Urinary *chiro*-Inositol and *myo*-Inositol Excretion Is Elevated in the Diabetic db/db Mouse and Streptozotocin Diabetic Rat

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Inositol phosphoglycan molecules containing either D-*chiro*-inositol or *myo*-inositol have been isolated from various mammalian tissues and are putative mediators of insulin action. Urinary excretion of inositols appears to be altered in diabetes mellitus; however, the relationships with different types of diabetes are unclear. The objective of this study was to determine the urinary excretion of *chiro*- and *myo*-inositol in diabetic animal models, including streptozotocin (STZ) rats, db/db mice, and fa/fa Zucker rats. In STZ rats (type 1 diabetes), 12-hr urinary excretion of *chiro*-inositol was elevated 336-fold and *myo*-inositol excretion was elevated 47-fold compared with their nondiabetic counterparts. When corrected for creatinine, *chiro*-inositol excretion was 259-fold higher and *myo*-inositol excretion was 36-fold higher in STZ rats than in normal rats. The same pattern was observed in db/db mice (type 2 diabetes), where 12-hr urinary *chiro*-inositol excretion was elevated 247-fold compared with normal mice. When corrected for creatinine, *chiro*-inositol excretion was 2455-fold higher and urinary *myo*-inositol excretion was elevated 8.5-fold in db/db mice compared with normal mice. The fa/fa Zucker rats (impaired glucose tolerance) had a pattern of urinary inositol excretion that was similar to the nondiabetic animals (lean Zucker rats, C57BL/6 mice, and Sprague-Dawley rats). In summary, urinary *chiro*-inositol and *myo*-inositol excretion was elevated in animal models of type 1 and type 2 diabetes mellitus, concomitant with hyperglycemia and glucosuria. Exp Biol Med 228:907–914, 2003

Key words: *chiro*-inositol; *myo*-inositol; urine; diabetes; streptozotocin rats; db/db mice; fa/fa Zucker rats.

New insight into the understanding of insulin action has emerged from the identification and partial characterization of two separate inositol phosphoglycan (IPG) molecules (1, 2). IPGs are hydrolyzed from glycosylphosphatidylinositols (GPIs) in cell membranes in response to insulin and are considered putative insulin mediators. Although the structures of the IPGs have not been completely elucidated, one contains *myo*-inositol and the other contains D-*chiro*-inositol, a rare inositol isomer. Both *myo*-inositol and *chiro*-inositol are incorporated into mammalian tissues and cells as free inositols or exist as inositol phosphates and inositol phospholipids (3, 4). GPIs found on the outer cell surface are derived from inositol phospholipids in cell membranes (3). Beside dietary origin, *myo*-inositol can be synthesized from glucose and *myo*-inositol can be converted to *chiro*-inositol (3, 5). After GPI hydrolysis by phospholipases, IPGs are incorporated into the cell where they activate enzymes involved in glucose and lipid metabolism. The *in vitro* and *in vivo* insulin-like effects of both IPG mediators have been reviewed elsewhere (6–9).

Experimental evidence suggests that IPGs are important in insulin signaling and the pathogenesis of diabetes. Insulin-induced generation of the *chiro*-inositol-containing IPG was reduced in hepatocytes and adipocytes of diabetic Goto-Kakizaki rats compared with normal rats (10). Impaired GPI-IPG-dependent insulin-signaling systems have been reported in hepatocytes of streptozotocin (STZ)-induced diabetic rats (11) and obese fa/fa Zucker rats (12). Decreased bioactivity of the *chiro*-inositol-containing IPG, measured by stimulation of pyruvate dehydrogenase phosphatase, has been documented in humans with type 2 diabetes (13, 14). Increased activity of the *myo*-inositol-containing mediator, determined by the stimulation of lipogenesis, has been described in individuals with type 1 or type 2 diabetes (15).

Urinary excretion of free inositols appears to be altered in diabetes mellitus compared with individuals with normal glucose tolerance (16–18); however, there is disagreement regarding the direction of the change. Reduced urinary *chiro*-inositol and elevated *myo*-inositol excretion have

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been reported in humans with type 2 diabetes mellitus (16, 18) and impaired glucose tolerance (IGT) (18) as well as in animal models of type 2 diabetes (10, 19). Kennington *et al.* (16) stated that analyses of urine from individuals with type 1 diabetes revealed a wide variation in *chiro*-inositol excretion. In contrast, Ostlund *et al.* (17) found that both type 1 and type 2 diabetic patients had increased urinary D-*chiro*- and *myo*-inositol excretion compared with normal subjects. The discrepancies between these reports have not yet been resolved. Ostlund *et al.* (17) reported urinary excretion of both D-*chiro*-inositol and L-*chiro*-inositol isomers, and the majority (>86%) of *chiro*-inositol was the D isomer.

The purpose of the present investigation was to determine the pattern of urinary inositol excretion in diabetic animal models. Twelve-hour urinary excretion of *myo*-inositol and *chiro*-inositol (the sum of D and L isomers) was analyzed in three different diabetic animal models and their nondiabetic counterparts. We chose STZ-induced diabetic rats (characterized by hyperglycemia and hypoinsulinemia) as a model of type 1 diabetes (DM-1), db/db mice (characterized by hyperglycemia, hyperinsulinemia, and obesity) as a model of DM-2, and fa/fa Zucker rats (characterized by hyperinsulinemia and IGT) as a model for the early stages of DM-2.

Materials and Methods

Standards and Chemical Reagents. The *myo*-inositol standard, phenyl-α-D-glucoside (internal standard), trimethylsilylimidazole, pyridine, and STZ were purchased from Sigma Chemical (St. Louis, MO). Reagent ethyl alcohol was purchased from Fisher Scientific (Ontario, Canada). D-*chiro*-inositol standard was a gift from Dr. S.G. Angyal (University of New South Wales, Australia).

Animals and Diet. A protocol for animal care procedures was approved by the University of Manitoba Protocol Management and Review Committee. Animals were maintained in a controlled environment of 21° to 23°C, 55% humidity, and a 14-hr light, 10-hr dark cycle.

