LOW URINARY *chiro*-INOSITOL EXCRETION IN NON-INSULIN-DEPENDENT DIABETES MELLITUS

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Abstract *Background and Methods.* Inositol is a major component of the intracellular mediators of insulin action. To investigate the possible role of altered inositol metabolism in non-insulin-dependent diabetes mellitus (NIDDM), we used gas chromatography and mass spectrometry to measure the *myo*-inositol and *chiro*-inositol content of urine specimens from normal subjects and patients with NIDDM. The study subjects were whites, blacks, and Pima Indians. The type of inositol and its concentration in insulin-mediator preparations from muscle-biopsy specimens from normal subjects and diabetic patients were also determined.

Results. The urinary excretion of *chiro*-inositol was much lower in the patients with NIDDM (mean [±SE], 1.8±0.8 μmol per day) than in the normal subjects (mean, 84.9±26.9 μmol per day; $P<0.01$). In contrast, the mean urinary *myo*-inositol excretion was higher in the diabetic patients than in the normal subjects (444±135 vs. 176±46 μmol per day; $P<0.05$). There was no correlation between *chiro*-inositol excretion and the age, sex, or weight of the diabetic patients, nor was there any correlation between urinary *chiro*-inositol and *myo*-inositol excretion in either group. The results were similar in a primate model of NIDDM, and *chiro*-inositol excretion was decreased to a lesser extent in animals with prediabetic insulin resistance. *chiro*-Inositol was undetectable in insulin-mediator preparations from muscle-biopsy samples obtained from patients with NIDDM. Similar preparations from normal subjects contained substantial amounts of *chiro*-inositol. Furthermore, the *chiro*-inositol content of such preparations increased after the administration of insulin during euglycemic–hyperinsulinemic-clamp studies in normal subjects but not in patients with NIDDM.

Conclusions. NIDDM is associated with decreased *chiro*-inositol excretion and decreased *chiro*-inositol content in muscle. These abnormalities seem to reflect the presence of insulin resistance in NIDDM. (N Engl J Med 1990; 323:373-8.)

THE existence of intracellular chemical mediators of the action of insulin was proposed in 1974 by Larner et al.[1] Since then, progress has been made in the identification of such mediators.[2] Putative mediators that regulate adenylate cyclase and cyclic AMP (cAMP)–dependent protein kinase,[3,4] cAMP phosphodiesterase,[4,5] pyruvate dehydrogenase,[7,8] and other insulin-controlled enzymatic systems[9] have been identified and purified to various degrees of homogeneity, although the structural relations among the individual mediators have not been elucidated. These mediators have been isolated from whole liver and muscle tissues,[3,4] liver membranes,[4,7,8] and a variety of other sources.[4,6,10]

Two separate mediators have been isolated in our laboratory from rat-liver tissue.[11] These mediators have been identified as inositol glycans and found to contain inositol, nonacetylated amino sugars, and neutral sugars as the carbohydrate constituents. One mediator contains D-*chiro*-inositol, whereas the second contains *myo*-inositol. Both D-*chiro*-inositol and *myo*-inositol (Fig. 1) have similar structures, differing only in the stereochemistry of one hydroxyl group. Since inositol is a major component of the insulin-mediator preparations, we decided to analyze the potential role of alterations in inositol metabolism on the formation and turnover of mediators in postreceptor insulin resistance. Therefore, we measured *chiro*-inositol and *myo*-inositol in urine specimens from normal subjects and patients with non-insulin-dependent diabetes mellitus (NIDDM) and in normal, obese, and diabetic monkeys. We found that the excretion of *chiro*-inositol in urine was very low in patients with NIDDM as compared with that in normal subjects; the same was true in diabetic as compared with normal monkeys.

