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ORIGINAL ARTICLE

Effects of glutamine supplementation on kidney of diabetic rat

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Abstract Glutamine is the most important donor of NH_3 in kidney playing an important role in acid–base buffering system. Besides this effect, glutamine presents many other relevant functions in the whole body, such as a precursor of arginine in adult and neonates. In addition to these effects, some studies have shown that glutamine can potentiate renal disease. In the present study, the effect of short-term treatment (15 days) with glutamine on control and diabetic rats was investigated. Using biochemical, histological and molecular biology analysis from control and diabetic rats we verified that glutamine supplementation increase in pro-inflammatory interleukins (IL)-1β and IL-6 content in renal cortex and induce alteration in glomerular characteristics. This study showed that short-term treatment with glutamine in association with increased glucose levels could cause important alterations in glomerular morphology that may result in fast progression of kidney failure.

Keywords Kidney · Diabetes · Glutamine supplementation

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Introduction

Kidney glutamine metabolism plays an essential role for acid–base buffering in plasma (Gstraunthaler et al. 2000; Curthoys and Gstraunthaler 2000). Glutamine is the most important donor of NH_3 in the kidney through phosphate-dependent glutaminase activity that is increased by low pH (Gstraunthaler et al. 2000). Glutamate formed from glutamine is converted to 2-oxoglutarate, and then to succinate, fumarate, malate, oxaloacetate and phosphoenolpyruvate (or malate to pyruvate directly) that enters the gluconeogenesis pathway. Renal gluconeogenesis is especially important in conditions where blood concentration of ketone bodies is increased (acidosis), such as long periods of hypoglycemia and diabetes (Eid et al. 2006). Glucose produced by this pathway provides up to 25% of plasma glucose levels (Stumvoll et al. 1999).

High dietary intake of protein has been associated with increased circulating amino acids levels, glomerular hyperfiltration and hypertension and, consequently, glomerular extracellular matrix (ECM) expansion and progressive renal injury (Brenner et al. 1982; Zatz et al. 1985). High concentration of an amino acid mixture [10% Travasol mixed amino acid solution (Baxter, Deerfield, IL, USA)] and L-arginine, which increased individual amino acid concentrations by 1.5- to 6-fold and raised the osmolarity to 13.3 mOsM increased expression of glomerulosclerosis markers and mesangial cell (MC) proliferation in vitro (Meek et al. 2003).

The importance of glutamine for the synthesis of proteins of the ECM was also evaluated in cultured MCs (Singh and Crook 2000; Singh et al. 2001, 2003, 2007; Pithon-Curi et al. 2006). Glutamine at 2 mM elicited an increase in smooth muscle cell alpha-actin (alpha-SMA), alpha1-type IV collagen and fibronectin transcripts (by

19.0-, 16.7- and 4.3-fold, respectively) being accompanied by an increase (by 12-fold) in alpha-SMA stress fibers compared to cells grown in absence of glutamine. This implies that glutamine is required for expression of important proteins of the ECM in cultured MCs (Pithon-Curi et al. 2006). Recently we have demonstrated that high glutamine concentrations (2, 10, 15 and 20 mM) in combination with high glucose levels (25 mM) increase MC proliferation in vitro in a dose-dependent manner. This effect was associated with glutamine: fructose 6-phosphate amidotransferase (GFAT) activation and cAMP production (Lagranha et al. 2008). Under hyperglycemic conditions, a high proportion of fructose 6-phosphate is converted into glucosamine (GlcN) 6-phosphate by the action of GFAT, the first and the rate-limiting enzyme of the hexosamine biosynthetic pathway. This pathway is involved in the glucose induced mesangial production of transforming growth factor beta (TGF-β), leading to increased ECM formation (Kolm-Litty et al. 1998; Singh and Crook 2000). Glutamine plays an important role in this process by providing the amine group to fructose 6-phosphate for glucosamine 6-phosphate production (Singh and Crook 2000). These findings suggest that high glutamine load may activate the initial steps of glomerulosclerosis.