DM-1. Six-week-old male Sprague-Dawley rats were obtained from Central Animal Holding (Winnipeg, Manitoba, Canada) and were maintained on standard laboratory chow (Prolab RMH 3000; Purina Mills, Richmond, IN). After a 1-week acclimatization period, diabetes was induced by intraperitoneal injections of 60 mg STZ/kg body weight/day on two consecutive days. Three days postinjection, hyperglycemia was confirmed by measuring serum glucose. Urine and blood samples were obtained from control animals (Sprague-Dawley rats) and STZ rats 1 week postinjection.

DM-2. Four-week-old female db/db mice (Jackson Laboratories, Bar Harbor, ME) were maintained on a semipurified diet based on the AIN-93G formulation for 7 weeks (20). Control mice were female C57BL/6 mice (Central Animal Holding, Winnipeg, Manitoba, Canada) fed standard laboratory chow (Prolab RMH 3000; Purina Mills).

IGT. Five-week-old male fa/fa and lean Zucker rats (Charles River Laboratories, Wilmington, MA) were maintained on a semipurified diet based on the AIN-93G formulation for 3 weeks (20). To confirm IGT in fa/fa compared with lean Zucker rats, an oral glucose tolerance test (OGTT) was administered after a 5-hr fast. For the OGTT, blood samples were collected from the saphenous vein immediately before and 15, 30, and 60 min after an oral dose of a 70% glucose solution (1 g glucose/kg body weight).

Blood Collection. Blood samples were obtained from animals after a 12-hr fast with the exception of STZ rats, which were in the fed state. A blood sample was collected via the saphenous vein from fa/fa and lean Zucker and STZ rats. Trunk blood was collected from Sprague-Dawley rats, C57BL/6, and db/db mice after sacrifice by CO_2 asphyxiation and decapitation. Blood samples were stored on ice until centrifuged to obtain serum. Serum was stored at −20°C until analyzed.

Urine Collection. Twelve-hour urine specimens were collected during an overnight fast in polycarbonate metabolic cages (Nalgene, Fisher Scientific), except that urine from STZ rats was collected during the day. During urine collection, animals were given free access to water, but not feed, to ensure that urine samples were not contaminated by the diets. The volume of each 12-hr urine sample was calculated using weight, and aliquots were stored at −20°C until analyzed.

Preparation of Urine for Inositol Analysis. The *chiro*-inositol and *myo*-inositol content was determined in 12-hr urine specimens collected from C57BL/6 and db/db mice, lean and fa/fa Zucker rats, Sprague-Dawley, and STZ-treated rats. One volume of ethanol was added to an equal volume of urine (0.1–0.5 ml). Samples were vortexed and evaporated to dryness under nitrogen at 40°C. Dried samples were sonicated for 5 min with 1 ml of trimethylsilylmidazole:pyridine (1:1, v/v), which contained 200 μg of phenyl-α-D-glucoside as an internal standard, and were derivatized for 1 hr at 80°C.

Preparation of Diet Samples for Inositol Analysis. The *chiro*-inositol and *myo*-inositol content was determined in the diets according to a modification of the method used for urine samples. Briefly, 1 g of ground diet sample was thoroughly homogenized with 5 ml of ethanol:water (1:1, v/v). The homogenate was vacuum filtered and the remaining residue was re-extracted with the same volume of solvent. An aliquot of the combined filtrates was evaporated and derivatized as described for urine specimens.

Inositol Analysis by Gas Chromatography. Two microliters of derivatized samples or standards were injected into a gas chromatograph (model GC-17A; Shimadzu, Columbia, MD) equipped with a flame ionization detector and split injector. Inositols were separated on a RTX-5MS capillary column (25 m in length, 0.25 mm ID, and 0.25-μm film thickness; Restek, Bellefonte, PA). Column temperature was programmed from 150° to 200°C at the rate of 3°C/min, and then to 325°C at the rate of 7°C/

min. Initial and final temperatures were held for 5 and 20 min, respectively. The injector and detector temperatures were held at 270° and 350°C, respectively. The carrier gas was hydrogen at 1.5 ml/min, and the split ratio used was 1:40. Inositols were quantified using phenyl-α-D-glucoside as the internal standard. Standard curves for D-*chiro*-inositol and *myo*-inositol were linear from 1 to 100 μg with r^2 values of 0.999 and an average error of 3.18% and 3.68%, respectively. D-*chiro*-inositol standard was added to selected samples to confirm peak identification. Urine and diet samples were analyzed in duplicate.

Determination of Recovery and Detection Limits for Inositols. Recovery was determined using two levels (1.4 and 20 μg) of D-*chiro*- and *myo*-inositol standards added to 0.5-ml aliquots of rat urine. To evaluate the recovery, total D-*chiro*- and *myo*-inositol was calculated for each sample and was compared with a urine aliquot without added inositol standards. Three repetitions were done at each level. The detection limit for *chiro*- and *myo*-inositol was defined when peaks of these inositols were no longer detected in decreasing concentrations of rat urine aliquots (0.1–0.005 ml).

Biochemical Analyses. Creatinine in urine was analyzed in duplicate using a colorimetric assay (procedure no. 555; Sigma Chemical). Glucose in the serum and urine was assessed in triplicate using an enzymatic colorimetric kit (procedure no. 315; Sigma Chemical). Insulin in the serum was analyzed in duplicate using a Sensitive Rat Radioimmunoassay kit (Linco, St. Charles, MO).

Statistical Analysis. Statistical significance between each set of diabetic rats and their normal counterpart was determined by Student's *t* test (SAS v.8.2; SAS Institute, Cary, NC). Time-course data was analyzed by analysis of variance (ANOVA) and by Duncan's multiple range test for means testing. Correlations were analyzed using Pearson's correlation coefficient. Differences were accepted as significant at $P < 0.05$. Data are expressed as the mean ± SEM.

