Augmented arginine uptake, through modulation of cationic amino acid transporter-1, increases GFR in diabetic rats

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Background. It is suggested that either arginine or its metabolites, nitric oxide and polyamines play a role in the renal hemodynamic alterations observed in the early stages of diabetes. Yet, the regulation of arginine transport in diabetic kidneys has never been studied.

Methods. Arginine uptake was determined in glomeruli harvested from control rats; diabetic rats (2 weeks following an intraperitoneal injection of streptozotocin, 60 mg/kg body weight); rats, 4 days following left nephrectomy (a nondiabetic model of hyperfiltration); diabetes + lysine (0.5% in the drinking water to attenuate arginine uptake); and control + lysine.

Results. Glomerular arginine transport was significantly increased in diabetic rats, but remained unchanged following uninephrectomy. Lysine abolished the increase in arginine uptake in diabetic rats but had no effect in controls. The increase in creatinine clearance observed in diabetes was completely abolished by lysine. Using reverse transcription-polymerase chain reaction (RT-PCR), Northern blotting, and immunohistochemistry, we found a significant increase in glomerular cationic amino acid transporter-1 (CAT-1) expression in diabetic animals, which was unaffected by lysine. When human endothelial cells were incubated with arginine end products no effect on arginine transport was observed. However, only in the presence of 0.5mmol/L sodium nitroprusside (SNP) an augmented steady-state CAT-1 mRNA was demonstrated by RT-PCR.

Conclusion. In a rat model of early diabetes, glomerular arginine uptake is elevated through modulation of CAT-1 expression, thus, contributing to the pathogenesis of hyperfiltration. Increased nitric oxide formation may play a role in this process.

Hyperfiltration secondary to renal vasodilatation is characteristic of early diabetes [1]. Cumulative evidence in the literature suggests that either arginine or its metabolites, namely, nitric oxide and polyamines play a role in the renal hemodynamic alterations observed in the early stages of diabetes. Excessive nitric oxide production in diabetic animals was previously documented and suggested to induce renal vasodilatation and increased glomerular filtration rate (GFR) [2–4]. In contrast, arginine administration prevented glomerular hyperfiltration in diabetic rats [5], and inhibition of renal ornithine decarboxylase activity (the rate-limiting step in polyamines biosynthesis) has been shown to prevent kidney hypertrophy in experimental diabetes [6]. Present knowledge about regulation of arginine transport in diabetic kidneys is fragmentary. Although renal tubular cells have the capacity to synthesize arginine, the cellular uptake route is believed to be of major importance [7, 8].

In the kidney, L-arginine transport is mediated by the y+ system carrier. Several transporters have been identified, and three transporters, termed cationic amino acid transporter-1, -2, and -3 (CAT-1, CAT-2, and CAT-3) exhibiting the y+ system properties have been cloned. System y+ is characterized by high affinity for cationic amino acids, saturability, sodium independence, stimulation of transport by substrate on the opposite (trans) side of the membrane, and inhibition of transport by substrate on the same (cis) side of the membrane [9–14]. CAT-1 is ubiquitously expressed, and appears to have a greater capacity than CAT-2 for transstimulation [15]. CAT-2 has been initially detected in activated murine macrophages and lymphocytes. CAT 2A, an alternately spliced transcript of CAT-2, encodes a kinetically distinct low affinity transporter, and is highly expressed in liver of adult rodents [15, 16]. CAT-3 expression was found to be present in adult brain and only recently in rat renal inner medullary collecting duct [17–19]. We have recently demonstrated that both glomerular and mesangial arginine transport systems are sodium independent, subjected to cis inhibition by lysine but not methionine, and constitutively express the two major arginine transporters, CAT-1 and CAT-2. These findings support the notion that arginine
uptake in renal tissue occurs largely via y+ system and that the above transporters play a major role in glomerular arginine traffic [20].

Our current experiments were designed to determine the effect of early diabetes on glomerular arginine uptake and on the expression of CAT-1 and CAT-2, and to elucidate whether these changes relate to glomerular hyperfiltration. In addition, we aimed to relate the changes observed in glomerular arginine transport in diabetes to the end products of arginine metabolism, namely, nitric oxide, agmatine, and polyamines.