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METHODS

Urine Collection and Preparation

The *chiro*-inositol and *myo*-inositol content was determined in 24-hour urine specimens collected from normal subjects (group 1) and patients with NIDDM (group 2) who were living in Virginia and from normal Pima Indians and whites in Arizona (group 3) and similar persons with NIDDM (group 4). Overall, urine samples from 26 normal subjects (17 men and 9 women) and 31 patients with NIDDM (14 men and 17 women) were analyzed. The clinical characteristics of the four groups of subjects are shown in Table 1. The protocols for the studies in Virginia and Arizona were approved by the appropriate institutional review committees, and informed consent was obtained from each subject.

The subjects in group 1 were recruited from the staff of the Health Sciences Center at the University of Virginia. All 14 were white. All were in good health, none were taking any medication known to affect carbohydrate metabolism, and none had a family history of diabetes. All had fasting venous plasma glucose values of less than 6.4 mmol per liter and glycated hemoglobin values within the normal range. The subjects in group 2 were chosen at random from nonhospitalized and hospitalized patients with diabetes who were treated at the University of Virginia Health Sciences

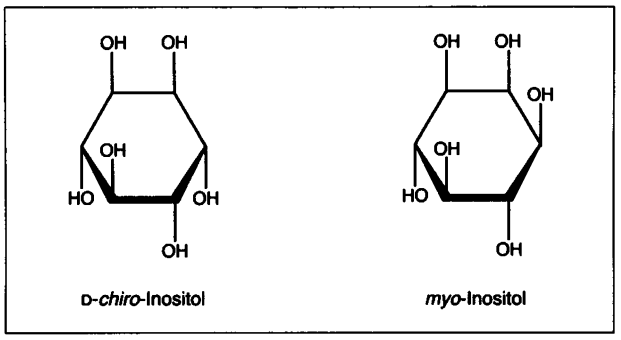

Figure 1. Chemical Structure of D-*chiro*-Inositol and *myo*-Inositol.

Center. Eight were black, and 16 were white. All fulfilled the criteria of the National Diabetes Data Group for NIDDM.[12] Ten were being treated with insulin, and 14 were being treated with an oral hypoglycemic agent. Their mean (±SE) creatinine clearance was 1.9±0.1 ml per second; four had values of less than 1.3 ml per second, the lowest being 0.8 ml per second, and three had values in excess of 2.3 ml per second. Protein was measured in 24-hour urine specimens from all but one patient in group 2. The values were below the detection limit of 60 mg per liter in all but four patients and exceeded the normal limit of 149 mg per day in only two patients.

Group 3 consisted of six Pima Indians and six whites, and group 4 contained seven Pima Indians. None of the subjects in group 4 had received any therapy for diabetes for at least two weeks, and all the subjects in both groups had followed a diet containing approximately 45 percent fat, 40 percent carbohydrate, and 15 percent protein for at least 48 hours before the urine samples were collected.

We also collected urine samples from rhesus monkeys (*Macaca mulatta*) maintained in the Obesity and Diabetes Research Center at the University of Maryland at Baltimore. On reaching "middle age," these monkeys frequently become obese, and overt diabetes develops in some of the obese monkeys.[13] This model of spontaneous adult-onset obesity-associated diabetes meets all the criteria for NIDDM as defined by the National Diabetes Data Group.[12] The animals were individually caged and provided unrestricted access to Purina Monkey Chow for eight hours each day. Under these conditions, some monkeys over the age of 10 years gradually become obese (body fat content >22 percent) without notable overfeeding. This obesity is associated with various degrees of peripheral insulin resistance as measured by the euglycemic-clamp technique.[14] Both increased peripheral insulin resistance and decreased insulin release in response to glucose precede overt NIDDM in these animals.[15] Urine specimens were collected from four normal lean monkeys, seven obese monkeys (four of which were progressing toward diabetes), and four overtly diabetic monkeys. Among the four diabetic monkeys, one had had diabetes for six years and the others for less than one year. Two of the four diabetic monkeys were receiving insulin treatment. The urine specimens were obtained over a 24-hour period with the use of metabolic pans that funneled the urine into iced bottles containing toluene and dilute sodium azide.