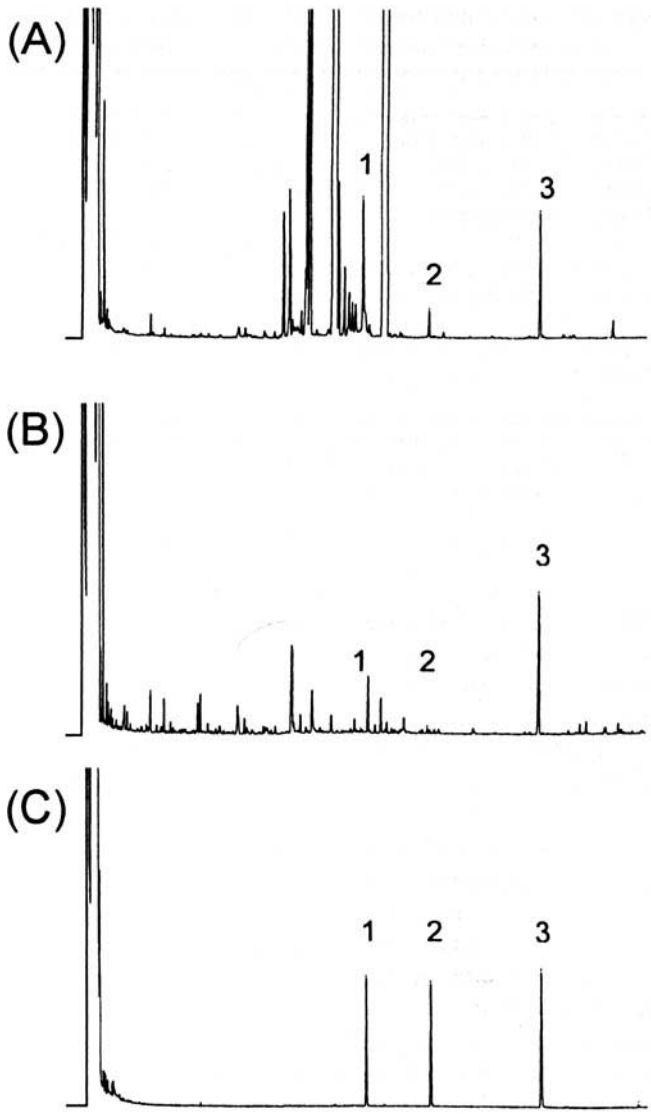

Figure 1. Chromatograms of silylated components in urine of Streptozotocin rat (A) and Sprague-Dawley rat (B), and the standards (C). 1, *chiro*-inositol; 2, *myo*-inositol; 3, internal standard (phenyl-α-D-glucoside).

Results

Recovery and Detection Limit of Inositols. Recovery of 1.4 μg of D-*chiro*- and *myo*-inositol added to 0.5 ml of urine was 98.2% ± 3.0% and 101% ± 4%, respectively. Recovery of 20 μg of D-*chiro*- and *myo*-inositol added to 0.5 ml of urine was 88.9% ± 3.5% and 83.0% ± 1.1%, respectively. For the recovery experiment, samples were done in triplicate. The detection limit for *chiro*-inositol and *myo*-inositol was 2 ng or 1 μg/ml urine. Typical chromatograms from a diabetic STZ rat, normal Sprague-Dawley rat, and inositol standards are shown in Figure 1.

Pattern of Inositol Excretion in STZ Rats (DM-1). Administration of STZ to normal rats destroys insulin-producing β cells of the pancreas, resulting in hypoinsulinemia and hyperglycemia. In this study, STZ-diabetic rats had hyperglycemia and glucosuria compared with their normal counterparts (Table I). Urinary *chiro*-inositol excretion was elevated 336-fold in STZ rats compared with their nondiabetic counterparts (Table I). Excretion of *myo*-inositol in STZ rats was also 47-fold higher than in normal rats (Table I). When expressed per creatinine, *chiro*-inositol excretion was elevated 259-fold and *myo*-inositol was 36-fold higher in STZ rats compared with normal rats (Table I and Fig. 2A). STZ rats excreted more *chiro*- than *myo*-inositol, whereas the reverse was observed in normal rats. As a result, the ratio of *myo*-inositol/*chiro*-inositol was 7.4-fold lower in STZ rats compared with nondiabetic rats (Table I and Fig. 3).

We also examined the relationships between urinary excretion of inositols and diabetic indices. In STZ rats, urine glucose was positively correlated with 12-hr excretion of both *chiro*-inositol (Fig. 4) and *myo*-inositol ($r^2 = 0.95$, $P \leq 0.0001$). When corrected for creatinine, both urinary excretion of *chiro*-inositol and *myo*-inositol were also posi-

Table I. Characteristics of STZ Rats (DM-1 Animal Model) Compared with Control Counterparts[a]

	Normal[b]	DM-1[c]
Serum glucose (mmol/L)	6.12 ± 0.32	30.4 ± 0.9****
Urine glucose (μmol/12 hr)	1.23 ± 0.15	19,100 ± 2,850***
Urine creatinine (μmol/12 hr)	28.4 ± 3.3	38.4 ± 3.7
Urine *chiro*-inositol		
nmol/12 hr)	120 ± 14	40,300 ± 7,340***
μmol/L/mmol/L creatinine	4.41 ± 0.63	1,140 ± 274**
Urine *myo*-inositol		
nmol/12 hr	209 ± 12	9,850 ± 1,790***
μmol/L/mmol/L creatinine	7.69 ± 0.84	273 ± 62**
Ratio *myo*-inositol/*chiro*-inositol	1.86 ± 0.29	0.25 ± 0.03**

[a] Values are means ± SEM; *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$, ****$P < 0.0001$ as determined by t test.
[b] $n = 5$ for 7-week-old Sprague-Dawley rats; mean body weight = 185 ± 2 g.
[c] $n = 9$ for 8-week-old STZ rats; mean body weight = 272 ± 4 g.

tively correlated with urine glucose ($r^2 = 0.73$, $P = 0.0264$ and $r^2 = 0.82$, $P = 0.0063$, respectively). No significant relationships were observed between urinary excretion of inositols and diabetic indices in normal Sprague-Dawley rats.

Pattern of Inositol Excretion in db/db Mice (DM-2). Diabetic db/db mice had hyperglycemia, hyperinsulinemia, and glucosuria (Table II). In db/db mice, urinary *chiro*-inositol excretion was elevated 247-fold compared with their nondiabetic counterparts (Table II). When expressed per creatinine, urinary *chiro*-inositol in db/db mice was 2455-fold higher than in normal mice (Table II and Fig. 2B), despite lower creatinine excretion in db/db mice (Table II). Urinary *myo*-inositol excretion of db/db mice was also elevated 8.5-fold compared with normal mice when corrected for creatinine (Table II and Fig. 2B). The db/db mice excreted more *chiro*-inositol than *myo*-inositol, whereas the reverse was observed in nondiabetic mice. As a result, the ratio of *myo*-inositol/*chiro*-inositol was 175-fold lower in db/db mice compared with normal mice (Table II and Fig. 3).