Alterations in plasma and intracellular amino acid levels have been described in chronic renal failure (Deferrari et al. 1985a, b) and nearly 45% of incident kidney failure occurs in diabetes (USRDS 2005). We postulated that glutamine may play a pro-inflammatory role in the initial steps of the pathogenesis of glomerular dysfunction in vivo. To investigate this proposition, control and diabetic rats were supplemented with glutamine for 2 weeks and the following parameters were measured: plasma and urine glucose levels, plasma creatinine, cholesterol, triacylglycerol, free fatty acid, glutamine levels, histological analysis of the renal cortex and expression of interleukins (IL)-1β and IL-6 genes in renal cortex.

Methods

Animals

Male Wistar rats, weighing 220 ± 20 g, obtained from the Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, were maintained at 23 ± 2°C under a cycle of 12-h light/12-h darkness, and were allowed free access to food and water. The animal ethical committee of the Institute of Biomedical Sciences approved the experimental procedure of this study.

Induction of diabetes

The experimental type 1 diabetes was induced by intravenous injection of 65 mg/kg b.w. streptozotocin (STZ) dissolved in citrate buffer (pH 4.2). Control rats were injected with buffer only. After 48 h of the STZ injection, the diabetic state was confirmed by blood glucose levels above 200 mg/dl estimated with the aid of a glucose meter (Accu-Chek Active®, Roche Diagnostics GmbH, Mannheim, Germany). Blood samples were obtained from the cut tip of the animal's tail.

Oral glutamine supplementation

An aqueous solution of glutamine (Gln), freshly prepared, was given by gavage once a day for 15 days. The daily dosage of 1 g glutamine per kilogram body weight was the same used by Shewchuk et al. (1997). Gln was dissolved in PBS and non-supplemented rats received PBS only.

Experimental procedure

The animals were divided into four groups: (1) control (C), injection of citrate buffer (vehicle of STZ) and supplemented with PBS (glutamine vehicle); (2) control plus glutamine (CGln), injection of citrate buffer and supplemented with Gln; (3) diabetic (D), injection of STZ and supplemented with PBS and (4) diabetic plus glutamine (DGln) rats, injected with STZ and supplemented with Gln. All animals were kept under similar conditions and were killed by decapitation without anesthesia always between 09:00 and 11:00 a.m. Blood was collected for metabolite measurements. Kidneys were collected for histological analysis.

Metabolite measurements

Blood and urine glucose levels were determined by utilizing the method of Barham and Trinder (1972) and blood creatinine levels were evaluated as described by Biggs and Cooper (1961) and Martinez and Doolan (1960). Blood cholesterol and triacylglycerol levels were measured as described by Pearson et al. (1952) and Fossati and Prencipe (1982), respectively. Plasma free fatty acid concentration was determined by an enzymatic colorimetric assay—NEFA C kit (Wako Chemical, Neuss, Germany) following the manufacturer's instructions. Blood glutamine levels were determined using the method described by Windmueller and Spaeth (1974) and Bernt and Bergmeyer (1974).

Histological analysis

Kidneys were fixed on 4% paraformaldehyde for 24 h at 4°C. After washing, the tissue was dehydrated and

embedded in Paraplast (Oxford, St Louis, MO, USA) at 56°C. Five-micron-thick sections were longitudinally cut and adhered to glass slides. After deparaffinization, samples were stained with Sirius red. The samples were examined by conventional light microscopy using a Nikon Eclipse E600 microscope (Nikon Eclipse E600, Nikon Corporation, Kanagawa, Japan). Images were captured using the Image Pro Plus software computer program (Ipwin 32, Media Cybernetics, L.P., MD, USA).

Glomerular geometry

The total area of glomeruli was determined using the Image Pro Plus software (Ipwin 32, Media Cybernetics, L.P., MD, USA) and the volume density of glomeruli was determined according to the equation: $v = \beta/K \times A_{\mathrm{T}}^{1.5}$ with $\beta = 1.382$, $K = 1$ and $A =$ total area (Schwarz et al. 1998; Amann et al. 2000). The glomerular cellularity was determined using a point counting method of 20 glomeruli per kidney at a magnification of 200×.

Indices of renal damage

The degree of sclerosis in the glomerular tuft was determined from Sirius-stained sections adopting the semi-quantitative scoring system proposed by El Nahas et al. (1991). Grade 0, normal glomeruli; grade 1, presence of mesangial expansion/thickening of the basement membrane; grade 2, mild/moderate segmental sclerosis involving less than 50% of the glomerular tuft; grade 3, diffuse glomerulosclerosis involving more than 50% of the tuft; grade 4, diffuse glomerulosclerosis with total tuft obliteration and collapse.

