Myoinositol Metabolism in Diabetes Mellitus

Effect of Insulin Treatment

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SUMMARY

The metabolism of myoinositol has been studied in 10 nondiabetic subjects and in six patients with diabetes mellitus before and after insulin therapy. While dietary myoinositol intake and fecal myoinositol excretion were similar in both groups, urinary myoinositol excretion was increased 10-fold in the untreated diabetic and accounted for a significant fraction of his dietary myoinositol intake. Insulin treatment restored the urinary myoinositol excretion toward normal. Despite increased myoinositol excretion, plasma myoinositol concentrations were significantly higher in the diabetics following the ingestion of a standard diet or of a 3.0-gm. myoinositol load. This abnormality in oral myoinositol tolerance was also corrected by insulin treatment. The size of the rapidly equilibrating myoinositol pool was significantly decreased in the untreated diabetic and returned to normal following a brief period of insulin treatment.

The elevated plasma myoinositol concentrations observed following myoinositol ingestion in the uncontrolled diabetic presumably represents a combination of enhanced gastrointestinal absorption and impaired intracellular transport of myoinositol. The decreased space of distribution of myoinositol also suggests an impairment of intracellular myoinositol transport in the untreated diabetic. These observations are consistent with the speculation that hyperglycemia may condition a widespread relative intracellular myoinositol deficiency in man and suggest that restoration of normal intracellular myoinositol concentrations might prove to be of benefit in the prevention and treatment of certain of the complications associated with human diabetes mellitus. DIABETES *26*:215-21, **March, 1977.**

It has been recognized for more than a century that human diabetics excrete large amounts of myoinositol in their urine.[1] However, the physiologic significance of this phenomenon has not been explained and its potential pathologic consequences have not been investigated.

Myoinositol (a hexahydroxy derivative of cyclohexane) is present in all plant and animal tissues that have been examined by adequate techniques.[2] Although a dietary requirement has not been established for man, exogenous myoinositol is an essential nutrient for all human cells in tissue culture.[3] Systems for its active intracellular transport have been demonstrated in mammalian lens,[4] kidney,[5] and intestine,[6] and most mammalian tissues maintain intracellular free myoinositol concentrations several orders of magnitude higher than those of plasma.[7] While the physiologic significance of this gradient is unknown, it may serve to facilitate the incorporation of myoinositol into membrane phospholipids, since the Km for myoinositol of CDP-diglyceride:inositol transferases has been found to be as high as 1.5 mM.[8] Impaired synthesis of phosphoinositides could have pathologic consequences, since the turnover of these myoinositol-containing membrane phospholipids has been speculated to play important roles in such diverse cellular functions as electrolyte transport,[9] amino acid transport,[10] nerve impulse transmission,[11,12] and the stimulated secretion of various intracellular products.[13-15]

Since elevated glucose concentrations inhibit the active transport of myoinositol,[5,6,16] it is not surprising that the induction of experimental diabetes results in increased urinary myoinositol excretion[17] and decreased intracellular myoinositol concentrations in the

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lens and nerve of experimental animals.[18,19] Recently it has been demonstrated that dietary myoinositol supplementation can normalize the myoinositol concentration of the sciatic nerve and prevent the decrease in sciatic motor nerve conduction velocity that follows the induction of streptozotocin diabetes in the rat.[18] Since it is possible that an analogous conditioned intracellular myoinositol deficiency in the peripheral nerve could be a factor in the pathogenesis of diabetic peripheral neuropathy in man, we have analyzed the effect of insulin treatment on the metabolism of myoinositol in the human diabetic.

MATERIALS AND METHODS

Patient Studies

Nondiabetic male subjects (fasting and postprandial plasma glucose concentrations of less than 105 mg./dl. and a negative family history for diabetes mellitus) were recruited from the student body of the University of Alabama in Birmingham. Newly diagnosed patients with stable but uncontrolled diabetes mellitus were obtained from the Diabetes Research and Education Hospital and from the Birmingham Veterans Administration Hospital. All inpatient subjects were admitted to the Clinical Research Center and provided fully informed consent prior to their participation in the studies described below. The mean age of the nondiabetic inpatient study subjects was 25.9 ± 1.1 years and that of the diabetic group 41.2 ± 4.5 years. In addition to the inpatient studies, a number of nondiabetic and poorly controlled diabetic subjects were studied as outpatients.