A comparison of the urinary inositol excretion of db/db mice at 5, 8, and 11 weeks of age showed a significant increase in *chiro*-inositol excretion (Fig. 5). There was an 11-fold increase in *chiro*-inositol excretion by 8 weeks and a 21-fold increase at 11 weeks compared with *chiro*-inositol excretion at 5 weeks as expressed per creatinine. Furthermore, urinary *chiro*-inositol excretion of db/db mice was higher than normal mice at 8 and 11 weeks of age ($P = 0.0198$ and $P = 0.0022$, respectively). When expressed per 12 hr, the same pattern of increasing *chiro*-inositol excretion was observed in db/db mice where urinary *chiro*-inositol was 58.2 ± 58.2 nmol at 5 weeks of age, 437 ± 117 nmol at 8 weeks of age, and 2820 ± 1030 nmol at 11 weeks of age. These values were significantly higher than in normal mice where 12-hr *chiro*-inositol excretion was 3.26 ± 1.99 nmol at 8 weeks of age and 11.4 ± 4.3 nmol at 11 weeks of age ($P = 0.0209$ and $P = 0.0413$, respectively). The increase in

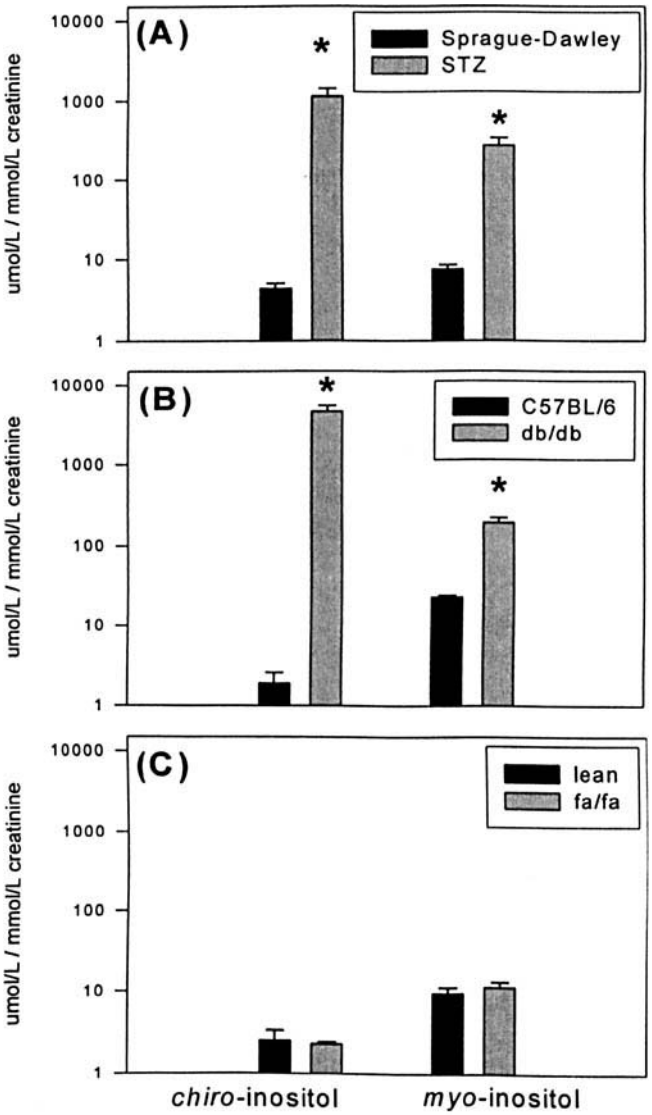

Figure 2. Urinary inositol excretion in Sprague-Dawley and streptozotocin-induced diabetic rats (A), C57BL/6 and db/db mice (B), and lean and fa/fa Zucker rats (C). An asterisk indicates differences ($P < 0.005$) in *chiro*-inositol and *myo*-inositol excretion for diabetic animals versus their normal counterparts.

chiro-inositol excretion in db/db mice was concurrent with an increase in urine glucose. Twelve-hour urinary glucose excretion in db/db mice was 10.9 ± 4.9 μmol at 5 weeks, 176 ± 37 μmol at 8 weeks, and 457 ± 115 μmol at 11 weeks. No difference in 12-hr urinary glucose excretion was observed in normal mice at 8 and 11 weeks with values of 0.04 ± 0.01 μmol and 0.11 ± 0.03 μmol, respectively. Furthermore, urine glucose excretion was significantly lower in normal mice compared with db/db mice at 8 weeks of age (0.11 ± 0.07 vs 176 ± 37; $P = 0.009$) and 11 weeks of age (Table II). This indicates that the increase in *chiro*-inositol excretion is related to the increasing severity of diabetes, as indicated by glucosuria, rather than an effect of age.

We also examined the relationships between urinary excretion of inositols and diabetic indices in normal and diabetic mice. In db/db mice, 12-hr urinary *chiro*-inositol

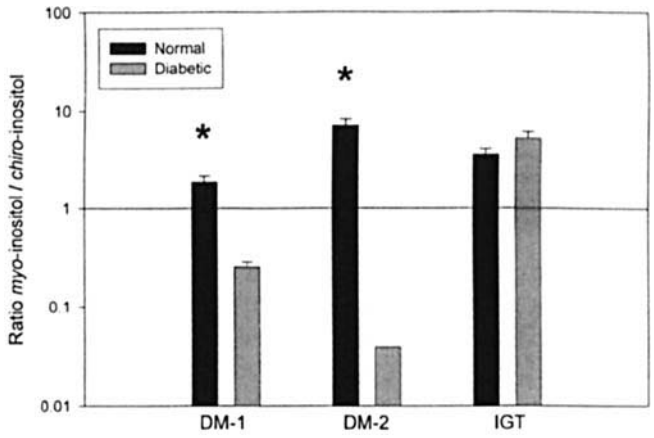

Figure 3. Ratio of *myo*-inositol/*chiro*-inositol excretion in normal and diabetic animal models. STZ rats for model of DM-1 versus normal Sprague-Dawley rats; db/db mice for model of DM-2 versus C57BL/6 mice; fa/fa Zucker rats for Model IGT versus lean Zucker rats. *Indicates differences ($P < 0.005$) in the ratio of urinary *myo*-inositol/*chiro*-inositol for diabetic animals versus their normal counterparts.