RNA extraction

Total RNA was obtained from 50 mg renal cortex by the guanidine isothiocyanate extraction method (Chomczynski and Sacchi 1987), using TRIzol® Reagent (Invitrogen Corporation, Carlsbad, CA, USA) that maintains the integrity of RNA while disrupting cells and dissolving cell components. Chloroform was added. After centrifugation, an aqueous phase with RNA was transferred to a fresh tube and precipitated by centrifugation with cold isopropyl alcohol (Chomczynski 1993). The RNA pellet was washed with 75% ethanol, dried in air, dissolved in RNase-free water and stored at −70°C. RNA was quantified by measuring absorbance at 260 nm. The ratio of absorptions at 260 vs 280 nm was calculated and used to assess the purity of DNA with respect to protein contamination, since protein tends to absorb at 280 nm.

Reverse transcription-polymerase chain reaction

Total RNA (1.5 µg) was treated with 1 U DNase I and inactivated with EDTA. cDNA was synthesized in 20 µl medium containing 10 mM dNTP Mix (dATP, dGTP, dCTP and dTTP), 0.1 M DTT and 200 U Superscript™ II RNase H − Reverse transcriptase (Invitrogen Corporation, Carlsbad, CA, USA).

Real-time polymerase chain reaction

Interleukin-1β and IL-6 gene expression was evaluated by real-time polymerase chain reaction (Higuchi et al. 1992) in a Rotor Gene 3000 (Corbett Research, Mortlake, Australia), using Platinum® SYBR® Green qPCR SuperMix UDG (Invitrogen Corporation, Carlsbad, CA, USA) that contains SYBR Green I as fluorescent dye. Quantification of gene expression was performed by $2^{-\Delta\Delta CT}$ (Livak and Schmittgen 2001; Pfaffl 2001), using tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta polypeptide, also known as phospholipase A_2 (YWHAZ) gene expression as inner control.

Biederman et al. (2004) verified that the best housekeeping gene for analyzing relative gene expression in glomeruli from diabetic rats or primary MCs chronically exposed to high glucose are β-actin and YWHAZ.

The sense and anti-sense sequences, annealing temperatures and fragment lengths of IL-1β, IL-6 and YWHAZ are shown in Table 1.

Table 1 The sequences of the primers, annealing temperature and fragment lengths of the genes studied

Gene	Primer	Annealing temperature (°C)	PCR fragment lengths (bp)
IL-1β (NM031512)	Sense: TGATGTTCCCATTAGACAGC	58	378
	Anti-sense: GAGGTGCTGATGTACCAGTT		
IL-6 (NM26744)	Sense: GAGCCCACCAGGAACGAAAG	49	303
	Anti-sense: TCTGACAGTGCATCATCGCTG		
YWHAZ (NM0130113)	Sense: AGAAGACGGAAGGTGCTGAG	56	249
	Anti-sense: GGTATGCTTGCTGTGACTGG		

Statistical analysis

All values are presented as mean ± SEM. One-way ANOVA was initially used to detect the presence of significant differences among the groups. Individual comparisons were performed using Bonferroni test. The level of significance was set at $P < 0.05$.

Results

As compared to control (C) and control supplemented with glutamine (CGln), STZ-induced diabetic rats (D) exhibited a significant increase in water and food intake, and significant reduction of body weight gain during the experimental period (Table 2). Blood glucose levels were marked elevated (by 5.9-fold) as also observed for urine glucose concentration (by 89.8-fold). Glutamine supplementation increased blood creatinine levels in diabetic rats (DGln) (Table 2). Plasma cholesterol and free fatty acid levels were not changed by either the diabetic state or glutamine supplementation. However, plasma triacylglycerol concentration was increased (by 2.6-fold) in the diabetic group as compared with control (Table 3). This change was abolished by glutamine supplementation.

A significant decrease (by 80%) of plasma glutamine concentration was found in the diabetic group, as compared with control. Dietary supplementation caused a significant increase (by 30%) of glutamine concentration in plasma of control rats (CGln group). Increased plasma levels of glutamine (by 3.2-fold) were also found in diabetic rats that received dietary supplementation as compared to diabetic rats. However, dietary supplementation was not able to increase the plasma glutamine levels in diabetic rats to the values observed in the control and CGln groups (Fig. 1).

