Effect of Aldose Reductase Inhibitor (Tolrestat) on Urinary Albumin Excretion Rate and Glomerular Filtration Rate in IDDM Subjects With Nephropathy

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OBJECTIVE — To explore the possible link between diabetic nephropathy and the enhanced activity of the polyol pathway, known to occur in IDDM subjects.

RESEARCH DESIGN AND METHODS — We studied the effects of the aldose reductase inhibitor tolrestat (200 mg/day) on urinary albumin excretion rate and glomerular filtration rate in 20 IDDM patients with diabetic nephropathy.

RESULTS — Six months of placebo treatment produced no significant changes in glomerular filtration rate, urinary albumin excretion rate, and renal plasma flow. Consequently, filtration fraction remained unchanged. During tolrestat treatment, glomerular filtration rate decreased from the basal value of 156 ± 14 ml · min^{-1} · 1.73 m^2 to 142 ± 13.7 ml · min^{-1} · 1.73 m^2 ($P < 0.001$) at 2 mo; 128 ± 12.4 ml · min^{-1} · 1.73 m^2 ($P < 0.001$) at 4 mo; and 123.7 ± 13.0 ml · min^{-1} · 1.73 m^2 at 6 mo. A significant decrease of urinary albumin excretion rate was observed during the trial (basal values 219 ± 32.5 vs. 196.9 ± 28.5 μg/min at 2 mo [$P < 0.05$]; 171.6 ± 25.5 μg/min at 4 mo [$P < 0.001$]; and 58.6 ± 19.3 μg/min at 6 mo [$P < 0.001$]). No significant change in renal plasma flow was seen during tolrestat treatment. Filtration fraction significantly decreased in the tolrestat group from the basal value of 0.23 ± 0.02 to 0.21 ± 0.01 at 2 mo ($P < 0.005$); 0.18 ± 0.02 at 4 mo ($P < 0.001$); and 0.17 ± 0.02 at 6 mo ($P < 0.001$).

CONCLUSIONS — The polyol pathway is implicated in hemodynamic changes associated with early diabetic nephropathy, and aldose reductase treatment can positively influence these parameters.

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IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; ARI, ALDOSE REDUCTASE INHIBITOR; UAER, URINARY ALBUMIN EXCRETION RATE; GFR, GLOMERULAR FILTRATION RATE; RPF, RENAL PLASMA FLOW; AST, ASPARTATE AMINOTRANSFERASE; ALT, ALANINE AMINOTRANSFERASE; BMI, BODY MASS INDEX; UNAR, UREA NITROGEN APPEARANCE RATE; DTPA, DIETHYL-TRIAMINO-PENTACETIC ACID; FF, FILTRATION FRACTION; RIA, RADIOIMMUNOASSAY; CV, COEFFICIENT OF VARIATION; UV, URINE VOLUME; BP, BLOOD PRESSURE; DBP, DIASTOLIC BLOOD PRESSURE; FBG, FASTING BLOOD GLUCOSE.

Aldose reductase, a member of the monomeric NADPH-dependent aldoketoreductase family, has been implicated in the development of various diabetic complications such as neuropathy (1,2), retinopathy (3,4), and cataracts (5,6). This enzyme catalyzes the reduction of several aldehydes, including the aldehyde form of glucose, which is reduced to the corresponding sugar alcohol, sorbitol. Sorbitol is subsequently metabolized to fructose by sorbitol dehydrogenase. The conversion of glucose to fructose by this means constitutes the polyol pathway of glucose. Under normal physiological conditions, this pathway plays a minor role in most tissues. In hyperglycemia associated with diabetes, however, cells undergoing insulin-independent uptake of glucose produce significant quantities of sorbitol. These cells accumulate sorbitol because of its poor penetration across cell membranes and its slow metabolism by sorbitol dehydrogenase. The resulting hyperosmotic stress to cells is postulated to be one of the primary causes of the development of diabetic complications (7,8).