METHODS

All animal experiments described in this study were conducted in accord with the protocol approved by the institutional committee on ethics in animal experiments. Studies were performed using male Wistar rats weighing 200 to 250 g. Animals were made diabetic by administration of streptozotocin (65 mg/kg intraperitoneally) (Sigma Chemical Co., St. Louis, MO, USA) suspended in sodium citrate buffer (pH 4.2). Blood glucose levels were determined using tail blood samples. Animals that exhibited blood glucose levels above 300 mg/dL were included in the studies. Diabetic rats were treated daily with protamine zinc insulin (PZI) insulin 2 to 4 IU in order to adjust blood glucose levels at approximately 350 mg/dL. Animals were allowed free access to regular rat chow and tap water. Experiments were performed 2 weeks following the administration of streptozotocin. Age-matched normal rats served as controls. Subsequently, rats were segregated into five groups: group 1, control, untreated rats; group 2, diabetes; group 3, uninephrectomy, in which animals underwent right nephrectomy and were allowed to recover 4 days, after which experiments were performed (this group served as a nondiabetic model of glomerular hyperfiltration); group 4, diabetes + lysine, in which diabetic rats were given L-lysine 0.5% in drinking water (starting from day 1) in order to attenuate arginine uptake; and group 5, control + lysine, in which untreated rats were given lysine as in group 4.

Measurement of GFR

Rats were anesthetized with Ketalar, 8 mg/100 g body weight, and xylazine, 0.25 mg/100 g body weight, intraperitoneally. The animals were placed on a servo-controlled heating table to maintain body temperature at 37°C. Tracheotomy was performed to facilitate free breathing. The right femoral artery was cannulated to obtain blood samples and monitor arterial pressure [HP 1290C Universal Quartz transducer (Tektronix, Inc., Beaverton, OR, USA) and a Mennen Med recorder (Clarence, NY, USA)]. The right jugular vein was cannulated for the infusion of isotonic NaCl-NaHCO3 containing 3 mg% creatinine. Maintenance solutions were administered at a rate of 1.5 mL/hour. Additional solution was infused to match urinary flow rate. The bladder was cannulated for urine collection. After completion of the surgical preparation, the animals were allowed to stabilize for 1 hour before clearance measurements were initiated.

Renal clearance experiments were conducted during three successive 20-minute periods. Arterial blood samples (160 μL) were withdrawn at the middle of each urine collection period. Creatinine clearances were calculated by standard equations.

Isolation of glomeruli

Additional animals were used for in vitro studies. Kidneys from all experimental groups were harvested, decapsulated, bisected, and the cortex was carefully dissected free. Glomeruli were prepared using a sieving technique. Cortices were minced to a fine paste with a razor blade and gently pressed through a 106 μm stainless steel sieve. The resulting material was suspended in HEPES buffer (KCl 5 mmol/L, CaCl2 0.9 mmol/L, MgCl2 1 mmol/L, D-glucose 5.6 mmol/L, HEPES 25 mmol/L, and NaCl 140 mmol/L), at 40°C, pH 7.4. The suspension was forced through a 20 gauge needle to decapsulate the glomeruli and then passed through a 75 μm sieve. Glomeruli which were trapped on the sieve were washed and pelletted by centrifugation at 1000 rpm for 1 minute. This was repeated three times. This fraction consisted of more than 95% glomeruli, the majority of which were decapsulated. The isolated glomeruli were used for RNA extraction and for arginine uptake measurements.

L-arginine uptake by freshly harvested glomeruli

Arginine uptake was determined essentially as described by Gazzola et al [21]. Glomerular suspensions from the various experimental groups were incubated and shaken for 10 minutes in HEPES buffer at pH 7.4, 37°C. L-[H3] arginine and L-arginine, in a final concentration of 1 mmol/L, were added to a total volume of 1 mL for additional 4 minutes. The duration of 4 minutes was chosen since it was within the linear portion of uptake curves (data not shown). Transport activity was terminated by rapidly washing the cells with ice-cold phosphate-buffered saline (PBS) buffer (four times, 2 mL/tube). The glomeruli where then dried and solubilized in 1 mL of 0.5% sodium dodecyl sulfate (SDS) in 0.5 N NaOH. Seven hundred microliters of the lysate were used to monitor radioactivity, by liquid scintillation spectrometry (Betamatic) (Kontron Instruments, Trappes, France). The remaining 300 μL were used for protein content determination by Lowry method. To correct for nonspecific uptake or cell membrane binding, glomeruli were incubated with 10 mmol/L unlabeled arginine in HEPES buffer, and the associated
radioactivity was subtracted from each data point. Results are expressed as mean ± SE of at least five different experiments.