All inpatient subjects received a standard diet (28.8 calories per kilogram body weight) that contained a previously determined amount of myoinositol. Daily 24-hour collections of urine and feces were stored at 4° C. prior to analysis of their myoinositol content. Heparinized samples of venous blood were collected on ice at 7:00 a.m., 11:00 a.m., 4:00 p.m., and 8:00 p.m. and rapidly centrifuged in the cold, and plasma samples were frozen prior to the analysis of their myoinositol and glucose content.

The size of the rapidly equilibrating myoinositol pool and the half-time of myoinositol disappearance was estimated in all inpatient study subjects. A bolus of 25 μCi. of sterile, pyrogen-free 2-^{3}H-myoinositol (2.84 Ci./mM, New England Nuclear, Boston, Mass.) dissolved in 1.0 ml. of isotonic saline was injected intravenously into an antecubital vein of the fasting subject. Heparinized venous blood samples were obtained from the contralateral antecubital vein at 10-minute intervals for one hour for the determination of plasma myoinositol specific activity.[20]

On the following day, oral myoinositol tolerance was determined. The subjects drank 250 ml. of iced tap water containing 3.0 gm. of myoinositol (Sigma, St. Louis, Mo.) over a two-minute period. Blood samples were obtained prior to the ingestion of myoinositol and at hourly intervals thereafter for four hours.

Diabetic subjects were then begun on a regimen of twice-daily injections of a mixture of NPH and crystalline insulin adjusted according to the plasma glucose concentrations. Oral myoinositol tolerance as well as myoinositol pool size and turnover rate was restudied after all plasma glucose concentrations had been maintained below 150 mg./dl. for at least 24 hours.

Laboratory Procedures

Plasma and urinary glucose concentrations were determined by the glucose oxidase technique.[21] Plasma urea nitrogen and creatinine concentrations were determined by AutoAnalyzer techniques.[22] The myoinositol content of plasma, urinary, fecal, and dietary samples was determined by gas-liquid chromatography.[23] Plasma samples (1.0 ml.) were added to a glass-stoppered tube containing α-methylmannose (10 μg., Sigma), 1.0 ml. of distilled water, and 4.0 ml. of zinc sulfate (5 per cent, Fisher Scientific, Pittsburgh, Pa.) and shaken. Barium hydroxide (4 ml., 0.3N, Fisher) was then added, the suspension was shaken and centrifuged, and a 5-ml. sample of the clear supernatant was lyophilized. The trimethylsilyl ethers of myoinositol were prepared and extracted into hexane as previously described,[24] and aliquots were analyzed on a 180-cm. column of 3 per cent SE-30 on Supelcoport (80/100 mesh, Supelco, Bellefonte, Pa.) isothermally at 185° C. in a Hewlett-Packard model 402 gas-liquid chromatograph. The peak area of myoinositol was determined by triangulation and quantified by comparison with a standard solution of hexa-O-trimethylsilyl myoinositol. Correction for losses was calculated by the recovery of α-methylmannose. This method was found to permit the determination of myoinositol concentrations in samples containing a minimum of 1.8 μg./ml. (10 μM) of myoinositol. With this technique, the recovery of myoinositol added to plasma samples was found to be 99.6 per cent and the variance of replicate samples 1.6 per cent.

Urinary free myoinositol was determined by the method of Clements and Starnes.[24] Samples of urine (1.0 ml.) were placed in vials containing approxi-

mately 9,000 cpm of 2-^{3}H-myoinositol. The mixture was acidified by the addition of 12N HCl (3 drops) and applied to a 1 × 5 cm. column of AG-501-X8 (Bio Rad, Richmond, Calif.) previously equilibrated with distilled water. The column was washed with distilled water and the first 8 ml. of the eluate was collected and lyophilized. The trimethylsilyl ethers of myoinositol were prepared and analyzed as described above. Correction for losses was calculated by the recovery of 2-^{3}H-myoinositol. The recovery of myoinositol added to urine samples was found to be 102 ± 5 per cent by this technique.