Relatively little is known about the involvement of the polyol pathway in the pathogenesis of diabetic nephropathy (6,9). Most reports concerning the possible prevention by ARIs of diabetic nephropathy are conflicting. Renal hypertrophy has been prevented by ARIs (10,11). However, an indirect measure of mean glomerular size, which is increased in experimental diabetes, has not been influenced by ARI treatment for 1 mo (12). Thickening of the glomerular basement membrane has been prevented in one study (13), but not significantly in another (10).

The aim of this study was to evaluate the effects of long-term treatment with an ARI (tolrestat) on UAER and GFR in IDDM patients with early nephropathy.

RESEARCH DESIGN AND METHODS

— This trial was carried out according to a double-blind place-

Table 1—*Baseline features and renal hemodynamics of 20 IDDM patients randomized to tolrestat or placebo groups*

	TOLRESTAT GROUP	PLACEBO GROUP
AGE (YR)	40 ± 4	43 ± 6
SEX	8M/2F	6M/4F
BMI (KG/M^2)	21.3 ± 1.3	23.0 ± 1.4
DURATION OF DIABETES (YR)	13 ± 6	16 ± 4
INSULIN DOSE (U/DAY)	48.5 ± 6.3	44.4 ± 5.8
PROTEIN INTAKE ($G \cdot KG^{-1} \cdot DAY^{-1}$)	1.6 ± 0.5	1.6 ± 0.5
ENERGY INTAKE (KCAL/DAY)	2000 (RANGE 1500–2000)	2000 (RANGE 1600–2000)
DBP (MMHG)	83 ± 3	78 ± 5
GFR ($ML \cdot MIN^{-1} \cdot 1.73M^2$ B.S.A.)	158 ± 14	160 ± 16
RPF ($ML \cdot MIN^{-1} \cdot 1.73M^2$ B.S.A.)	685.7 ± 60.6	675.3 ± 58
UAER (μG/MIN)	219 ± 32.5	223 ± 30.4
SERUM CREATININE (MG/DL)	0.91 ± 0.14	0.89 ± 0.10

Data are means ± SE. b.s.a., body surface area.

bo-controlled design. We selected 20 IDDM subjects from outpatients of The Center for the Treatment of Diabetes and Metabolic Diseases. Baseline features and renal hemodynamics are given in Table 1. The diagnosis of diabetic nephropathy was made in the presence of persistent proteinuria in patients with diabetes for >10 yr but without clinical or biochemical evidence of other renal disease or cardiac failure. None of the patients had symptomatic hypoglycemia during the examination. Besides having diabetes, the patients were healthy and received no medication other than insulin. We used the following criteria for inclusion: infertile men or postmenopausal or clinically or surgically sterile (hysterectomy or bilateral fallopian tube ligature) women; with 7–20 yr of IDDM and history of ketoacidosis with clinically significant symptoms; subcutaneous insulin treatment; UAER <300 μg/min; arterial mean dBP while supine <90 mmHg; and GFR >120 $ml \cdot min^{-1} \cdot 1.73\ m^2$. The following were reasons for exclusion: liver disease (indicated by elevated serum transaminases [AST or ALT]) or total blood bilirubin >34.2 μg/L), the presence of any renal disease not primary or secondary to diabetes; history of symptomatic ischemic heart disease or peripheral vascular disease; history or clinical evidence of alcohol or drug abuse, mental disease, or malignant tumors. The study protocol was approved by the Faculty Ethical Committee. All patients were examined for late complications involving eyes and nerves. When retinopathy was found, the patients underwent fluoroangiographic examination. Retinopathy was investigated by ophthalmologic examination. In its likelihood it was further studied by fluoroangiography. The diagnosis of autonomic or peripheral neuropathy was based on the history and clinical and specialized tests. After rigorous physical examination to assess Achilles and patellar reflexes, vascular trophism of the lower limbs, and muscle strength of foot extensors, patients underwent four cardiovascular function tests (Valsalva maneuver, deep breathing, lying to standing, and sustained handgrip). Each of these four tests were given a score of 0, 1, or 2 depending on whether the outcome was normal, borderline, or pathologic. In the case of two or more abnormal tests with an overall score >3, a diagnosis of autonomic neuropathy was made. Peripheral neuropathy was investigated by femur motor conduction velocity and median sensory nerve conduction velocity. In three patients we observed a significant reduction in motor and sensory conduction velocity; two of these also had proven autonomic neuropathy; 5 patients had background retinopathy (a few microaneurisms); and 3 had diminution of nerve conduction velocity as an expression of incipient neuropathy. The mean energy and protein intake of the usual diet was calculated from a daily dietary record. Estimated usual energy and protein intake is given in Table 1. Dietary compliance was assessed by the UNAR as calculated from the total urinary urea excreted in 24 h (14) (Table 2).