**L-arginine uptake in endothelial cells**

Bone marrow-derived transformed endothelial cells (a generous gift from A. Eldor, Hematology Department, Tel Aviv Medical Center) were seeded onto 6-well plates (Corning Incorporated, Corning, NY, USA), at a density of 10^6 cells per well. When confluent, they were washed with 2 mL HEPES buffer, 37°C, pH 7.4. Sodium nitroprusside (SNP), agmatine, or putrescine (all from Sigma Chemical Co.), in varying concentrations (0.1 to 1 mmol/L), were added for 24 hours.

Cells were then washed and transport assay was performed by adding, 1 mL of L-[H^3] arginine and L-arginine, in a final concentration of 1 mmol/L, for 1 minute (within the linear portion of uptake curve). Transport assays were performed as described above.

**Analysis of mRNA levels for CAT-1 and CAT-2 by reverse transcription-polymerase chain reaction (RT-PCR)**

Total cellular RNA was extracted from glomeruli or endothelial cells following the method described by Chomczynski and Sacchi [22]. RT was carried out for 1 1/2 hours at 42°C, and PCR in 10 × buffer for a total of 35 cycles, each of 94°C for 1 minute, 60°C for 1 minute, 72°C for 2 minutes, and 7 minutes (final cycle).

The first pair of primers was designed to bind to a portion of the rat CAT-1 gene: forward 21-mer, 5′-GCCATCGTATCCTCCTCTG-3′ and reverse 21-mer, 5′-CCCTCCCTACCGGTATTTCAC-3′ expression. A second pair of primers, which hybridize to a sequence, common to both CAT-2 and CAT-2A were forward 24-mer, 5′-AACGTGTCTTTATGCTTGT-3′ and reverse 23-mer 5′-GGTGACCTGGGACTCGCTT-3′ [23]. A third pair of primers was designed to bind to a portion of the human CAT-1 gene: forward 21-mer, 5′-CCAGTACTTCCGACGAGTTAG-3′ and reverse 22-mer, 5′-CATCCACACAGCAAAACCGACC-3′.

Cells were then washed and transport assay was performed by adding, 1 mL of L-[H^3] arginine and L-arginine, in a final concentration of 1 mmol/L, for 1 minute (within the linear portion of uptake curve). Transport assays were performed as described above.

**Northern blot analysis**

CAT-1 mRNA level was determined by northern hybridization. Fifteen micrograms of total RNA were denatured and fractionated by size on 1.3% formaldehyde-agarose gel. RNA was transferred overnight, by capillary action, to a nylon membrane (Hybond-N, Amersham International, Buckinghamshire, UK), and cross-linked by short-wave ultraviolet illumination. Purified end products of CAT-1 and GAPDH cDNA (High Pure™ PCR Product Purification Kit) (Boehringer Mannheim, Mannheim, Germany) were used directly for radiolabeling with [32P] deoxycytidine triphosphate (dCTP) by a random primer labeling method (Random Primers DNA Labeling System Kit) (Gibco BRL, Gaithersburg, MD, USA). After prehybridization for 1 hour at 50°C, membranes were hybridized overnight at 50°C with the 32P-labeled cDNA. Membranes were sequentially washed twice in 1 × standard sodium citrate (SSC), 0.1% SDS for 15 minutes, at room temperature, once in 1 × SSC, 0.1% SDS for 15 minutes at 50°C, followed by 0.5 × SSC, 0.1% SDS at 55°C for 30 minutes, and then washed at high stringency in 0.1 × SSC, 0.1% SDS at 57°C for 15 minutes. Autoradiography was carried out with Kodak XAR film (Kodak, Rochester, NY, USA) for 24 to 48 hours at −80°C. Relative mRNA abundance was quantified by measuring the density of the exposed film with a densitometer (B.I.S. 202D) (Fuji, Tokyo, Japan). CAT-1 mRNA level was normalized to GAPDH mRNA and expressed in arbitrary units as the ratio of CAT-1 to GAPDH expression in three different experiments.