The effect of glutamine supplementation on expression of the two important pro-inflammatory interleukins (IL-1β and IL-6) was examined. Renal cortex of the DGln group presented increased expression of IL-1β and IL-6 as compared with control, CGln and diabetic rats (Fig. 2a, b).

As observed by histological analysis, total area of the glomeruli was decreased (by 10%) in kidneys from CGln

Table 2 Food intake, water intake, body weight gain, blood glucose and creatinine levels and urine glucose levels in control, CGln, diabetic and DGln rats

Groups	Food intake (g/day)	Water intake (ml/day)	Body weight gain (g)	Blood glucose levels (mg/dl)	Blood creatinine levels (mg/dl)	Urine glucose levels (mg/dl)
Control	3.1 ± 0.3	7 ± 1[c]	103 ± 7[c]	95 ± 1[c]	0.8 ± 0.1[d]	8 ± 3[c]
CGln	3.3 ± 0.3	8 ± 1[d]	104 ± 5[c]	92 ± 3[c]	1.4 ± 0.5[e]	11 ± 3[c]
Diabetic	6.0 ± 0.4[a]	41 ± 4[a,c]	−13 ± 3[a]	562 ± 6[a]	1.9 ± 0.6	719 ± 21[a]
DGln	4.7 ± 0.3[b]	24 ± 2[a]	−23 ± 8[a]	568 ± 21[a]	3.5 ± 0.1	665 ± 63[a]

Rats were rendered diabetic by streptozotocin injection (65 mg/kg, i.v.) and the measurements were carried out 2 weeks later. Aqueous solution of glutamine was administered by gavage during the experimental period. Values are presented as mean ± SEM from at least four animals per group

[a] $P < 0.001$ vs control and CGln

[b] $P < 0.05$ vs control and CGln

[c] $P < 0.001$ vs DGln

[d] $P < 0.01$ vs DGln

[e] $P < 0.05$ vs DGln

Table 3 Plasma cholesterol, triacylglycerol and free fatty acid levels in control, CGln, diabetic and DGln rats

Groups	Cholesterol (mg/dl)	Triacylglycerol (mg/dl)	Free fatty acids (mEq/l)
Control	94 ± 3	49 ± 8	0.50 ± 0.04
CGln	99 ± 5	52 ± 10	0.48 ± 0.11
Diabetic	104 ± 11	128 ± 8[a,b]	0.44 ± 0.06
DGln	98 ± 4	69 ± 8	0.43 ± 0.03

Rats were rendered diabetic by streptozotocin injection (65 mg/kg, i.v.) and the measurements were carried out 2 weeks later. Aqueous solution of glutamine was administered by gavage during the experimental period. Values are presented as mean ± SEM from five animals per group

[a] $P < 0.001$ vs control and CGln

[b] $P < 0.01$ vs DGln

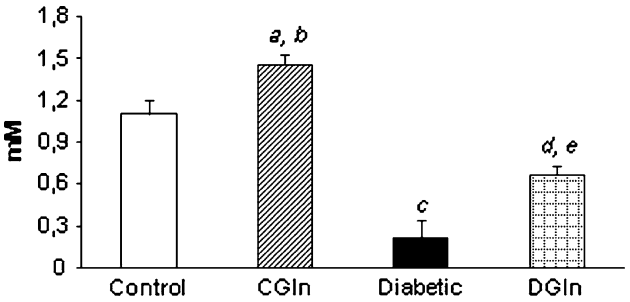

Fig. 1 Blood glutamine levels in control, CGln, diabetic and DGln rats. The values are presented as mean ± SEM of two determinations from at least four animals per group. [a]$P < 0.01$ vs control, [b]$P < 0.0001$ vs diabetic and DGln, [c]$P < 0.01$ vs control, [d]$P < 0.01$ vs control and diabetic, [e]$P < 0.0001$ vs CGln