After giving informed consent, the patients were randomly allocated to the tolrestat (200 mg/day) group or the placebo group. One tablet of each was given daily. The placebo and the tolrestat tablets were identical in appearance. For 3 consecutive days before entrance into the study, the patients were examined for blood pressure, HbA_{1c}, blood glucose, serum creatinine, and UAER. GFR and RPF were determined the day before launching the study. All examinations were repeated after 2, 4, and 6 mo of treatment with placebo or tolrestat. FBG was determined by the glucose oxidase method. Serum HbA_{1c} (normal range 3.5–6.2%) was measured by liquid chromatography (Bio-Rad, Richmond, Ca) (15). Serum creatinine was analyzed by using the Jaffè reaction (16). Right brachial supine pressure was also recorded by the same observer before each study with a standard sphygmomanometer. GFR evaluation on renal scintigraphy was carried out by DTPA injection at 200 MBq. A characteristic of this tracer is its exclusive elimination by renal filtration without tubular reabsorption and its capacity to give a complete picture of renal vessels and function. GFR was expressed in $ml \cdot min^{-1} \cdot 1.73m^2$ body surface area. Tracer uptake and excretion was monitored by computerized γ-counter, recording 1 frame/s for 30 s to study the vascular phase and 2 frames/min for 20 min to study the excretion-filtration phase. The γ-counter was set up to use Gates' (17) model software to

Table 2—*FBG, HbA_{1c}, BMI, dBP, UNAR, and FF before and during treatment with tolrestat or placebo*

	Tolrestat (N = 10)				Placebo (N = 10)			
	Baseline	2 mo	4 mo	6 mo	Baseline	2 mo	4 mo	6 mo
FBG (mM)	9.0 ± 1.2	8.9 ± 1.0	9.1 ± 1.3	9.0 ± 1.0	8.9 ± 1.1	8.7 ± 0.9	9.0 ± 1.1	8.8 ± 1.0
HbA_{1c} (%)	6.7 ± 0.8	6.8 ± 0.9	6.8 ± 0.8	6.6 ± 0.7	6.5 ± 0.7	6.7 ± 0.6	6.9 ± 0.7	6.8 ± 0.9
BMI (kg/m^2)	21.3 ± 1.3	21.6 ± 1.4	22.0 ± 1.4	21.4 ± 1.3	23.0 ± 1.4	22.8 ± 1.3	23.2 ± 1.2	22.6 ± 1.6
dBP (mmHg)	83 ± 3	81.5 ± 5	84 ± 6	82 ± 5	78 ± 5	80 ± 6	82 ± 5	78 ± 6
UNAR (mg/min)	9.75 ± 2.2	9.80 ± 2.1	10.1 ± 2.6	9.6 ± 2.0	9.54 ± 2.4	9.72 ± 2.3	9.40 ± 2.1	9.60 ± 2.1
FF	0.23 ± 0.02	0.21 ± 0.001*	0.17 ± 0.02†	0.17 ± 0.02†	0.23 ± 0.02	0.24 ± 0.02	0.24 ± 0.02	0.24 ± 0.02

