chiro*-inositol derivative) that is obtained from dietary foods.[12] Myo-inositols are exclusively converted into *chiro*-inositols in the inositol phospholipids forms.[13] In the study which radioisotope tagged *myo*-inositols are injected into peritoneum, *myo*-inositols are largely converted to *chiro*-inositols in insulin sensitive tissues.[7] However, Goto-Kakizaki diabetic rats showed a low conversion rate from *myo*-inositols into *chiro*-inositols in insulin sensitive tissues. In contrast, inositol phospholipids in Goto-Kakizaki diabetic rats were highly elevated rather than in non-diabetic Wistar rats.[14]

According to previous reports, insulin re-

Table 3. Comparison of 24-Hour Urinary Inositols among Korean, Japanese and American Groups

	Korean	Japanese	American
Control group	(N = 39)	(N = 10)	(N = 44)
Myo-inositol (μmol/day)	193.3 ± 60.7	192 ± 54	91 ± 11
Chiro-inositol (μmol/day)	78.5 ± 31.7	96.0 ± 17.6	36.1 ± 6.6
Myo-/*chiro*-inositol	2.7 ± 0.9	2.5	2.5
Type 1 diabetes	(N = 11)		(N = 35)
Myo-inositol (μmol/day)	665.5 ± 278.6		251 ± 32
Chiro-inositol (μmol/day)	65.5 ± 23.0		18.5 ± 5.7
Myo-/*chiro*-inositol	10.3 ± 3.2		13.6 ± 4.1
Type 2 diabetes	(N = 39)	(N = 18)	(N = 50)
Myo-inositol (μmol/day)	601.5 ± 299.1	499 ± 112	270 ± 55
Chiro-inositol (μmol/day)	46.1 ± 26.0	32.3 ± 16	13.2 ± 3.6
Myo-/*chiro*-inositol	17.3 ± 14.3	15.5	20.4

sistance and *chiro*-inositol deficiency were correlated in both Rhesus monkeys and humans.[6,11] Streptozotocin-induced diabetic rats injected intramuscularly by *chiro*-inositols showed a decrease of plasma glucose levels and no hypoglycemia. Therefore, *chiro*-inositols might play a role as insulin sensitizers rather than as a direct mediator in the insulin action. The pinitol-mediated hypoglycemia was inhibited by a phosphatidylinositol 3-kinase inhibitor, LY294002, in streptozotocin-induced diabetic rats, suggesting that pinitol might improve the phosphatidylinositol 3-kinase's activity that is required for the translocation of glucose transporter (GLUT4) to the cell membrane in insulin sensitive tissues.[15]

Insulin resistance might play a more important role in the development of type 2 diabetes than in type 1 diabetes. In fact, this study showed that type 2 diabetes patients had a more significant decrease of 24-hour urinary *chiro*-inositols and the *myo*-/*chiro*-inositol ratio than patients with type 1 diabetes. This study has two drawbacks. First, all subjects were not on controlled diets, so dietary pinitols might influence the inositol level in urine samples. Secondly, we did not determine insulin resistance of each person in this study. However it has been suggested that the *myo*-/*chiro*-inositol ratio is closely related to insulin resistance according to many other studies.[5,6,9,11]

Nestler et al.[16] showed that insulin resistance is a main pathogenetic cause of polycystic ovary syndrome. In this study, nineteen patients showed their improvement in insulin resistance and successful ovulation (19/22, 86%) after oral medication with *chiro*-inositols. Moreover, two other insulin-sensitizers, thiazolidinedione or metformin, also affected ovulation by improving insulin resistance.[17,18]

In conclusion, we present that diabetes patients have highly elevated *myo*-/*chiro*-inositol ratios greater than the control group did, and the correlation between its ratio and diabetes incidence is statistically acceptable. However, its physiological implication in the molecular mechanism of diabetes is not clear. Therefore, we need to further investigate how *chiro*-inositols or pinitols improve insulin resistance and lower plasma glucose level in type 1 and type 2 diabetes patients.

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