For the determination of the water-soluble myoinositol content of food and feces, 0.2-gm. portions were homogenized in the cold in glass homogenizing vessels containing a mixture of 5 ml. of distilled water, 10 μg. of α-methylmannose, and 2 ml. of zinc sulfate (5 per cent). A 2-ml. portion of barium hydroxide (0.3N) was added and the mixture was rehomogenized and centrifuged. The myoinositol content of the clear supernatant was determined as described above. Total myoinositol content was determined by using 0.2-gm. portions of food and feces weighed into 15 × 150 mm. Pyrex test tubes containing tracer amounts of 2-^{3}H-myoinositol. A 2-ml. portion of 6N HCl was added, and the tubes were sealed with a torch and heated at 125° C. for 40 hours. The hydrolysate was filtered and lyophilized, and the trimethylsilyl ethers of myoinositol were prepared and analyzed as described above. Correction for losses during the hydrolysis and derivatization procedures was calculated by the recovery of labeled myoinositol. The myoinositol hexaphosphate content of food samples was determined by the method of Oshima, Taylor, and Williams.[25]

For statistical analyses, the Student's *t* test for paired data or for two groups was employed.[26]

RESULTS

Dietary Myoinositol Content

The daily dietary myoinositol intake determined in aliquot diets for the 10 nondiabetic and the six diabetic subjects studied is given in table 1. The mean body weight of the diabetic subjects was slightly less than that of the control subjects and, therefore, their total caloric and myoinositol intake was less. In the individual diets of both the control and the diabetic subjects, 41.3 ± 3.3 per cent of the total myoinositol content was found to be in the form of water-soluble myoinositol and the remainder was assumed to be in the form of lipid-bound myoinositol. In contrast to the high concentrations of phytate present in the diets of populations in which a large proportion of the diet consists of unprocessed cereal grains, in the present study less than 0.06 per cent of the myoinositol content of the standard diet was found to be in the form of myoinositol hexaphosphate.[27] No significant differences in the total myoinositol content or its distribution were found between the control and diabetic diets.

TABLE 1

Dietary myoinositol content

	Controls (n = 10) (Body weight 81.0 ± 3.8 kg.)	Diabetics (n = 6) (Body weight 75.0 ± 7.4 kg.)
Water-soluble myoinositol (mg.)	395.8 ± 62.1	238.6 ± 41.0
Lipid-bound myoinositol (mg.)	504.4 ± 86.0	449.7 ± 78.4
Myoinositol-hexaphosphate (mg.)	0.50 ± 0.11	N.D.
Total myoinositol (mg.)	900.7 ±131.5	688.3 ±107.6

All values expressed as the means ± S.E.M.
N.D.—Not determined

Fasting Plasma Myoinositol Concentrations in Diabetics and Nondiabetics

Myoinositol concentrations were determined in fasting plasma samples obtained from 73 nondiabetic and 54 diabetic outpatients. As shown in table 2, a statistically significant elevation in the mean plasma myoinositol concentration was observed in the diabetic group. However, the mean plasma urea nitrogen concentration was also found to be elevated in the diabetic group. Since we have previously observed a linear relationship between urea nitrogen and myoinositol concentrations in patients with varying degrees of renal failure,[28] the plasma urea nitrogen and myoinositol concentrations were compared in the present group of subjects. A positive linear correlation between the urea nitrogen and myoinositol concentra-

TABLE 2

Fasting plasma myoinositol and urea nitrogen concentrations in nondiabetics and diabetics

	Myoinositol (μM)	Urea nitrogen (mg./dl.)
Nondiabetics (n = 73)	29.0 ± 1.9	14.2 ± 0.5
Diabetics (n = 54)	40.0 ± 2.9	17.1 ± 0.8
p value	< 0.005	<0.005

tion was observed ($r = +0.60$, $p < 0.001$), which suggests that the elevated mean fasting plasma myoinositol concentrations observed in the diabetics may be explained by the increased prevalence of impaired renal function in this group. In support of this speculation is the observation that the mean fasting plasma myoinositol concentration in the 38 diabetics whose serum urea nitrogen was less than 20 mg./dl. was observed to be 35.6 ± 3.0 μM, which was not statistically different from that of the controls.

Diurnal Variations in Plasma Myoinositol Concentrations

The mean plasma myoinositol concentrations observed at 7:00 a.m., 11:00 a.m., 4:00 p.m., and 8:00 p.m. in the 10 nondiabetic and six diabetic inpatient subjects are shown in figure 1. Renal function was normal in all subjects in both groups, and no significant difference between the mean fasting plasma myoinositol concentration of the normal and diabetic groups was observed. While no diurnal change in the plasma myoinositol concentration was observed in the nondiabetic group, the plasma myoinositol concentrations were found to rise significantly during the day in the diabetics. This rise was presumed to result from the ingestion and gastrointestinal absorption of dietary myoinositol.