Data are means ± SE. No significant changes in FBG, HbA_{1c}, BMI, dBP, and UNAR were seen during the study in any of the study population. A significant decrease in FF was seen in the tolrestat group after 2 ($P < 0.005$), 4 ($P < 0.001$), and 6 mo ($P < 0.001$) of treatment.
*$P < 0.005$.
†$P < 0.001$.

calculate total (both kidneys) and separate (right or left) GFR. RPF was evaluated by rapid intravenous infusion of L-Hippuran at 30 μCi (18). FF represents the ratio of GFR to RPF using the standard formula and values corrected to 1.73 m^2 body surface area. No adverse reactions were observed in either the tolrestat or the placebo group, and in consequence, none of the patients left the study. UAER was assayed by RIA (19) (Sclavo Kit, Milan, Italy) in three overnight urine samples per subject, collected on successive days. Values of renal albumin excretion were expressed as UAER in μg/min.

All samples were sterile. Finally, we determined CVs of UAER and UV to study daily variability of UAER and urine volumes.

Statistical analysis

This was a double-blind, randomized, noncross-sectional study. Statistically significant differences were calculated by two-tailed unpaired Student's *t* test and validated by nonparametric test (Wilcoxon's rank-sum test). For β or type II error, $P < 0.05$ was chosen as the level of significance. Data are means ± SE.

RESULTS

Glycemia and arterial pressure

Glycemic control as assessed by mean blood glucose and serum HbA_{1c} concentrations was similar during tolrestat or placebo treatment. In fact, as depicted in Table 2, no significant differences between basal concentration of plasma glucose and HbA_{1c} and corresponding values at 2, 4, and 6 mo of treatment with placebo or tolrestat were found. Neither was a significant difference in mean BP recorded at the start of the study nor during treatment with placebo or tolrestat (Table 2).

Renal hemodynamics

Six months of placebo treatment produced no significant changes in GFR or UAER (Figs. 1, 2). Consequently, FF remained unchanged (Table 2). During tolrestat treatment, GFR decreased from the basal value of 158 ± 14 to 142 ± 13.7 ml · min^{-1} · 1.73m^2 at 2 mo ($P < 0.01$), 128 ± 12.4 ml · min^{-1} · 1.73m^2 at 4 mo ($P < 0.001$), and 123.7 ± 13.0 ml · min^{-1} · 1.73m^2 at 6 mo ($P < 0.001$) (Fig. 1). No significant change in RPF was observed during tolrestat treatment (Fig. 4). In fact, clearance of P-aminohippuric acid remained unchanged during the tolrestat study (685.7 ± 60.6 vs. 674.5 ± 35.3 ml · min^{-1} · 1.73m^2 at 2 mo, 687 ± 54.7 ml · min^{-1} · 1.73m^2 at 4 mo, and 705 ± 34.9 ml · min^{-1} · 1.73m^2 at 6 mo, all NS). FF (defined as GFR divided by RPF) significantly decreased in the tolrestat group from the basal value of 0.23 ± 0.02 to 0.21 ± 0.001 at 2 mo ($P < 0.005$), 0.18 ± 0.02 at 4 mo ($P < 0.001$), and 0.17 ± 0.02 at 6 mo ($P < 0.001$) (Table 2).