**Immunohistochemical analysis**

For immunostaining, renal tissues were fixed in 4% buffered formaldehyde for 24 hours and embedded in Paraplast (Oxford Labware, St. Louis, MO, USA). The paraffin blocks were cut and mounted on slides and processed by labeled streptavidin-biotin method using Histostain Plus Kit (Zymed Laboratories, San Francisco, CA, USA). Sections were then incubated for 30 minutes with 1:400 diluted rabbit polyclonal anti-CAT-1 antibody (synthesized by Dr Leitner, Weizmann Institute, Rehovot, Israel). A biotinylated secondary antibody was applied for 10 minutes, followed by incubation with horseradish peroxidase-conjugated streptavidin (HRP-SA) for 10 minutes. Slides were washed thoroughly with Optimax wash buffer (Biogenex, San Ramon, CA, USA) following each incubation. The immunoreaction was visualized by an HRP-based chromogen/substrate system (Liquid DAB Substrate Kit) (Zymed). Sections were then counterstained with Mayer’s hematoxylin, dehydrated in ascending ethanol concentrations, cleared in xylene, and mounted for microscopic examination.
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Fig. 1. Effects of glomerular arginine uptake in diabetic rats. (A) Sodium independence and cis-inhibition by lysine. Uptake of radiolabeled arginine (L-arginine) by freshly harvested glomeruli from diabetic animals in the presence of either 140 mmol/L sodium chloride (Na) or 140 mmol/L choline chloride and lysine or methionine 10 mmol/L. Data are presented as the mean ± SEM of four different experiments. *P < 0.05 vs. control. (B) Concentration dependence of L-arginine. Uptake of [3H]-arginine was measured for 4 minutes in freshly harvested glomeruli from diabetic rats over a range of concentrations (0 to 1 mmol/L). Data are presented as the mean ± SEM of four different experiments.

Fig. 2. Uptake of radiolabeled arginine ([3H] L-arginine) by freshly harvested glomeruli. (A) Uptake from controls (CTL), diabetes (D), uninephrectomy (Neph), diabetes + lysine (D + L), and control + lysine (CTL + L). Data are presented as the mean ± SEM of at least five different experiments. *P < 0.05 vs control. (B) Uptake from diabetic rats pretreated with different concentrations of lysine in the drinking water. Data are presented as the mean ± SEM of four different experiments *P < 0.05 vs. control.

Statistical analysis

Means ± SE one-way analysis of variance (ANOVA) for comparison between groups and Student t test between two groups were computed and used to assess statistical significance. P values < 0.05 were considered to be statistically significant.

RESULTS

Initially, we wished to explore the possibility that early diabetes changes the characteristics of y+ system, the predominant arginine uptake system in glomeruli. Figure 1A demonstrates that in glomeruli harvested from diabetic animals arginine transport system remains sodium independent. Excess concentration of lysine strongly inhibited L-arginine uptake while the neutral amino acid methionine was found to be a poor inhibitor. In addition, we characterized the kinetics of L-arginine transport in diabetic glomeruli by measuring saturable uptake of L-arginine (0 to 1 mmol/L). The plot of L-arginine uptake as a function of extracellular L-arginine concentration is shown in Figure 1. A high affinity transporter was found to be present with a K_m of 135 µmol/L and V_max of 14.5 nmol arginine/µg protein/4 minutes. These data establish that in glomeruli from diabetic rats system y+ remains the predominant arginine transport system and the kinetic properties of the system resemble those of CAT-1 and CAT-2.

The next set of experiments was designed to explore a possible effect of diabetes on glomerular y+ system. When compared to normal rats, diabetes induced a significant increase in glomerular arginine uptake. In contrast, we found that arginine traffic in rats subjected to uninephrectomy, a different model of glomerular hyperfiltration, remained unchanged (Fig. 2A). In order to study a possible relation between glomerular arginine transport and hyperfiltration, we performed experiments...
Regulation of CAT-1 and CAT-2 gene expression in diabetes

To determine whether the observed diabetes-induced changes in arginine uptake are associated with similar directional changes in mRNA levels for CAT family of transporters, total glomerular RNA was analyzed by RT-coupled PCR to amplify portions of CAT-1 and CAT-2. We identified cDNA encoding both CAT-1 and CAT-2 in glomeruli harvested from control rats. Diabetes significantly augmented the expression of CAT-1 mRNA while CAT-2 remained unchanged (Fig. 4). To further confirm this observation, Northern blotting was performed (Fig. 5). Diabetic rats exhibited a significant increase in steady-state glomerular CAT-1 mRNA level, which was unaffected by cotreatment with lysine (0.5%) in the drinking water. Lysine had no effect on glomerular CAT-1 mRNA in control rats.