Table 1. Clinical Characteristics of the Normal Subjects (Groups 1 and 3) and the Patients with NIDDM (Groups 2 and 4).*

Characteristic	Group 1	Group 2	Group 3	Group 4
No. of patients	14	24	12	7
Sex (M/F)	5/9	15/9	4/8	2/5
Age (yr)	45.7±15.5	57.9±12.3	33.9±6.9	25.6±4.0
Weight (kg)	70.5±17.8	92.2±21.8	93.1±27.9	115.7±45.0
Fasting plasma glucose (mmol/liter)	5.0±0.4	10.0±4.4	5.1±0.3	11.2±3.2
Glycated hemoglobin (%)	5.2±0.6	10.9±2.3	—	—
Duration of disease (yr)	—	7.6±10.8	—	4.5±5.85

*Plus–minus values are means ±SD.

Twenty milliliters of urine from the subjects and animals was stored frozen until processed for analysis. At that time, 5-ml aliquots were passed through anion (Amberlite IR 120plus; hydrogen) and cation (Amberlite IRA 410; hydroxide) exchange resins to remove ionic substances, then through a C18 Sep-Pak cartridge (Waters) to remove nitrogenous and peptide-like material. The inositols and other neutral hydrophilic materials were eluted from the Sep-Pak cartridge with 2 ml of water. A volume of the combined eluates equivalent to 0.6 ml of urine was lyophilized before quantitative analysis by gas chromatography and mass spectrometry. The lyophilized samples were derivatized with 100 μl of heptafluorobutyrylimidazole (Pierce) and heated at 55°C for five hours in a modification of the procedure of Leavitt and Sherman.[16] The samples were then extracted into 200 μl of *n*-hexane suitable for gas chromatography and mass spectrometry (Burdick and Jackson). The inositol content was quantitated by comparison with standard curves for each inositol from the peak heights of characteristic mass fragments. All samples were analyzed twice, and the results reported are the average of the two analyses (rate of error, <6 percent). The limit of detection for the analytic method was 0.5 μmol per liter; any sample in which *chiro*-inositol was not detected was assigned this value, corrected for the 24-hour urine volume.

Urinary glucose was measured by the oxygen-rate method with use of a Beckman oxygen electrode. Serum and urinary creatinine levels were measured by an automated enzymatic method, and an automated benzethonium chloride method was used to measure urinary protein.

Muscle Biopsy and Mediator Preparation

Muscle samples were obtained by percutaneous needle biopsy during euglycemic–hyperinsulinemic-clamp studies in nine subjects in Arizona. The biopsies for the *chiro*-inositol and *myo*-inositol assays were performed just before and 15 and 20 minutes after the beginning of the insulin infusion. The clamp studies were performed at plasma glucose concentrations of approximately 5.6 mmol per liter and plasma insulin concentrations of more than 14,350 pmol per liter, and biopsy of the vastus lateralis muscle was performed as previously described.[17] The biopsy samples were frozen in liquid nitrogen and stored until analyzed. Seven of the nine subjects were Pima Indians, and two were white. Five were patients with NIDDM, and the remaining four were normal subjects.

The muscle-biopsy samples, which weighed approximately 200 mg each, were homogenized and extracted in three volumes (in milliliters) of 50 mM formic acid containing 1 mM EDTA and 1 mM 2-mercaptoethanol and boiled at 100°C for five minutes according to published methods.[11] After cooling and centrifugation, the extracts were neutralized to pH 6.0 with ammonium hydroxide, absorbed on Dowex 1 by 8, and eluted at pH 2.0 and 1.3.[11,18] The eluates containing mediators were lyophilized and redissolved in water for bioassay or hydrolyzed in 2 N trifluoracetic acid for six hours at 100°C for subsequent analysis by gas chromatography and mass spectrometry. Two mediator assays were performed. The mediator that eluted at pH 2.0 was assayed by the activation of mitochondrial pyruvate dehydrogenase phosphatase,[8] and the mediator that eluted at pH 1.3 was assayed by the inactivation of cAMP-dependent protein kinase.[4] The samples for gas chromatography and mass spectrometry were lyophilized and derivatized with a mixture of pyridine, trimethylchlorosilane, and trimethylsilylimidazole (10:1:2) to form the trimethylsilylethers. In tissue extracts from normal subjects, *myo*-inositol predominates in the pH 1.3 eluate and *chiro*-inositol in the pH 2.0 eluate. *myo*-Inositol and *chiro*-inositol were measured in both eluates, and the results are given as the sum of the values in each eluate.