Tolrestat treatment was responsible for a significant decrease in UAER (Fig. 2) (basal values 219 ± 32.5 vs. 196 ± 28.5 μg/min at 2 mo [$P < 0.001$], 172.6 ± 25.5 μg/min at 4 mo [$P < 0.001$], and 158.6 ± 19.3 μg/min at 6 mo [$P < 0.001$]). No significant

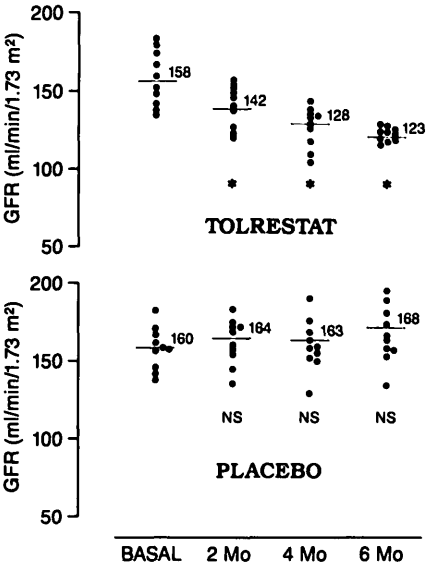

Figure 1—*Trends in GFR (ml · min^{-1} · 173m^2) before and at 2, 4, and 6 mo of treatment with tolrestat or placebo in 20 IDDM patients. *$P < 0.001$ vs. baseline.*

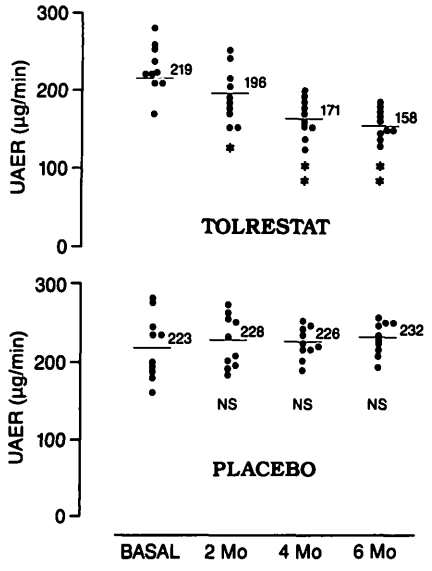

Figure 2—*Trends in UAER (µg/min) before and at 2, 4, and 6 mo of treatment with tolrestat or placebo in 20 IDDM patients. *P < 0.05 and **P < 0.001 vs. baseline.*

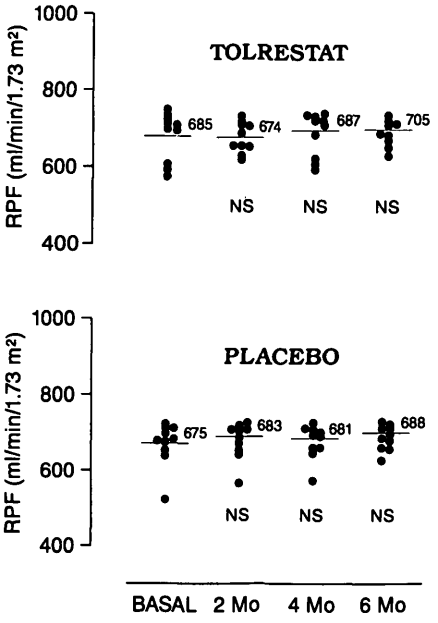

Figure 4—*Trends in RPF (ml · min^{-1} · 173m^2) before and at 2, 4, and 6 mo of treatment with tolrestat or placebo in 20 IDDM patients.*

correlation between GFR and UAER in the placebo group was found. In the tolrestat group, a significant correlation between GFR and UAER was found (r = 089; $2P < 0.001$) (Fig. 3). No change in dBP, BMI, or UNAR was seen in either the placebo or the tolrestat group (Table 2). Serum creatinine remained unchanged during the study in the placebo and tolrestat groups.