Oral Myoinositol Tolerance

The effect of a 3.0-gm. oral load of myoinositol on plasma myoinositol concentrations was studied in 23

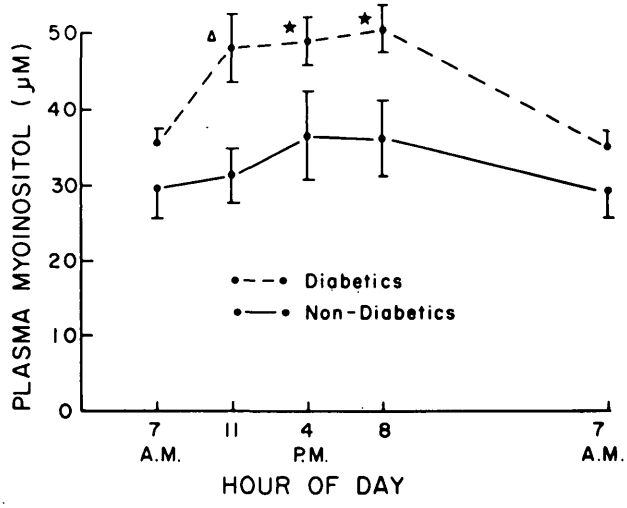

FIG. 1. Variations in plasma myoinositol concentrations during the day in six diabetic and 10 nondiabetic subjects ingesting a standard diet. Each value represents the mean ± S.E.M. Paired comparisons of the plasma myoinositol concentration at 11:00 a.m., 4:00 p.m., and 8:00 p.m. with that observed at 7:00 a.m. that were statistically significant are indicated by the symbols – $p < 0.05$ (triangle), $p < 0.01$ (stars).

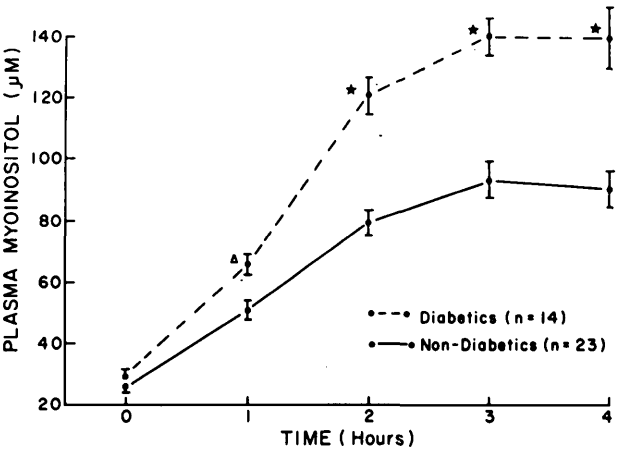

FIG. 2. Plasma myoinositol concentrations following a 3.0-gm. oral myoinositol load in nondiabetic and uncontrolled diabetic males. Each value represents the mean ± S.E.M. Significant differences between the groups are indicated by the symbols – $p < 0.01$ (triangle), $p < 0.001$ (stars).

nondiabetic and 14 untreated diabetic males with normal renal function. As shown in figure 2, following the ingestion of myoinositol, the plasma myoinositol concentrations rose sharply in the diabetic subjects and were significantly higher than those of the controls at all time intervals studied.

The effect of insulin therapy on oral myoinositol tolerance was studied in six diabetic males. Prior to therapy, the mean plasma glucose concentration throughout the day in these subjects was 334 ± 52 mg./dl. Following insulin therapy, the mean plasma glucose concentration in the same subjects was 128 ± 9 mg./dl. during the 24-hour period prior to myoinositol tolerance testing. As shown in figure 3, insulin therapy restored the oral myoinositol tolerance of these subjects to normal. When compared to the pretreatment concentrations, the plasma myoinositol concentrations were significantly lower in the treated group at the second, third, and fourth hours following the oral load.