Gas Chromatography and Mass Spectrometry

All samples were analyzed on a Varian 3400 gas chromatograph with a Finnigan Incos 50B mass spectrometer. An injection of 1 μl was made into a split/splitless injector with a 0.8-minute split time.

The urine samples were analyzed on a 30-m Chirasil Val III column (Alltech Associates) with an internal diameter of 0.25 mm at a flow rate of 11 lb per square inch. The temperature program consisted of a 2-minute initial holding period at 55°C, followed by an increase of 10°C per minute to 225°C — a temperature that was then maintained for 10 minutes. The mass spectrometer was operated in the electron-impact mode with a 70-eV input.

The muscle samples were analyzed on a 30-m DB-5 column (J and W Chromatography) with an internal diameter of 0.25 mm at a flow rate of 11 lb per square inch. The temperature program consisted of a 2-minute initial holding period at 70°C, followed by an increase of 10°C per minute to 250°C — a temperature that was then maintained for 10 minutes. The mass spectrometer was operated in the electron-impact mode with a 70-eV input.

Chemical and Biologic Control Studies

Sample storage and treatment were found to be critical for the proper analysis of *chiro*-inositol. The concentration of *chiro*-inositol increased in urine samples kept at room temperature for more than 48 hours, presumably because of bacterial action. This increase was prevented by the addition of dilute aqueous sodium azide or toluene.

To verify that the urine samples from the diabetic patients did not contain substances that masked *chiro*-inositol, two types of experiments were performed. In one, known amounts of *chiro*-inositol were added to urine samples before sample purification, and the recovery of *chiro*-inositol was verified quantitatively. In a second study, glucose (which was present in the urine samples from most diabetic patients) was removed from the urine by treatment with glucose oxidase. The *chiro*-inositol content was similar before and after such treatment. *chiro*-Inositol was also shown not to be formed during the hydrolysis or derivatization procedure. The overall recovery of both *chiro*-inositol and *myo*-inositol throughout the purification procedure was greater than 95 percent.

Results

Urinary *chiro*-Inositol Excretion in Humans

The mean (±SE) urinary excretion of *chiro*-inositol was 84.9±26.9 μmol per day in the 26 normal subjects (groups 1 and 3) and 1.8±0.8 μmol per day in the 31 diabetic patients (groups 2 and 4; $P<0.01$). The mean daily excretion of *myo*-inositol was 176±46 μmol in the normal subjects and 444±135 μmol in the diabetic patients ($P<0.05$).

Figure 2 shows the 24-hour urinary excretion of *chiro*-inositol and *myo*-inositol in the four groups of subjects. The mean urinary excretion of *myo*-inositol in group 1 was 179±75 μmol per day, similar to the values reported in the literature.[19,20] The mean urinary excretion of *chiro*-inositol in group 1 was 65±12 μmol per day. In contrast, in the patients with NIDDM in group 2, the mean *chiro*-inositol excretion was 2.2±0.9 μmol per day, and the mean excretion of *myo*-inositol was 460±115 μmol per day. The results in groups 3 and 4 were similar to those in groups 1 and 2, respectively. The mean urinary excretion of *myo*-inositol in group 3 was 160±44 μmol per day, and the mean *chiro*-inositol excretion was 108±44 μmol per day. In contrast, in the patients with NIDDM (group 4), the mean urinary excretion of *myo*-inositol was 231±58 μmol per day, and the mean *chiro*-inositol excretion was 0.6±0.5 μmol per day. Twenty-four of the 31 diabetic patients had no detectable *chiro*-inositol in their urine.