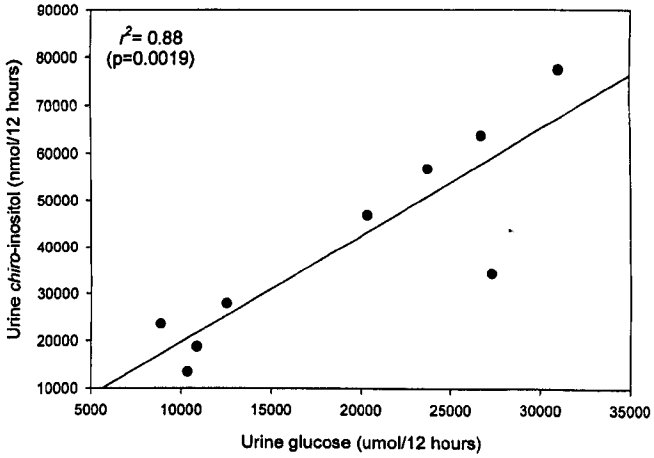

Figure 4. Scatter plot of urine glucose excretion versus urine *chiro*-inositol excretion in STZ rats. Data points are the values obtained from individual animals.

excretion was positively correlated with urine glucose ($r^2 = 0.83$, $P = 0.0409$). When corrected for creatinine, *myo*-inositol excretion was elevated compared with normal mice (Table II and Fig. 2B) and was positively correlated with serum glucose ($r^2 = 0.88$, $P = 0.0224$). No significant relationships were observed in normal mice.

Pattern of Inositol Excretion in fa/fa Zucker Rats (IGT). The fa/fa Zucker rats were hyperinsulinemic (Table III) and had IGT during an OGTT when compared with lean rats (Fig. 6). Diabetes is defined as a fasting serum glucose concentration greater than 7 mmol/L (21). Although fasting serum glucose concentrations of fa/fa rats were significantly higher than lean rats (Table III, 6.66 ± 0.30 vs 4.32 ± 0.36, $P = 0.0019$), fa/fa rats were not hyperglycemic according to the definition of diabetes. Urinary glucose excretion was 1.4-fold higher in fa/fa rats compared with lean rats (Table III). However, fa/fa rats did not have glucosuria when compared with the db/db mice and STZ rats where urine glucose excretion was elevated 4,155- and

Table II. Characteristics of db/db Mice (DM-2 Animal Model) Compared with Control Counterparts[a]

	Normal[b]	DM-2[c]
Serum glucose (mmol/L)	8.25 ± 0.49	40.6 ± 2.3***
Serum insulin (ng/mL)	0.15 ± 0.05	2.59 ± 0.28**
Urine glucose (μmol/12 hr)	0.11 ± 0.03	457 ± 115*
Urine creatinine (μmol/12 hr)	4.74 ± 0.98	0.57 ± 0.15*
Urine *chiro*-inositol		
nmol/12 hr	11.4 ± 4.3	2820 ± 1030*
μmol/L/mmol/L creatinine	1.87 ± 0.68	4590 ± 797**
Urine *myo*-inositol		
nmol/12 hr	106 ± 21	117 ± 40
μmol/L/mmol/L creatinine	23.0 ± 1.4	196 ± 28**
Ratio *myo*-inositol/*chiro*-inositol	7.00 ± 1.13	0.04 ± 0.00**

Note. In 8-week-old C57BL/6 mice fed the semipurified diet, urinary *chiro*- and *myo*-inositol excretion was 3.26 ± 1.99 and 47.1 ± 17.5 n*M*/12 hr, respectively ($n = 4$).

[a] Values are means ± SEM; *$P < 0.05$, **$P < 0.003$, ***$P < 0.0001$ as determined by *t* test.

[b] $n = 6$ for 11-week-old C57BL/6 mice; mean body weight = 18 ± 1 g.

[c] $n = 6$ for 11-week-old db/db mice; mean body weight = 31 ± 1 g.

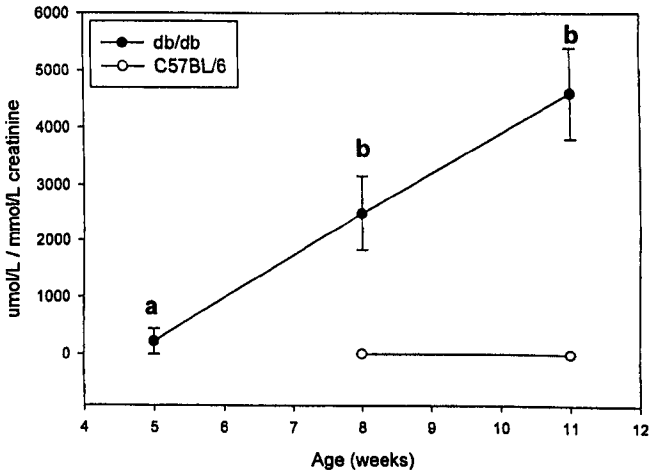

Figure 5. Urinary *chiro*-inositol excretion of db/db mice (●) at 5, 8, and 11 weeks of age compared with C57BL/6 mice (○) at 8 and 11 weeks of age. For db/db mice, data points with different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple range test ($n = 4$, $n = 5$, and $n = 6$ at 5, 8, and 11 weeks, respectively). Urinary *chiro*-inositol excretion of db/db mice at 8 and 11 weeks of age was higher than in C57BL/6 mice where *chiro*-inositol excretion was 5.49 ± 3.44 ($n = 4$) at 8 weeks and 1.87 ± 0.68 ($n = 6$) at 11 weeks, with significance at $P = 0.0198$ and $P = 0.0022$, respectively, as determined by *t* test.