Urinary and Fecal Myoinositol Excretion

As has been described by others,[1,29-31] we found the urinary excretion of myoinositol to be significantly elevated in the uncontrolled diabetics (table 3). Following insulin therapy, urinary myoinositol excretion decreased significantly but did not reach normal values. During the 24-hour period following the ingestion of a 3.0-gm. myoinositol load, nondiabetics were found to excrete an average of 1.2 per cent of the administered myoinositol in the urine. In contrast, the diabetics excreted nearly 6 per cent of the ingested myoinositol load in their urine both before and after insulin therapy. Fecal myoinositol excretion was

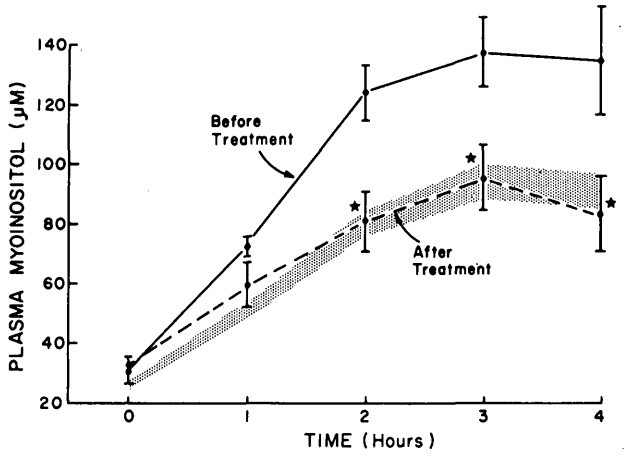

FIG. 3. Plasma myoinositol concentrations following a 3.0-gm. oral myoinositol load in six diabetic subjects before and after insulin therapy. Each value represents the mean ± S.E.M. The shaded area represents the range of myoinositol concentrations observed in nondiabetic subjects under similar conditions. Paired differences in which the p was < 0.01 are indicated by the stars.

and the amount of myoinositol excreted in the urine ($r = + 0.48$, $p < 0.001$).

Myoinositol Half-time and Pool Size

Analysis of the decrease in specific activity of plasma myoinositol following the intravenous administration of 2-^{3}H-myoinositol was employed to estimate the half-time of myoinositol disappearance in the study subjects.[20] The half-time of myoinositol disappearance averaged 18.2 minutes in the diabetic subjects and was similar to that of the nondiabetics (table 4). Following treatment, the myoinositol half-time increased in all diabetic subjects. In the untreated diabetic, the size of the rapidly equilibrating myoinositol pool was less than half that observed in the nondiabetic subject but returned to normal following insulin treatment.

DISCUSSION

The present studies demonstrate a number of abnormalities in the metabolism of myoinositol by the

TABLE 3

Urinary and fecal myoinositol excretion in nondiabetic and diabetic males before and after a 3.0-gm. oral myoinositol load

	Urinary myoinositol excretion (mg./24 hr.)		Fecal myoinositol excretion (mg./24 hr.)	
Subjects	Standard diet	Standard diet + 3.0 gm. myoinositol	Standard diet	Standard diet + 3.0 gm. myoinositol
Nondiabetics (n = 10)	29.8 ± 4.9	65.3* ± 11.1	1.4 ±0.3	6.0 ±4.3
Untreated diabetics (n = 6)	316.1† ± 61.4	491.0* ±121.1	1.2 ±0.5	4.2 ±2.4
Treated diabetics (n = 6)	69.8* ± 13.0	245.8 ± 29.0	N.D.	N.D.

N.D.—Not determined
* p vs. controls < 0.025.
† p vs. controls < 0.001.
‡ p vs. untreated diabetics < 0.005.

found to be similar in both diabetics and nondiabetics (less than 0.2 per cent of the amount ingested) and did not increase significantly on the day following the oral myoinositol load.

The relationships between the excretion of urinary myoinositol and the 24-hour urine volume, urine glucose content, and mean plasma glucose concentrations were studied on 66 occasions in the 10 nondiabetic and six diabetic subjects. Barely significant correlations were found to exist between urinary myoinositol excretion and both the urine volume ($r = + 0.30$, $p < 0.05$) and the urinary glucose content ($r = + 0.31$, $p < 0.05$). A statistically significant relationship was found between the mean plasma glucose concentration

TABLE 4

Myoinositol half-time and pool size in nondiabetics and in diabetics before and after treatment

Subjects	2-^{3}H-myoinositol half-time (min.)	2-^{3}H-myoinositol pool size (ml./kg. body weight)	2-^{3}H-myoinositol pool size (mg./kg. body weight)
Nondiabetics (n = 10)	21.9 ± 1.0	442.5 ± 31.8	2.46 ±0.26
Untreated diabetics (n = 6)	18.2 ± 2.1	200.1* ± 56.5	1.25† ±0.36
Treated diabetics (n = 6)	33.8†‡ ± 5.7	385.7‡ ± 83.0	2.59‡ ±0.62

* p vs. controls < 0.005.
† p vs. controls < 0.025.
‡ p of paired differences < 0.005.

uncontrolled diabetic. In addition to the previously documented myoinositoluria, we have observed that the plasma myoinositol concentrations are significantly elevated following the ingestion of a normal diet or of a large oral myoinositol load and that the myoinositol pool size is significantly decreased in the untreated diabetic. All of these abnormalities are restored toward normal following a brief period of careful insulin therapy.