Statistical analysis of the urinary *chiro*-inositol values was performed with a general-linear-model procedure developed by the SAS Institute. Assuming a multivariate model with *chiro*-inositol excretion as the dependent variable and age, sex, body weight, and a clinical diagnosis of diabetes as independent variables, the analysis demonstrated a significant correlation between *chiro*-inositol excretion and the diagnosis of NIDDM ($P<0.001$) and between *chiro*-inositol excretion and body weight ($P<0.05$). Excluding the result in a single obese, normal subject whose *chiro*-inositol excretion was eight times higher than the mean value in normal subjects did not alter the correlation between urinary *chiro*-inositol excretion and diabetic status. In contrast, the correlation with body weight was eliminated after the exclusion of this subject's data. No correlation was found with age, sex, or urinary *myo*-inositol excretion. There was also no correlation between either urinary glucose concentration and *chiro*-inositol excretion or between urinary *chiro*-inositol and glycated hemoglobin values in group 2. Neither urinary glucose nor glycated hemoglobin was measured in group 4.

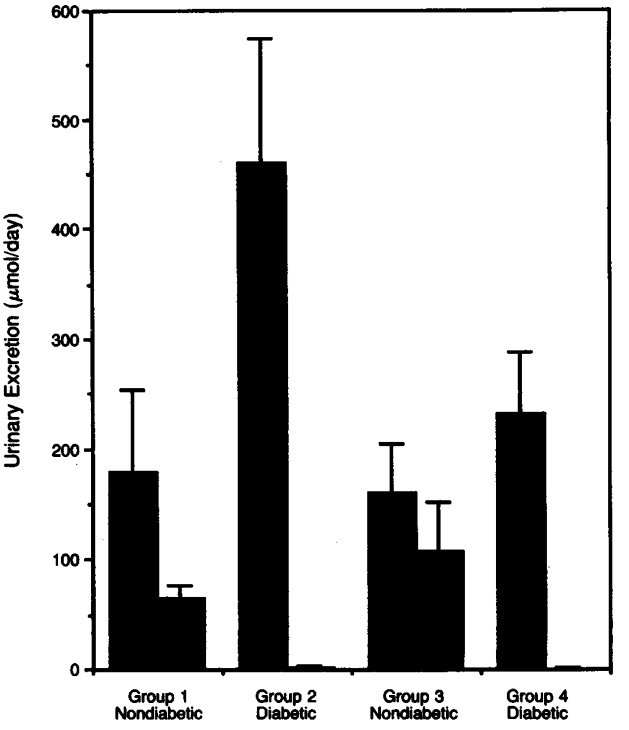

Figure 2. Mean 24-Hour Urinary Excretion of *chiro*-Inositol (Hatched Bars) and *myo*-Inositol (Solid Bars) in the Four Groups.

Group 1 consisted of nondiabetic whites from Virginia; group 2, diabetic blacks and whites from Virginia; group 3, nondiabetic whites and Pima Indians from Arizona; and group 4, diabetic Pima Indians from Arizona. T bars denote standard errors.

Urinary *chiro*-Inositol Excretion in Monkeys

We measured urinary *chiro*-inositol excretion in several animal models of diabetes: Zucker rats, rats with streptozocin-induced diabetes, partially pancreatecto-

mized dogs, and rhesus monkeys. Only the diabetic monkeys had a decrease in urinary *chiro*-inositol excretion that was analogous to that in humans. The mean urinary excretion of *chiro*-inositol was 6.7±2.3 μmol per day in the normal, lean monkeys, 1.2±0.2 μmol per day in the obese monkeys, and 0.2±0.1 μmol per day in the diabetic monkeys (Table 2). These results indicate that low urinary *chiro*-inositol excretion is a feature of diabetes in these animals and that the obesity and its associated insulin resistance, which precedes diabetes, are associated with a smaller decline in *chiro*-inositol excretion.