15,528-fold, respectively (Tables I and II). There was no significant difference in the urinary *chiro*- or *myo*-inositol excretion between fa/fa and lean rats (Table III and Fig. 2C). There was also no difference in the ratio of *myo*-inositol/*chiro*-inositol between lean and fa/fa rats (Table III and Fig. 3). Similar to the normal Sprague-Dawley rats and normal C57BL/6 mice, both lean and fa/fa rats excreted more *myo*-inositol than *chiro*-inositol (Table III and Fig. 2C). In fa/fa Zucker rats, 12-hr urinary *chiro*-inositol excretion was positively correlated with urine glucose ($r^2 = 0.97$, $P = 0.029$); however, no significant relationships

Table III. Characteristics of fa/fa Zucker Rats (IGT Animal Model) Compared with Control Counterparts[a]

	Normal[b]	IGT[c]
Serum glucose (mmol/L)	4.32 ± 0.36	6.66 ± 0.30**
Serum insulin (ng/mL)	0.47 ± 0.04	7.33 ± 0.70**
Urine glucose (μmol/12 hr)	0.86 ± 0.07	1.21 ± 1.13*
Urine creatinine (μmol/12 hr)	32.7 ± 1.7	31.6 ± 4.2
Urine *chiro*-inositol		
nmol/12 hr	78.6 ± 24.3	72.3 ± 7.0
μmol/L/mmol/L creatinine	2.52 ± 0.81	2.32 ± 0.10
Urine *myo*-inositol		
nmol/12 hr	314 ± 49	372 ± 57
μmol/L/mmol/L creatinine	9.79 ± 1.82	11.92 ± 1.65
Ratio *myo*-inositol/*chiro*-inositol	3.55 ± 0.54	5.21 ± 0.84

[a] Values are means ± SEM; *$P < 0.05$, **$P < 0.003$ as determined by *t* test.
[b] $n = 5$ for 8-week-old lean Zucker rats; mean body weight = 255 ± 9 g.
[c] $n = 4$ for 8-week-old fa/fa Zucker rats; mean body weight = 414 ± 13 g.

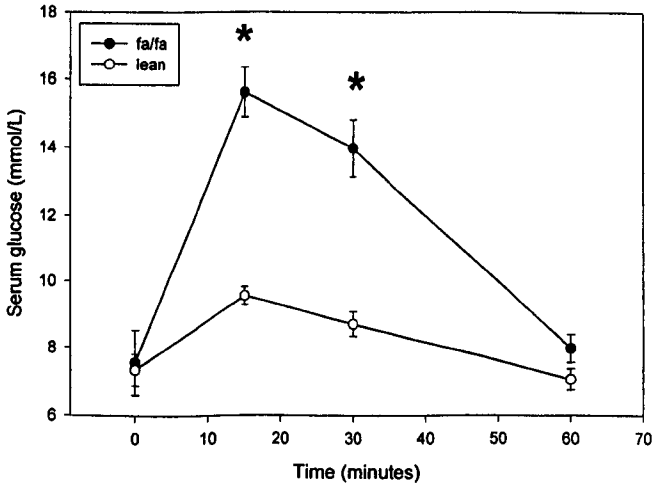

Figure 6. Serum glucose concentrations of fa/fa (●) and lean (○) Zucker rats during a oral glucose tolerance test. An asterisk indicates differences ($P < 0.002$) in serum glucose concentrations, as determined by *t* test.

were demonstrated between excretion of urine inositols and diabetic indices in lean rats.

Discussion

The data presented show that urinary inositol excretion is substantially altered in animal models of DM-1 and DM-2, but not IGT. Table IV provides a summary of the patterns of inositol excretion and diabetic indices in the different animal models. In the animal models of DM-1 and DM-2, STZ rats and db/db mice, respectively, urinary excretion of both *chiro*- and *myo*-inositol was elevated and the ratio of *myo*-inositol/*chiro*-inositol was reduced compared with their normal counterparts. However, in the fa/fa Zucker rats, a model for IGT, urinary inositol excretion and the ratio of *myo*-inositol/*chiro*-inositol were not altered. The fa/fa Zucker rats had hyperinsulinemia, but not hyperglycemia

Table IV. Pattern of Diabetic Indices and Urinary Excretion of Inositols in Animal Models of Type 1 and Type 2 Diabetes Mellitus and Impaired Glucose Tolerance Compared with Their Control Counterparts[a]

	DM-1	DM-2	IGT
Hyperglycemia	✓	✓	
Hyperinsulinemia		✓	✓
Hypoinsulinemia	✓		
Glucosuria	✓	✓	
Elevated urinary *chiro*-inositol[b]	✓	✓	
Elevated urinary *myo*-inositol[b]	✓	✓	
Reduced *myo*-inositol/*chiro*-inositol	✓	✓	

[a] STZ rats for model of DM-1 versus Sprague-Dawley rats; db/db mice for model of DM-2 versus C57BL/6 mice; fa/fa Zucker rats for model of IGT versus lean Zucker rats.
[b] Expressed as micromoles per liter per millimole per liter of creatinine.

(fasting serum glucose >7 mmol/L) or glucosuria as was observed in the STZ rats and db/db mice. The pattern of urinary inositol excretion observed in fa/fa Zucker rats was similar to the nondiabetic animals (lean Zucker rats, normal C57BL/6 mice, and Sprague-Dawley rats). In all diabetic animal models, urinary *chiro*-inositol excretion was positively correlated with urine glucose. In contrast, no relationships were observed between *chiro*-inositol excretion and urine glucose in normal animals.

The present study compares inositol excretion in animal models of DM-1 and DM-2. Urinary excretion of both *chiro*-inositol and *myo*-inositol were elevated in STZ rats and db/db mice, whereas inositol excretion was normal in fa/fa Zucker rats. Suzuki *et al.* (10) reported elevated urinary *myo*-inositol excretion in diabetic Goto-Kakizaki (GK) rats, but reduced *chiro*-inositol excretion compared with normal Wistar rats. GK rats are nonobese, mildly hyperglycemic (8.5 mmol/L), hyperinsulinemic, and have impaired intravenous glucose tolerance due to a poor insulin secretory response (22). To date, this is the first study to publish data on the urinary inositol excretion in db/db mice. Although a comment in the paper of Kennington *et al.* (16) indicated that the pattern of inositol excretion in STZ and fa/fa Zucker rats is different from diabetic humans, no data were reported. Our data indicate elevated inositol excretion in diabetic animal models characterized by hyperglycemia and glucosuria, regardless of circulating insulin and body weight (hypoinsulinemic nonobese STZ rats versus hyperinsulinemic obese db/db mice).