Glomerular CAT-1 protein in diabetes

Immunohistochemical analysis with an anti-CAT-1 antibody was performed to elucidate the extent of CAT-1 protein in kidneys taken from our experimental groups. Figure 6 depicts a dramatically augmented staining in diabetes, predominantly detected in glomerular capillary endothelial cells. Similar findings were obtained in lysine-treated diabetic animals.

Effect of early diabetes on arginase I and arginase II mRNA expression

RT-PCR was utilized to examine the changes in arginase I and II in early stages of diabetes. The expression for both isoenzymes was significantly increased as compared to controls (Fig. 7).

Effect of L-arginine metabolites on CAT-1 mRNA

Since all three arginine metabolites-nitric oxide, agmatine, and polyamines have a potential for both
augmenting GFR and altering arginine uptake, we tested the effect of these substances on arginine uptake and CAT expression in endothelial cells. Incubating cells with increasing concentrations of either SNP, agmatine, or putrescine (0.1 to 1 mmol/L) had no effect on arginine uptake (data not shown). However, analyzing the level of CAT-1 mRNA in endothelial cells by RT-PCR revealed that 0.5 mmol/L of SNP induced a significant increase in CAT-1 expression while no effect was obtained with the effect of these substances on arginine uptake and CAT expression in endothelial cells. Incubating cells with increasing concentrations of either SNP, agmatine, or putrescine (0.1 to 1 mmol/L) had no effect on arginine uptake, while an effect was obtained with similar concentrations of either putrescine or agmatine (Fig. 8).

DISCUSSION

The current studies were designed to determine whether glomerular arginine transport is modulated in the early stages of diabetes and to define a possible consequence impact upon glomerular function. Three principal issues were addressed: (1) whether arginine uptake is altered in the diabetic kidney, and by which transporter/s, (2) do changes in arginine uptake observed in diabetes bear relationship to glomerular hyperfiltration, and (3) to try and define a possible mechanism.

We have demonstrated that diabetic rats exhibit augmented glomerular arginine uptake with characteristics corresponding to the y+ system. This phenomenon was associated with modulation of both CAT-1 mRNA and protein. In order to determine whether changes in glomerular filtration have a potential to affect arginine uptake, we measured arginine transport in rats subjected to uninephrectomy, as a different model of hyperfiltration. These animals exhibited normal arginine uptake, implying that increased intracellular arginine requirements are not essential for the development of hyperfiltration. Next, we wished to examine whether this latter finding plays a specific role in the pathogenesis of hyperfiltration in the early stage of diabetic nephropathy. Diabetic rats were given lysine in the drinking water in order to chronically attenuate arginine consumption by cells. We have chosen to use lysine instead of an arginine analogue to avoid any possible effect of such a substance on nitric oxide synthase (NOS) enzymes. Attenuating arginine uptake by chronic in vivo administration of lysine prevented the hyperfiltration in diabetic rats. These findings support the postulate that the increase in arginine uptake, observed in diabetes is related to glomerular function.

Interestingly, the effect of lysine was not associated with prevention of CAT-1 up-regulation, implying that augmentation of glomerular arginine uptake in diabetes is a primary event and is not a result of changes in glomerular hemodynamics. Indeed, increased arginine uptake by other tissues in diabetes has been reported previously.

Contreras et al [24] demonstrated that diabetes stimulates arginine transport in isolated rabbit gastric glands and Sobrevia et al [25], have shown that gestational diabetes is associated with activation of system y+ in human endothelial cells. To further consolidate our main findings, we studied the expression of arginase I and II, which activity may diminish intracellular arginine availability through metabolizing L-arginine into L-ornithine and urea. Up-regulation of these isoforms was suggested as a mechanism to prevent overproduction of nitric oxide [26]. We found increased expression of both isoforms supporting the observation of augmented intracellular arginine content in early diabetes.

Our data suggest that (1) early diabetes augments arginine uptake via modulation of CAT-1 and (2) increased glomerular arginine traffic by CAT-1 impacts glomerular hemodynamics. In contrast, Reyes et al [5], have shown that arginine administration ameliorated the development of hyperfiltration and proteinuria in diabetic rats. Their findings contradict our conclusions since providing excess arginine to diabetic glomeruli cannot be implicated in both enhancing and preventing glomerular hyperfiltration. These discrepancies may well reflect...
different phases of the disease: While we studied animals that were diabetic for only 2 weeks, their rats were kept for 14 weeks before experiments were performed. Alternatively, increased arginine uptake via modulation of a specific transporter, as found here, could alter intracellular arginine metabolism in a different manner than merely the effect of excess extracellular arginine.