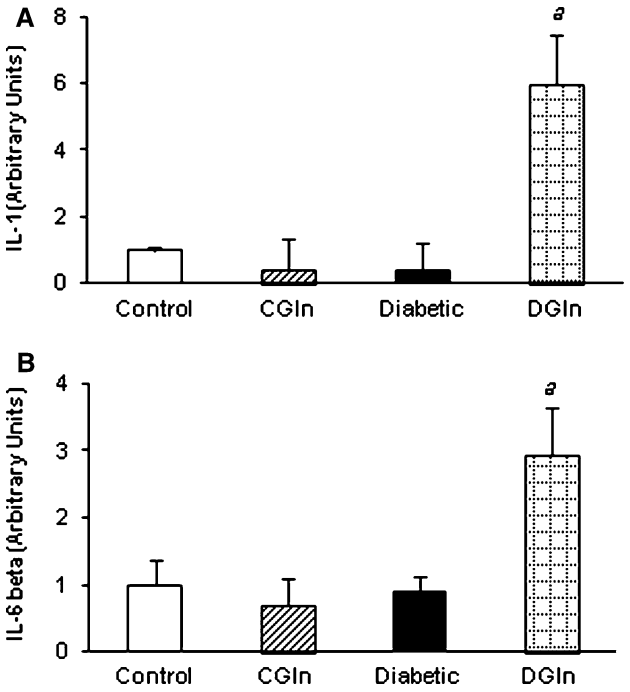

Fig. 2 Expression of IL-1 beta (**a**) and IL-6 (**b**) genes was evaluated by real-time PCR levels in kidneys from control, CGln, diabetic and DGln rats. The values are relative to control and are presented as mean ± SEM from at least four animals in each group. [a]$P < 0.001$ vs control, CGln and diabetic

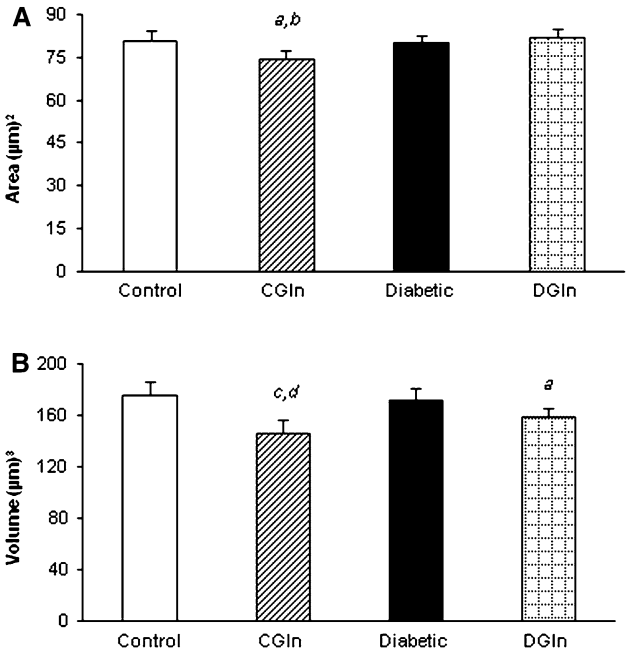

Fig. 3 Glomerular area (**a**) and glomerular volume (**b**) in kidneys from control, CGln, diabetic and DGln rats. The values are presented as mean ± SEM from at least five animals per group. [a]$P < 0.05$ vs control, [b]$P < 0.01$ vs DGln, [c]$P < 0.001$ vs control and [d]$P < 0.01$ vs diabetic

rats as compared with the C, D and DGln groups (Fig. 3a). Volume of the glomeruli from CGln and DGln was decreased (by 15 and 11%, respectively) as compared with glomeruli from C and D rats (Fig. 3b). The histological analysis showed that the glomerular morphology was modified by both the diabetic state and glutamine supplementation. Glomeruli of the CGln group presented irregular capillaries and modest Bowman's capsule attachment to glomerular wall (Fig. 4b) as compared to C rats (Fig. 4a). Glomeruli of diabetic rats presented irregular capillaries and modest MC proliferation (Fig. 4c), however, glutamine supplementation promoted modest attachment of Bowman's capsule and MC proliferation in diabetic rats (Fig. 4d).

As observed by the analysis of renal damage index, kidneys from glutamine supplemented diabetic rats revealed a moderate increase of mild segmental sclerosis involving less than 50% of the glomerular tuft (grade 2: 0.70 ± 0.09) as compared to the control (grade 0: 0.34 ± 0.06) and CGln (grade 0: 0.31 ± 0.03) groups, the values as shown as mean ± SEM of 20 determinations per kidney from five animals each group. Glomeruli of diabetic rats presented mesangial expansion of the basement membrane and the renal damage index was 0.50 ± 0.11 (grade 1). As mentioned above, kidneys from DGln rats exhibited features of glomerulosclerosis (Fig. 5a), as increased mesangial matrix and adhesions to Bowman's capsule. On the other hand, glomerulus from kidney of DGln rats, which did not present indications of glomerulosclerosis, exhibited signals of glomerulonephritis, such as decreased cellularity in mesangial areas (Fig. 5b).