Analyses of Human Muscle-Biopsy Samples

We measured insulin-mediator bioactivity in extracts of muscle obtained by biopsy from the normal subjects before and during insulin administration (but not the extracts from the diabetic patients). Insulin-mediator activity was detected in the deproteinized, partially purified extracts of muscle by its ability to stimulate pyruvate dehydrogenase phosphatase (the pH 2.0 eluate)[8] or inhibit cAMP-dependent protein kinase[4] (the pH 1.3 eluate) in assays in vitro.[18] Both insulin mediators were found in the extracts of muscle tissue from normal subjects; these findings were similar to the results for rat-liver extracts. The activity of both mediators increased in the muscle tissue of normal subjects during the administration of insulin. The mediator activity was maximal after 8 to 15 minutes, and it declined to basal levels at 30 minutes despite continued insulin administration (data not shown).

The results of the assays for *chiro*-inositol and *myo*-inositol are shown in Table 3. Insulin mediators have been shown to be inositol glycans containing 1 mol of inositol per mole of mediator.[21] The presence of carbohydrate constituents of inositol glycan mediators, including mannose and galactose, in roughly stoichiometric amounts is evidence that the inositols detected are a reliable chemical measure of the partially purified insulin mediator (data not shown). Before the administration of insulin, both *myo*-inositol and *chiro*-inositol were detectable in the mediator preparations from the muscle-biopsy specimens obtained from the normal subjects (Table 3). During the administration of insulin, the concentrations of both *myo*-inositol and *chiro*-inositol increased fivefold to ninefold in 15 minutes and then decreased. *chiro*-Inositol was not detected in mediator preparations from muscle-biopsy specimens obtained from the patients with NIDDM either before or during insulin administration, whereas *myo*-inositol concentrations increased and then decreased during insulin administration, as in the normal subjects.

Table 2. Urinary Excretion of *chiro*-Inositol and *myo*-Inositol in Monkeys.

Monkey No.	Diagnosis	*chiro*-Inositol*	*myo*-Inositol
		μmol/day	
1	Normal	18.5	21.9
2	Normal	3.1	5.9
3	Normal	1.8	6.7
4	Normal	3.7	3.5
Mean ±SE	—	6.7±2.3	9.5±2.4
5	Obese	1.7	7.0
6	Obese	3.6	6.5
7	Obese	2.9	11.3
8	Obese	0.3	3.1
9	Obese	ND	72.2
10	Obese	ND	63.2
11	Obese	ND	4.9
Mean ±SE	—	1.2±0.2	24.0±4.7
12	Diabetic	1.0	169.0
13	Diabetic	ND	61.4
14	Diabetic	ND	312.5
15	Diabetic	ND	61.3
Mean ±SE	—	0.2±0.1	151.1±34.3

*ND denotes not detectable (limit of detection, <0.5 μmol per liter).

Table 3. Mean Concentrations of *chiro*-Inositol and *myo*-Inositol in Mediator Preparations from Muscle-Biopsy Specimens Obtained during Euglycemic–Hyperinsulinemic-Clamp Studies in Normal Subjects and Patients with NIDDM.*

Group	Time from Start of Clamp Study to Biopsy	*myo*-Inositol Concentration	*chiro*-Inositol Concentration
	min	*ng/mg wet weight of tissue (no. of samples)*	
Normal subjects	0	0.28±0.11 (4)	0.09±0.03 (4)
	15	2.58±0.41 (2)	0.52±0.14 (2)
	20	0.10±0.02 (2)	0.03±0.02 (2)
Patients with NIDDM	0	0.57±0.33 (5)	ND (5)
	15	6.48±3.10 (2)	ND (2)
	20	1.32±0.81 (3)	ND (3)

*Plus–minus values are means ±SE. Four normal subjects and five patients with NIDDM were studied immediately before the insulin infusion as described in Methods. Samples were obtained 15 minutes after the beginning of the insulin infusion in two normal subjects and two patients with NIDDM and 20 minutes after the beginning of the infusion in two normal subjects and three patients with NIDDM. ND denotes not detectable.