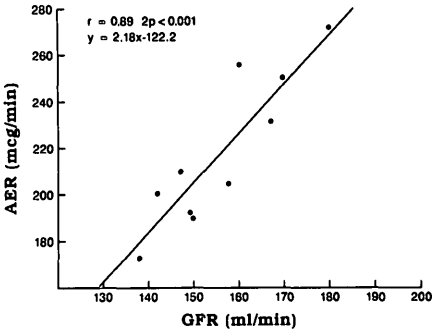

Figure 3—*Correlation between GFR and UAER in 10 IDDM patients treated with tolrestat. r = 0.89, 2P < 0.001.*

CONCLUSIONS— These findings confirm previous observations in animal models (20–22) regarding effects of diabetes and ARIs on vascular albumin permeation and GFR. In contrast, they demonstrate that RPF is markedly increased in diabetic subjects with early nephropathy, and this increase in blood flow is not prevented by ARIs. Sorbitol is a nonperturbing osmotically active organic solute that increases cellular osmolality without the damaging effects on macromolecules that would be caused by increasing intracellular NaCl or KCL (23,24). Thus, these solutes are believed to help cells function and survive under hyperosmotic conditions by regulating cell volume while maintaining a favorable intracellular milieu (25). Localization of aldose reductase, the enzyme that produces sorbitol, parallels the concentrations and distribution of sorbitol in kidneys of dogs (26), rats (27), and rabbits (28,29). High levels of aldose reductase have been reported in the renal inner medulla within the cells of collecting ducts, loops of Henle, and the pelvic papillary epithelium (26,27,30–32). Thus, sorbitol metabolism is linked to renal osmoregulation. In contrast to its normal physiological role in the kidney, sorbitol at high concentrations causes osmotic damage from swelling. The altered metabolic state of the diabetic patient, with increasing sorbitol production, is probably related to characteristic changes in the structure of the basement membrane. Recently, the basement membrane thickness, marker of diabetic nephropathy, was shown to be influenced by the polyol pathway (33,34). Moreover, in animal models polyol metabolism also has been linked to the development of morphological and functional changes in microvasculature (33,34). The observations that thickening occurs not only in diabetic humans but also in galactosemic rats suggested that aldose reductase was involved (33,35–37). That the glomerular polyol accumulation occurring in diabetic nephropathy, is prevented by treatment with an ARI (33) strongly suggests that aldose reductase is involved in the process of capillary basement membrane thickening in the kidney. Human glomerular microvessels exhibit aldose reductase activity (38), and ARIs are the only compounds known to inhibit capillary basement membrane thickening (39).

Our findings confirm those from animal models in which early diabetes-induced hemodynamic changes and increased GFR and UAER are aldose reductase–linked phenomena and are reversible by aldose reductase inhibition (20–22). In our patients, in fact, chronic tolrestat administration leads to a significant fall in UAER and GFR. That this effect is likely to be mediated by tolrestat administration is supported by the experimental evidence of insignificant changes in GFR and UAER in the placebo group. Concerning the suggested link between GFR reduction and aldose reductase inhibition, changes in protein intake and glycemic control might act as