The effect of early diabetes on arginine transport was largely mediated by modulation of CAT-1 expression. Could this finding enlighten us about the role of arginine in diabetic hyperfiltration? It has recently been appreciated, at least with regard to nitric oxide generation, that each arginine transporter serves a different NOS isoform. We have confirmed and extended these findings by demonstrating that in both sepsis and ischemic acute renal failure the induction of intact NOS (iNOS) is accompanied by up-regulation of CAT-2 [20, 27]. Furthermore, Nicholson et al [28] have recently reported that iNOS activity was reduced in macrophages from CAT-2 knockout mice. Taken together, one can assume that CAT-2 is the source of intracellular arginine, which is consumed by iNOS. CAT-1, on the other hand, has recently been shown to colocalize with endothelial NOS (eNOS) in the caveolar complex [29]. Such a complex has been suggested to serve as a mechanism for channeling of newly acquired extracellular arginine to eNOS for nitric oxide synthesis. Having illustrated CAT-1 protein to be almost restricted to the capillary endothelium lends additional support to this assumption. To our knowledge, these findings provide the first evidence that CAT-1 can be up-regulated in vivo. This leads us to speculate that excess arginine provided by CAT-1 in early diabetes is being used to increase activation of glomerular eNOS. Indeed, the increase in L-arginine uptake, observed in human umbilical vein
endothelial cells from patients with gestational diabetes, is inhibited by blocking NOS [25].

The possible role of nitric oxide in diabetic hyperfiltration was dealt in depth by numerous papers and further discussion is beyond the scope of the present study. In brief, high urinary levels of NO\textsubscript{2}/NO\textsubscript{3} and an exaggerated renal hemodynamic response to NOS antagonists were interpreted to reflect excessive nitric oxide generation, which results in diabetic hyperfiltration [2–4]. In contrast, decreased ex vivo generation of nitric oxide by glomeruli harvested from diabetic rats has been reported [30]. In addition, data regarding expression of various NOS isoforms is confusing [31, 32].

Nevertheless, it is always tempting to relate the hemodynamic changes in diabetes to nitric oxide. If increased glomerular arginine uptake in diabetes reflects a greater demand for the substrate by NOS, one could then speculate that increased nitric oxide generation would up-regulate CAT-1 in order to meet the increasing requirements for substrate. To test this hypothesis, we have incubated endothelial cells with increasing SNP, a spontaneous nitric oxide donor, as well as with two other arginine metabolites previously suggested to augment GFR: agmatine and putrescine (a representative of the polyamine family). None affected arginine transport velocity. Only SNP induced a significant increase in CAT-1 mRNA expression when administered in supraphysiologic concentrations. Therefore, at this juncture we are unable to provide solid evidence to support the hypothesis that arginine uptake is increased to compensate for increased nitric oxide generation. A plausible interpretation of our findings views the increase in renal arginine uptake as an effect of diabetes on amino acid metabolism, resulting in undesired augmented nitric oxide generation, causing impaired renal hemodynamics. Alternatively, arginine could induce hyperfiltration via a nitric oxide-independent mechanism yet to be discovered. Possible mechanisms could involve protein kinase C (PKC), which is activated in diabetes. Both PKC and CAT-1 are located in the caveola. CAT-1 protein contains three putative sites for phosphorylation by PKC. Recently, Krotora, Zharikov, and Block [33] demonstrated that classical isoforms of PKC are involved in regulation of CAT-1.
transport activity in pulmonary artery endothelial cells by altering the phosphorylation state of the transporter. Alternatively, Flores et al [34] have shown that D-glucose infusion activates arginine uptake in human umbilical vein endothelial cells, an effect which was mediated by stimulation of p42 and p44 mitogen-activated protein kinases [34].

CONCLUSION

Arginine uptake is increased in glomeruli of diabetic rats due to modulation of CAT-1 mRNA and protein and might play a role in the pathogenesis of hyperfiltration in the early stages of the disease. Increased nitric oxide formation may have a contributing role in this process.

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