Discussion

We have previously shown that *myo*-inositol and *chiro*-inositol are the main inositol components of the putative chemical mediators of insulin action in the liver of rats.[11] The two alternative sources for these inositols are endogenous biosynthesis and dietary intake. *myo*-Inositol is synthesized from glucose by a head-to-tail condensation of the hexose.[22] *chiro*-Inositol may be formed by an analogous mechanism or by the inversion of one hydroxyl of *myo*-inositol.[23] However, nothing is known about the formation of *chiro*-inositol in humans.

Variations in dietary intake cannot explain the dramatic differences in the urinary excretion of *chiro*-inositol in the normal subjects and the patients with NIDDM and in the diabetic and nondiabetic monkeys. All subjects in group 3 (normal subjects) and group 4 (patients with NIDDM) ate the same diet before sample collection. Similarly, all monkeys were fed the same diet throughout the studies.

Another potential explanation for these results is altered cellular metabolism of inositol, induced by hyperglycemia. However, we found no correlation be-

tween urinary *chiro*-inositol excretion and either urinary glucose concentration or long-term diabetic control, as reflected by glycated hemoglobin values, in the patients with NIDDM. Alternatively, the low *chiro*-inositol excretion in the patients with NIDDM could have been due to increased renal tubular reabsorption. This explanation is unlikely because the *chiro*-inositol content in the muscle-biopsy specimens from the patients with NIDDM was also reduced. Thus, the urinary excretion of *chiro*-inositol in both normal subjects and patients with NIDDM appears to be correlated with the *chiro*-inositol content in tissues. In nondiabetic patients with chronic renal disease, Niwa et al. found a 5.8-fold increase in *chiro*-inositol excretion in comparison with values in normal subjects.[24] This increase has been confirmed in our laboratories (unpublished data). In addition, it is unlikely that the decreased urinary excretion of *chiro*-inositol in our patients with NIDDM was due to renal disease specifically related to diabetes, since most patients in group 2 had normal renal function.

It could also be argued that the insulin or oral hypoglycemic drugs used in the treatment of the patients with NIDDM induced the alteration in the metabolism of *chiro*-inositol, but urinary *chiro*-inositol excretion was low not only in the patients who were receiving insulin or an oral hypoglycemic drug (group 2) but also in those who had received no therapy for at least two weeks before sample collection (group 4). Although we cannot rule out possible important effects of insulin and other agents on the metabolism of *chiro*-inositol, it is clear from our data that these effects were not the primary cause of the low *chiro*-inositol excretion in patients with NIDDM. We can also rule out obesity as a primary cause of reduced urinary *chiro*-inositol excretion. Some obese, normal subjects had normal urinary excretion of *chiro*-inositol, and lean patients with NIDDM had low *chiro*-inositol excretion. Multivariate analysis of our data failed to reveal any correlation between *chiro*-inositol excretion and body weight. Therefore, we propose that the virtual absence of *chiro*-inositol in the urine of patients with NIDDM results from a biochemical defect associated with the biosynthesis of the inositol, its absorption in the gastrointestinal tract, its enhanced metabolic removal, or a combination of these factors.