confounding factors. In this study, protein intake and metabolic control were stable during the study. Therefore, the amelioration of glomerular permeaselectivity came without changes in diabetic control (Table 2) or RPF (Fig. 4), excluding the potential confounding effects of different blood glucose levels or renal vascular resistence levels on renal hemodynamics. A modulation of renal hemodynamics mediated by a direct effect of tolrestat may not be excluded. The possible role of hyperglycemia is suggested by evidence that GFR increased with glucose infusion in both normal subjects and IDDM patients (40), and that raised GFR and RPF were lowered in IDDM patients after glucose levels were normalized with acute (41) and chronic (42) insulin therapy. Furthermore, under conditions of hyperglycemia, both fructose and triose phosphates, more potent glycating agents than glucose, may play a major role in the glycation process. Fructose and other glycating agents are, in fact, formed in greater amounts than under normal conditions because of polyol pathway activation (43,44). Likewise, systematic confounding changes in other well-known GFR modulators, such as growth hormone and glucagon (45,46), are improbable. Finally, an increased production of vasodilatory prostaglandins (47,48) mediated by tolrestat was untenable. The mechanism by which tolrestat may mediate deleterious effects of diabetes on the kidney is unclear. Several hypotheses have been proposed to explain polyol-linked renal injury. One possibility is that the increased intracellular levels of sorbitol resulting from metabolism of excess serum glucose have hyperosmotic effects that lead (49) to cell damage. In addition, an increased polyol pathway causes intracellular *myo*-inositol depletion with a consequent decreased Na/K-ATPase activity. This enzyme is reduced in tissues that are sites of complications. This of course includes glomeruli. Such a reduction may be prevented by aldose reductase inhibition (50,51). The reduction in the albumin clearance also may result from the improvement in glomerular membrane selectivity because the filtration of this protein is restricted by both size and charge. Finally, it has been postulated that a decrease in the excretion of albumin could have occurred because of an enhanced reabsorption of these proteins. However, this explanation is unlikely because tubular reabsorption was probably saturated in these persistently proteinuric patients (52). Although the role of sorbitol accumulation in the pathogenesis of diabetic nephropathy remains speculative, note that enlargement of renal cortical podocytes, suggestive of intracellular edema, as well as abnormal cytoplasmic extensions of their cell membranes have been described frequently in diabetic glomerulosclerosis as well as in nondiabetic renal disease (53,54). Glomerular epithelial cells participate in basement membrane synthesis, and the proteinuria occurring in diabetes can be related to the morphological alterations in the epithelial foot processes (55). Note that the renal hypertrophy associated with galactosemia, which may be analogous to diabetic hypertrophy, is diminished with aldose reductase inhibition (11). The significantly reduced GFR in diabetic subjects treated with tolrestat compared with control subjects (Fig. 1), despite identical renal blood flow in both groups, is consistent with evidence that the increase in GFR is the consequence of imbalances between afferent and efferent arteriolar resistance, leading to increased transglomerular capillary hydraulic pressure rather than to increased blood flow per se (56). Based on these data, it may be excluded that lowered GFR was mediated by a drop in RPF, which remained unchanged during the entire study. Goldfort et al. (57) reported that increased GFR in diabetic rats was reduced by sorbinil and by diets supplemented with 1% *myo*-inositol and that sorbinil had no effect on GFR in control rats. Because *myo*-inositol–supplemented diets also normalize sciatic nerve *myo*-inositol levels, Na^+-K^+ ATPase activity, and functional sciatic nerve impairment in diabetic rats without affecting sorbitol levels (58), it may not be sorbitol levels per se but the metabolic imbalances resulting from increased glucose flux through the sorbitol pathway (including decreased intracellular *myo*-inositol levels) that impair vascular function (seen in the kidney by increased blood flow and GFR). This increase in renal blood flow is related to the degree of metabolic derangement and develops in the face of mild to moderate hyperglycemia (59). Moreover, they are prevented by insulin therapy, proportional to the degree of glycemic control achieved (60,61) and are ameliorated by restoration of euglycemia with islet transplantation (62). Recently, we have reported (63) that chronic tolrestat administration was able to reduce UAER in IDDM patients with early nephropathy. Our observation was confirmed and extended by Pedersen et al. (64). These authors reported a significant reduction in GFR in a group of hyperfiltering normoalbuminuric IDDM patients without changes in RPF, UAER, or renal vascular resistance, suggesting a possible pathophysiological role of increased polyol pathway activity in early hemodynamic changes in diabetic kidney function.

In conclusion, our findings show that early diabetes-induced hemodynamic changes and increased GFR and UAER are aldose reductase–linked phenomena, and long-term treatment with the ARI tolrestat is able to positively influence these parameters in diabetic patients with kidney disease. Based on these considerations and the results presented here, the possibility that the polyol pathway is pathogenetically linked to diabetic nephropathy and influences GFR and UAER is evident. Further studies are in progress to define a possible therapeutic role for tolrestat in diabetic nephropathy.

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