Discussion

Glutamine supplementation in normoglycemic individuals may not induce or potentiate renal cortex injury. However,

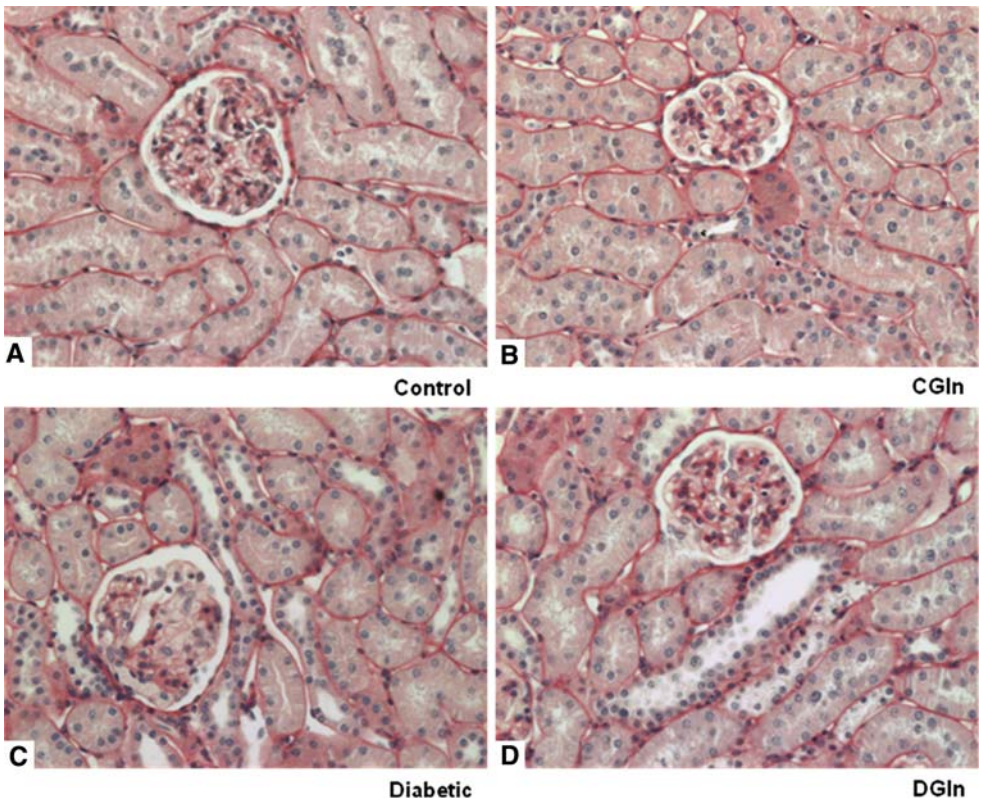

Fig. 4 Glomerular morphology analyzed by light microscopy (Sirius red stain, magnification × 200): **a** glomerulus from control rats, **b** glomerulus from CGln rats, **c** glomerulus from diabetic rats, **d** glomerulus from DGln rats