Insulin resistance is a hallmark of NIDDM in humans and monkeys and develops well before clinically overt diabetes. Bogardus et al.[25] and Shulman et al.[26] have shown that insulin resistance is associated with a decreased response of glycogen synthase to insulin in skeletal muscle. In addition, Kida et al.[17] have suggested that the defect in the activation of glycogen synthase in skeletal muscle is directly associated with a lack of activation of glycogen synthase phosphatase. Mandarino et al.[27] have shown that pyruvate dehydrogenase is also not stimulated by insulin in adipocytes from patients with NIDDM. Although insulin resistance has been associated in some cases with structural alterations of the insulin receptor, post-binding defects are the primary cause of insulin resistance in NIDDM.[28]

One of the mechanisms by which insulin regulates glycogen synthase, pyruvate dehydrogenase, and other metabolic responses is by regulating the generation of mediators. Previous observations from our laboratory have shown that *chiro*-inositol is a major component of one of these mediators.[11] In this study, we have demonstrated that *chiro*-inositol is virtually absent in the urine of patients with NIDDM and in partially purified mediator preparations from their muscle-biopsy samples. Thus, we suggest that the insulin resistance seen in such patients is related to the absence of one of the putative mediators of insulin action.

A most intriguing aspect of our observations is the widespread distribution of the *chiro*-inositol defect among patients with NIDDM and among nonhuman primates with NIDDM. When these studies were initiated, we expected to detect a subpopulation of patients with NIDDM who had deficiencies in the metabolism of inositol and insulin mediators. The data presented here suggest that defects in *chiro*-inositol metabolism are common to most patients with NIDDM and that these defects may have an important role in insulin resistance. In contrast, analyses of 24-hour urine specimens from 14 patients with insulin-dependent diabetes mellitus revealed a wide variation in values for *chiro*-inositol excretion, ranging from 170 μmol per day to undetectable values (in approximately one third) (unpublished data). This variation may reflect the heterogeneity in the degree of insulin resistance previously reported in insulin-dependent diabetes.[29] Thus, decreased urinary excretion of *chiro*-inositol may be a biochemical marker for insulin resistance.

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ACTIVE LUNG FIBROSIS UP TO 17 YEARS AFTER CHEMOTHERAPY WITH CARMUSTINE (BCNU) IN CHILDHOOD

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Abstract *Background.* Carmustine (BCNU) is an anticancer drug known to produce pulmonary fibrosis as a side effect within three years of treatment. It is not known whether pulmonary fibrosis can appear later.

Methods. To investigate the clinical range of this side effect, we studied the survivors among 31 children treated with carmustine for brain tumors between 1972 and 1976. Fourteen had died of their tumor; of the remaining 17, 6 had died of lung fibrosis — 2 within 3 years of treatment and 4 from 8 to 13 years after treatment. This report focuses primarily on the 11 survivors, 8 of whom were available for detailed study 13 to 17 years (mean, 14) after treatment.

Results. Of the eight survivors studied, six had abnormal chest radiographs showing predominantly upper-zone fibrotic changes. These patients also had abnormal CT scans, showing a previously undescribed pattern of upper-zone fibrosis. All the survivors studied had restrictive spirometric defects (mean [±SD] vital capacity, 54±19 percent of the predicted value). Bronchoalveolar-lavage fluid contained abnormal proportions of specific macrophage subgroups. Light and electron microscopy in six patients revealed interstitial fibrosis and elastosis with damage to epithelial and endothelial cells. Four patients had symptoms (shortness of breath, cough, or both).

Conclusions. Carmustine chemotherapy in childhood causes lung fibrosis that may remain asymptomatic for many years or become symptomatic at any time. (N Engl J Med 1990; 323:378-82.)

CARMUSTINE (BCNU) is a nitrosourea drug with activity against brain tumors, lymphomas, malignant melanoma, and certain other neoplasms. It is known to be capable of causing lung fibrosis within three years of treatment. Weiss et al. have reviewed this condition, and they report an incidence of 20 to 30 percent,[1] although an incidence as low as 1 percent has been reported.[2]

We became aware of six deaths due to lung fibrosis among a group of persons who had survived childhood brain tumors that were treated by surgery, radiotherapy, and carmustine chemotherapy. Two of these deaths occurred within 3 years of the start of chemotherapy, but the other deaths occurred 8 to 13 years after treatment. We therefore undertook to review all the surviving patients who had received carmustine

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