Analysis of dietary myoinositol content by gas-liquid chromatography is in agreement with earlier studies employing yeast bioassay techniques.[29,32] In the present study, the total daily dietary myoinositol content of both diabetic and nondiabetic diets was found to average 813 mg., with a range of from 366 to 1,654 mg. This wide range of dietary myoinositol content suggests that individual foodstuffs may differ considerably in their myoinositol content and raises the possibility that diets can be developed that will provide either a high or a low myoinositol content.

That, as has been demonstrated in the rat,[17] virtually all of the ingested myoinositol is absorbed from the gastrointestinal tract of man is indicated by the observation that human feces contain little myoinositol. However, the present studies do not preclude the possibility that substantial amounts of myoinositol may be degraded by enteric organisms. The greater rise in plasma concentrations following the ingestion of myoinositol by the uncontrolled diabetic suggests that hyperglycemia may enhance gastrointestinal myoinositol absorption or decrease the rates of myoinositol uptake by various tissues. The former speculation is supported by animal studies in which the induction of alloxan diabetes in rats has been found to accelerate the rate of disappearance of myoinositol from the gastrointestinal tract.[17] However, the present studies also suggest that the decreased myoinositol pool size may contribute to the elevated plasma concentrations observed following myoinositol ingestion by the uncontrolled diabetic.

Urinary excretion of myoinositol represents a significant fraction (one-third to one-half) of the myoinositol ingested by the untreated diabetic. The present studies suggest that myoinositoluria may be a function of the plasma glucose concentrations to which the kidney is exposed (and hence the filtered glucose load) and is less dependent on urine volume and total urinary glucose excretion. These observations support the suggestion of Daughaday and Larner that renal tubular reabsorption of myoinositol is inhibited by a high filtered glucose load.[29]

The size of the rapidly equilibrating myoinositol pool was found to be similar to the size of the estimated extracellular fluid space in the untreated diabetic. In contrast, the myoinositol pool size in the nondiabetic and in the treated diabetic was substantially larger than this space. This suggests that hyperglycemia may interfere with the movement of myoinositol into the intracellular space in the untreated diabetic. Such a suggestion is consistent with the in-vitro observations that elevated glucose concentrations inhibit the active intracellular transport of myoinositol.[5,6,16] While the specific tissues operative in the decreased intracellular transport of myoinositol in the untreated human diabetic have not been determined, the present studies suggest that hyperglycemia may condition a widespread impairment of intracellular myoinositol transport.

Although the mechanism by which a conditioned decrease in intracellular myoinositol concentrations could contribute to the functional abnormalities observed in the peripheral nerves of animals with experimental diabetes is unknown, the recent studies of Greene, de Jesus, and Winegrad indicate that these abnormalities can be prevented or corrected simply by restoring the nerve myoinositol concentrations to normal by means of dietary myoinositol supplementation.[18] Our observation that the plasma myoinositol concentration in man can be raised by the oral administration of myoinositol and that roughly 94 per cent of an oral myoinositol load is retained by the uncontrolled diabetic indicate that chronic oral myoinositol supplementation could be employed to elevate the plasma myoinositol concentrations in human diabetics. Whether the intracellular concentration of myoinositol is decreased in the nerves of the human diabetic and whether the nerve myoinositol concentration could be restored to normal by diet-induced elevation of the plasma myoinositol concentration is not known. The possibility that oral myoinositol supplementation could result in improvement in the peripheral nerve function of patients with diabetes mellitus will require further study. It should be pointed out that caution will be required in any study of the effect of dietary myoinositol supplementation on peripheral nerve function in man, since excessive elevation of the plasma myoinositol concentration has been found to be associated with impaired peripheral nerve function in both the normal and the diabetic rat.[18,28,31,33]

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