Urinary *chiro*-inositol excretion has been reported as both reduced in human subjects with DM-2 (16, 18) and elevated in humans with DM-1 and DM-2 (17). Urinary *myo*-inositol has been consistently demonstrated as higher among individuals with DM-2 (13–15) and DM-1 (17). In the work of Ostlund *et al.* (17), elevated urinary excretion of D-*chiro*-inositol was strongly related to urinary glucose, plasma glucose, and glycated hemoglobin. Findings of the present study suggest a positive relationship between *chiro*-

inositol excretion and glucosuria. In addition, the same authors found that urinary D-*chiro*-inositol excretion was particularly elevated among sulfonylurea-treated type 2 diabetic patients with poor glycemic control, and subsequent insulin treatment for 1 to 20 days reduced mean D-*chiro*-inositol excretion by 63%. Similarly, *chiro*-inositol excretion increases with the severity of diabetes as demonstrated in db/db mice from age 5 to 11 weeks (Fig. 5).

In the present study, free inositols were determined using an alternate method for urine preparation than was used in previous reports (10, 16–18). The method used in the present study does not apply any purification steps to avoid losses of inositols, but rather, it involves the derivatization of all components in urine samples using a silylation agent specific for hydroxyl groups. The pattern of *myo*-inositol excretion observed among diabetic animal models in the present study is similar to previous reports on humans and diabetic animal models (10, 16–18). Our results for *chiro*-inositol excretion in animal models of DM-1 and DM-2 support those of Ostlund *et al.* (17), but are different from those reported by Kennington *et al.* (13) and Suzuki *et al.* (10 and 18). Further characterization of these different animal models and types of diabetes is required to understand the observed differences.

Dietary intake is a possible reason for increased urinary excretion of *chiro*- and *myo*-inositol. We analyzed both the semipurified diet and the standard laboratory chow for presence of *chiro*-inositol and *myo*-inositol. Neither inositol was detected in the semipurified diet and only 0.032 mg of *chiro*-inositol and 0.122 mg of *myo*-inositol per gram of diet were detected in the laboratory chow. The pattern of inositol excretion in STZ rats and db/db mice reported in this study is not due to differences in dietary intake. The only animal pairs not fed the same diet were the db/db mice and normal C57BL/6 mice. To the contrary, inositol excretion was higher in db/db mice fed the semipurified diet in which no inositols were detected.

Beside dietary intake, other potential explanations for increased renal excretion of a substance include increased production, decreased tissue uptake, or altered renal metabolism. Pak *et al.* (4) demonstrated the incorporation of radioactive labeled *myo*-inositol and *chiro*-inositol into a wide range of tissues, with kidney being the highest. Incorporation into inositol phospholipids and inositol phosphates varied for each tissue (4). *Myo*-inositol can be synthesized from glucose in several tissues, including the liver and kidney (3). Furthermore, the conversion of *myo*-inositol to *chiro*-inositol has been demonstrated in various tissues, including liver, muscle, and fat, and kidney (4). In contrast, no or minimal conversion of *chiro*-inositol to *myo*-inositol was reported. The major product of *myo*-inositol breakdown is glucuronic acid, with kidney as the main site of the oxidation (4). It is possible that increased urinary *chiro*- and *myo*-inositol excretion in STZ rats and db/db is related to the metabolism of inositols in these animals.

To date, the metabolism of inositols has not been fully elucidated, however, experimental evidence suggests that inositol metabolism is altered among diabetic animal models. Pak *et al.* (4) reported a difference in the metabolism of *myo*-inositol and *chiro*-inositol in diabetic GK rats compared with normal Wistar rats. GK rats demonstrated a defect in conversion of *myo*- to *chiro*-inositol in insulin-sensitive tissues and increased incorporation of *myo*-inositol and *chiro*-inositol into inositol phospholipids. However, the relationship of altered inositol metabolism with urinary excretion was not examined. It is possible that altered inositol metabolism also exists among STZ rats and db/db mice, leading to elevated inositol excretion. Altered renal metabolism may also explain the increased inositol excretion in these animal models. According to Niwa *et al.* (23), urinary excretion of *myo*-inositol and *chiro*-inositol was significantly increased in patients with chronic renal failure. Further studies are needed to investigate inositol metabolism and possible renal perturbations in STZ rats and db/db mice.

Elevated urinary *chiro*- and *myo*-inositol excretion in STZ rats and db/db mice is also consistent with reports of impaired GPI/IPG signaling systems in diabetic animal models. Sanchez-Arias *et al.* (11) reported that hepatocytes isolated from STZ rats had 60% less GPI, blocked hydrolysis of GPI in response to insulin, and markedly reduced uptake of IPG (40%) compared with control rats. Although the exact mechanism and the role of inositols in GPI/IPG signaling is not fully understood, increased renal excretion of *chiro*- and *myo*-inositol is in keeping with this observed defect. To date, no data on the GPI/IPG signaling system has been reported for db/db mice, however, a similar pattern of impaired GPI-dependent insulin signaling was demonstrated in 14- to 21-week-old fa/fa rats (12). Hepatocyte content of GPI and IPG uptake were reduced by approximately 30% in fa/fa rats compared with lean control animals. These findings bring into question why we did not observe the same pattern of increased inositol excretion in fa/fa rats as STZ rats, if both have impaired GPI/IPG signaling systems. In the present study, the fa/fa rats were only 8 weeks old and may not have a sufficiently impaired GPI/IPG signaling system. However, we did observe altered inositol excretion in STZ rats and db/db mice at 8 weeks of age. Another possible explanation is that the pattern of impaired GPI/IPG signaling in hepatocytes of fa/fa rats reported by Sánchez-Gutiérrez *et al.* (12) was less dramatic than the STZ rats (11) and is concomitant with normoglycemia of fa/fa rats. Furthermore, results from the present study indicate that urinary *chiro*-inositol and *myo*-inositol excretion increases with the severity of diabetes, as observed in the db/db mice. Nonetheless, if an impaired GPI/IPG signaling system is responsible for increased inositol excretion, then the impaired signaling system observed in both STZ rats (insulin deficient) and fa/fa rats (insulin resistant) can explain why urinary inositol excretion was greater in both STZ rats and insulin-resistant db/db mice.

The data presented here suggest that urinary *chiro*-inositol and *myo*-inositol excretion is elevated in animal

models of type 1 and type 2 diabetes mellitus, both characterized by hyperglycemia and glucosuria. It remains to be elucidated whether altered inositol metabolism of diabetic animal models relates to the pathogenesis of diabetes and the potential mechanisms involved. It is still unclear if the same pattern of urinary inositol excretion exists among humans with diabetes.