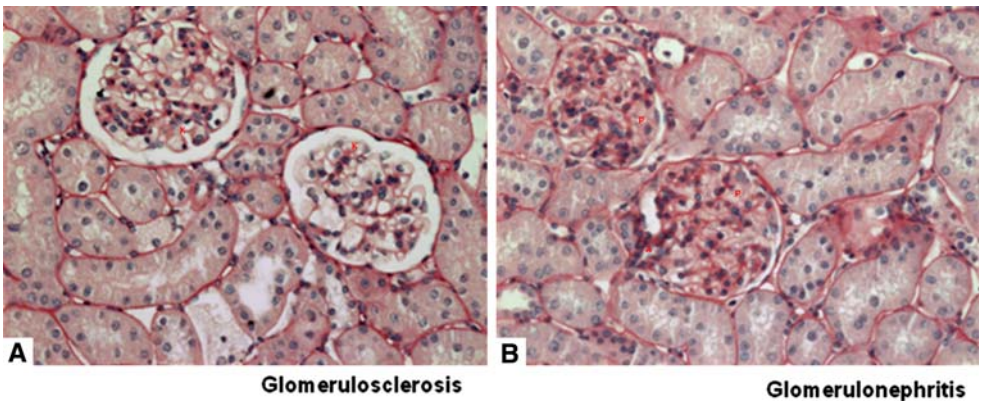

Fig. 5 *Micrographs* showing pathological changes in glomeruli from DGln kidneys. a Diabetic glomerulosclerosis. There is a decreased cellularity in mesangial areas, increased matrix acellular Kimmelstiel–Wilson nodules or nodular diabetic glomerulosclerosis, often with compressed mesangial cell nuclei pushed to cell periphery (K). **b** "Diabetic glomerulonephritis". There is a segmental proliferation in the glomerular tuft (P), largely as a result of increase in size and number of mesangial cells; that is accompanied by an increase in acellular mesangial material. Sometimes abnormal segments of the tuft adhere to Bowman's capsule to form a tuft adhesion (a). Glomerular morphology as analyzed by light microscopy (Sirius red stain, magnification × 200)

in subjects presenting increased plasma levels of glucose (i.e., diabetic patients) glutamine supplementation may potentiate the development of renal injuries. In our study, the association of high blood glucose levels and glutamine supplementation was responsible by the decrease of food intake by rats, consequently, decrease in body weight gain and triacylglycerol levels was observed. Moreover, glutamine supplementation promoted decrease water intake by diabetic rats that is an indicative of renal function alteration.

In fact, glucosamine and hexosamine pathways (HP) are involved in the development of diabetic nephropathy

(Masson et al. 2005). Glucosamine is widely used to mimic HP activation by high glucose because of its ability to readily enter the cells by the glucose transport system and the HP, bypassing the rate-limiting enzyme, GFAT. Masson et al. (2005) demonstrated that glucosamine (0.5 mM) treatment significantly decreases proliferation and induces hypertrophy of MCs by an increase of total protein/cell number ratio. The same group observed by flow cytometry that glucosamine raises the proportion of MCs at the G0/G1 cell cycle phase by flow cytometry, suggesting a blockade of the cell-cycle progression at this phase (Masson et al. 2006). Kolm-Litty et al. (1998) and Weigert et al. (2003) verified that glucosamine treatment and HP activation induce TGF-β1 expression and subsequent ECM protein synthesis in MCs.

High glutamine concentrations (2, 10, 15 and 20 mM) in combination with high glucose (25 mM) levels increased MC proliferation in vitro. This effect was associated with GFAT activation and increase of cAMP levels (Lagranha et al. 2008), which might play a role in the initial steps of the glomerulosclerosis as observed in kidneys from diabetic rats supplemented with glutamine. Glutamine is the most abundant amino acid found in plasma (Williamson and Brosnan 1974) and its concentration is maintained in a narrow range even under catabolic conditions (Curi et al. 2005). In the present study, a significant decrease of plasma glutamine levels was found in diabetic rats that were not fully recovered even under glutamine supplementation.

Various pro-inflammatory interleukins (such as IL-1, IL-6 and IL-8) are synthesized and secreted by MCs (Coleman and Ruef 1992; Sedor et al. 1992). In turn, IL-1 and IL-6 affect phenotype of cultured MCs (Abbott et al. 1990; Ruef et al. 1990; Sedor et al. 1992). MC-derived IL-1β exerts autocrine stimulatory effects on production of eicosanoids, collagen and cytokines such as IL-1, IL-6 and IL-8 (Horii et al. 1989; Abbott et al. 1990; Ruef et al. 1990; Coleman and Ruef 1992). IL-6 induces matrix protein transcription and autocrine growth of MCs in vitro (Horii et al. 1989; Ruef et al. 1990; Zoja et al. 1993). High expression of glomerular IL-6 has been reported in human glomerulonephritis, being observed in IgA nephropathy and some types of lupus nephritis, characterized by mesangial hypercellularity (Fukatsu et al. 1993; Horii et al. 1993; Waldherr et al. 1993; Yoshioka et al. 1993; Malide et al. 1995). In the present study, over expression of IL-1β and IL-6 was found in renal cortex from glutamine supplemented diabetic rats. This finding indicates the occurrence of an inflammatory process as observed in glomerulonephritis. Several studies in renal tissue from patients and experimental animals with glomerulonephritis have shown the high quantity of mRNA IL-1 and IL-6 or production of these cytokines by MCs (Horii et al. 1993; Sedor et al. 1992, 1993). Prostaglandins and leukotrienes may also contribute to this inflammatory process. In fact, the content of linoleic and arachidonic acids, precursors of lipidic inflammatory mediators, was increased in renal cortex from diabetic and DGln rats as compared to controls (data not shown).

Sassy-Prigent et al. (2000) and Gross et al. (2004) observed higher glomerular volume and mesangial expansion associated with glomerular hypertrophy in STZ-diabetic as compared to controls. In our study, kidneys from diabetic rats showed irregular capillaries but there was no alteration in glomerular volume. Supplementation of diabetic rats with glutamine promoted an alteration in glomerular volume. This feature is associated with glomerular hypertrophy and, consequently, glomerulosclerosis. IL-1β mRNA synthesis was increased in kidneys from glutamine supplemented diabetic rats. This cytokine is involved in the synthesis of type IV collagen and adhesion molecules that mediate macrophage tissue infiltration, important features of glomerulosclerosis (Sassy-Prigent et al. 2000).

The mesangial changes have been recognized as the main cause of declining renal function in diabetes nephropathy (Steffes et al. 1989). The expansion of mesangial matrix impinges on glomerular capillaries, reducing the surface available for filtration and narrowing or occluding the lumen. Impairment of glomerular function has been associated with the extent of these changes in both types of diabetes (Osterby et al. 2001, 2002). In our study, kidneys from diabetic rats presented higher index of renal damage (grade 1) than control and control glutamine (grade 0), being more pronounced when diabetic rats were supplemented with glutamine (grade 2). Blood creatinine levels were not different between control and diabetic rats as also observed by Gross et al. (2004). However, glutamine supplementation promoted an increase of plasma creatinine levels in diabetic rats that indicates the occurrence of renal damage.

Mesangial cells exposed to high glucose levels revealed increased expression of genes that potentially play a role in the development of glomerulosclerosis, including TGF-beta and connective tissue growth factor (Murphy et al. 1999; Wahab et al. 2001). This factor upregulates transcription of several matrix proteins and represses that of matrix metalloproteinases (Davies et al. 1990). These events lead to the establishment of glomerulosclerosis in vivo (Mason and Wahab 2003).

The development of glomerulosclerosis lesion is a consequence of systemic hypertension and glomerular capillary hypertension, hyperfusion and hyperfiltration (Hostetter et al. 1981; Anderson et al. 1986), hyperlipidemia (Kasiske et al. 1988; Diamond and Karnovsky 1998), mesangial dysfunction (Grond et al. 1984, 1985), genetic factors (Weening et al. 1986), glomerular hypertrophy (Yoshida et al. 1989) and altered glomerular permeability

to macromolecules (Remuzzi and Bertani 1990). Previous studies have shown that glutamine increase ammonia production and excretion (Matthews and Campbell 1992). Increased ammoniagenesis is associated with changes in the activities of a number of proteins, including the apical membrane Na^+/H^+ antiporter, glutaminase, glutamate dehydrogenase and phosphoenolpyruvate carboxykinase, which may affect intracellular substrate availability for protein turnover and gluconeogenesis (Curthoys and Watford 1995). In MCs, ammonia causes deposition of ECM and hypertrophy (Ling et al. 1998). In our study, analysis of kidneys from DGln revealed morphological alteration related with glomerulonephritis and glomerulosclerosis suggesting that glutamine not only induce metabolic alterations as shown previously but also morphological alterations. Carstens et al. (1982) verified that diabetic patients who present glomerulopathy and advanced renal insufficiency show glomerulonephritis superimposed with glomerulosclerosis. The same association was observed by Mazzucco et al. (1985), Monga et al. (1989) and Lui et al. (2002).

The results of this study suggest that high glutamine intake may increase the risk of chronic kidney disease. However, there is undeniable evidence that glutamine supplementation is beneficial when the endogenous supply of this amino acid is insufficient. Nevertheless, there is a need for increased awareness regarding the risks associated with high dietary glutamine supplementation especially among those who are diabetic or have transiently higher blood glucose levels.

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Conflict of interest The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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