1. Larner J, Huang LC, Schwartz CFW, Oswald AS, Shen T-Y, Kinter M, Tang G, Zeller K. Rat liver insulin mediator which stimulates pyruvate dehydrogenase phosphatase contains galactosamine and D-*chiro*-inositol. Biochem Biophys Res Commun **151:**1416–1426, 1988.
2. Larner J, Huang LC, Suzuki S, Tang G, Zhang C, Schwartz CFW, Romero G, Luttrell L, Kennington AS. Insulin mediators and the control of pyruvate dehydrogenase complex. Ann N Y Acad Sci **573:**297–305, 1989.
3. Beemster P, Groenen P, Steegers-Theunissen R. Involvement of inositol in reproduction. Nutr Rev **60:**80–87, 2002.
4. Pak Y, Hong Y, Kim S, Piccariello T, Farese R, Larner J. In vivo *chiro*-inositol metabolism in the rat: a defect in *chiro*-inositol synthesis from *myo*-inositol and an increased incorporation of *chiro*-[^{3}H]inositol into phospholipid in the Goto-Kakizaki (G.K.) rat. Molecules and Cells **8:**301–309, 1998.
5. Pak Y, Huang L, Lilley K, Larner J. *In vivo* conversion of [^{3}H]myo-inositol to [^{3}H]chiroinositol in rat tissues. J Biol Chem **267:**16904–16910, 1992.
6. Varela-Nieto I, Léon Y, Caro HN. Cell signalling by inositol phosphoglycans from different species. Comp Biochem Physiol **115B:**223–241, 1996.
7. Field M. Is there evidence for phospho-oligosaccharides as insulin mediators? Glycobiology **7:**161–168, 1997.
8. Jones DR, Varela-Nieto I. The role of glycosyl-phosphatidylinositol in signal transduction. Int J Biochem Cell Biol **30:**313–326, 1998.
9. Jones DR, Varela-Nieto I. Diabetes and the role of inositol-containing lipids in insulin signaling. Mol Med **5:**505–514, 1999.
10. Suzuki S, Taneda Y, Hirai S, Abe S, Saski A, Suzuki K, Toyota T. Molecular mechanism of insulin resistance in spontaneous diabetic GK (Goto- Kakizaki) rats. In: Saskamoto N, Angel A, Hotta N, Eds. New Directions in Research and Clinical Works for Obesity and Diabetes Mellitus. New York: Elsevier Science, pp197–203, 1991.
11. Sanchez-Arias J, Sanchez-Gutierrez J, Guadaño A, Alvarez J, Samper B, Mato J, Feliu J. Impairment of glycosyl-phosphatidylinositol-dependent insulin signaling system in isolated rat hepatocytes by streptozotocin-induced diabetes. Endocrinology **131:**1727–1733, 1992.
12. Sánchez-Gutiérrez J, Sánchez-Arias J, Valle J, Guadaño A, Samper B, Mato J, Felíu J. Insulin resistance in genetically obese (fa/fa) rats: changes in the glycosyl-phosphatidylinositol signaling system in isolated hepatocytes. Endocrinology **134:**1485–1492, 1994.
13. Asplin I, Galasko G, Larner J. *Chiro*-inositol deficiency and insulin resistance: a comparison of the *chiro*-inositol- and the *myo*-inositol-containing insulin mediators isolated from urine, hemodialysate, and muscle of control and type II diabetic subjects. Proc Natl Acad Sci U S A **90:**5924–5928, 1993.
14. Shaskin PN, Shashkina EF, Fernqvist-Forbes E, Zhou Y-P, Grill V, Katz A. Insulin mediators in man: effects of glucose ingestion and insulin resistance. Diabetologia **40:**557–563, 1997.
15. Kunjara S, Wang DY, Greenbaum AL, McLean P, Kurtz A, Rademacher TW. Inositol phosphoglycans in diabetes and obesity: urinary levels of IPG A-Type and IPG-P Type, and relationship to pathophysiological change. Mol Genet Metab **68:**488–502, 1999.
16. Kennington AS, Hill CR, Craig J, Bogardus C, Raz I, Ortmeyer HK, Hansen BC, Romero G, Larner J. Low urinary *chiro*-inositol excretion in non-insulin-dependent diabetes mellitus. N Engl J Med **323:**373–378, 1990.
17. Ostlund RE Jr, McGill JB, Herskowitz I, Kipnis DM, Santiago JV, Sherman W. D-*chiro*-inositol metabolism in diabetes mellitus. Proc Natl Acad Sci U S A **90:**9988–9992, 1993.
18. Suzuki S, Kawasaki H, Satoh Y, Ohtomo M, Hirai M, Hirai A, Hirai S, Onoda M, Matsumoto M, Hinokio Y, Akai H, Craig J, Larner J, Toyota T. Urinary *chiro*-inositol excretion is an index marker of insulin sensitivity in Japanese Type II Diabetes. Diabetes Care **17:**1465–1468, 1994.
19. Ortmeyer HK, Bodkin NL, Lilly L, Larner J, Hansen BC. *Chiro*-inositol deficiency and insulin resistance. I. Urinary excretion rate of *chiro*-inositol is directly associated with insulin resistance in spontaneously insulin-resistant Rhesus monkey. Endocrinology **132:**640–645, 1993.
20. Lepage LM, Giesbrecht J-AC, Taylor CG. Expression of T lymphocyte p56^{lck}, a zinc-finger signal transduction protein, is elevated by dietary zinc deficiency and caloric deficiency in mice. J Nutr **129:**620–627, 1999.
21. Meltzer S, Leiter L, Daneman D, Gerstein HC, Lau D, Ludwig S, Yale J-F, Zinman B, Lillie D. Steering and Expert Committees. 1998 Clinical Practice Guidelines for the Management of Diabetes in Canada. Can Med Assoc J **159**(Suppl 8):S1–S28, 1998.
22. Portha B, Serradas P, Bailbé D, Suzuki K-I, Goto Y, Giroix M-H. β-Cell insensitivity to glucose in the GK rat, a spontaneous nonobese model for type II diabetes. Diabetes **40:**486–491, 1991.
23. Niwa T, Yamamoto N, Maeda K, Yamada K. Gas chromatographic-mass spectrometric analysis of polyols in urine and serum of uremic patients. Identification of new deoxyalditols and inositol isomers. J Chromatogr **277:**25–39, 1983.