

Renal physiopathologic changes in diabetic Golden-Syrian hamsters (*Mesocricetus auratus*) fed with hypercaloric diet

Cambios fisiopatológicos renales en hámster sirio-dorado (*Mesocricetus auratus*) diabéticos alimentados con dieta hipercalórica

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ABSTRACT:

Background: Diabetic nephropathy is the single major cause of end stage renal failure. The increase of visceral adipose tissue may lead to glomerular hypertrophy and chronic kidney disease. Our objective was to determine renal changes in diabetic Golden-Syrian Hamster (*Mesocricetus auratus*) supplemented with a hypercaloric diet. **Methods:** One group of animals (n = 10) was fed with a standard diet (SD), and the other group (n = 10) was fed with a hypercaloric diet (HCD) for 1 month. Afterwards, both groups were treated with three doses of Streptozotocin. Hyperglycemia was determined throughout 73 d. The animal's weight, blood and kidney tissues were obtained for analysis. **Results:** Diabetic animals fed with HCD diet manifested hyperglycemia (250 - 350 mg/dL) with significant weight loss (40 g), and an important glomerular filtration rate decrement (0.491 mL/min). Regarding renal fibrosis, all animals showed an increase of glomerular, interstitial, and cortical extracellular matrix (36.3, 75.2 and 70.7 %, respectively). Diabetic animals that were SD-fed showed only mild hyperglycemia and slight increase of glomerular, interstitial, and cortical extracellular matrix. A group of animals (n = 5), fed exclusively with HCD, was also included in the study. **Conclusions:** Our finding suggests that HCD feeding can accelerate the progression of chronic kidney disease in a diabetic condition.

Keywords: Diabetic, Nephropathy, Hypercaloric diet, Histopathologic, Fibrosis.

RESUMEN

Antecedentes: la nefropatía diabética es la principal causa de insuficiencia renal terminal. El aumento del tejido adiposo visceral puede provocar hipertrofia glomerular y enfermedad renal crónica. El objetivo es determinar los cambios renales en hámster sirio dorado (*Mesocricetus auratus*) diabéticos suplementados con una dieta hipercalórica. **Métodos:** Un

grupo de animales (n = 10) alimentado con dieta estándar (SD) y otro (n = 10) con dieta hipercalórica (HCD) durante 1 mes. Posteriormente, ambos grupos fueron tratados con tres dosis de Estreptozotocina. La hiperglucemia se determinó durante 73 días. Se obtuvo el peso de los animales, sangre y tejido renal. **Resultados:** Los animales diabéticos alimentados con HCD manifestaron hiperglucemia (250-350 mg/dL) con pérdida de peso significativa (40 g), disminución del filtrado glomerular (0.491 mL/min) y aumento de la matriz extracelular glomerular, intersticial y cortical (36.3, 75.2 y 70.7 %, respectivamente). Los animales diabéticos que fueron alimentados con SD mostraron sólo una hiperglucemia leve y un ligero aumento de la matriz extracelular glomerular, intersticial y cortical. Un grupo de animales (n = 5) alimentados exclusivamente con HCD fue incluido en el estudio. **Conclusiones:** Nuestro hallazgo sugiere que la alimentación con HCD puede acelerar la progresión de la enfermedad renal crónica en una condición diabética.

Palabras clave: Diabético, Nefropatía, Dieta hipercalórica, Histopatológico, Fibrosis.

INTRODUCTION

End-stage renal disease (ESRD) has become the most common cause of diabetes, also known as diabetic nephropathy (DN), in Western countries. It remains an important clinical problem with substantial medical comorbidity (Kalantar-Zadeh *et al.*, 2021). The high incidence and prevalence of ESRD has a rapid impact on global health, and represent a health care burden; it also disproportionately affects low- and middle-income countries (Thurlow *et al.*, 2021). Demographics of ESRD diabetic patients indicate that the populations at highest risk are women, African American, Hispanic American, Asian American, Native American, and Pacific Islanders (Bleyer *et al.*, 2008).

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Received: August 16, 2024

Accepted: October 25, 2024

Published: November 27, 2024

The symptoms of DN are characterized by proteinuria, kidney hypertrophy, increased urinary albumin excretion, decreased kidney function, glomerulosclerosis, and accumulation of extracellular matrix proteins in the tubular-interstitial zone, which eventually results in ESRD (Uil *et al.*, 2018). In particular, urinary albumin is associated with histopathologic features such as progressive mesangial expansion. It is mainly due to the accumulation of type IV collagen, laminin, fibronectin, proteoglycans and other proteins, in the extracellular matrix (ECM). Albuminuria progress ensues in glomerulosclerosis, arteriolar hyalinosis, and tubulointerstitial fibrosis development. These pathologic features correlate to the glomerular filtration rate (GFR) in humans with diabetes and kidney disease (Dizin *et al.*, 2013; Breyer *et al.*, 2005).

There are several factors that lead to diabetes mellitus. For instance, pharmacology, diet, or more recently, SARS-CoV-2 (COVID-19) can trigger said disease. The use of antibiotics during childhood has been recently associated with pancreatic islet alterations, as well as modulating insulin secretion and the proliferation of β -cells. Likewise, antibiotic treatment in prenatal mice results in gut microbiome changes such as adiposity and hepatic metabolism of lipid and cholesterol (Cox *et al.*, 2014; Li *et al.*, 2009). Based on this information, the use of antibiotics can be related to diabetic conditions and kidney damage through increases in adiposity. Furthermore, a key factor in the development of diabetic renal injury is diet. Similar to humans, hamsters were hyper-responsive to increasing cholesterol levels (Zhang *et al.*, 2009). Adding to this, carbohydrates in the diet can induce changes in both glucose and lipid metabolism. Hence, carbohydrate-rich diets may increase the risk of the development of chronic kidney disease (CKD) in non-diabetic subjects (Nam *et al.*, 2019; Elsisy *et al.*, 2021). High-fat-high-carbohydrate diet induces signs of early and mild podocyte stress in animals, a characteristic of renal damage during metabolic syndrome and obesity (Seikrit *et al.*, 2021).

MATERIAL AND METHODS

Experimental design

Twenty-five male Golden-Syrian Hamsters (*Mesocricetus auratus*), weighing approximately 140 g each, were used divided into 3 groups. All of the subjects were housed in a room with a 12/12-h light/dark cycle, and an ambient temperature between 22 °C and 25 °C under specific pathogen-free conditions. The experiment took place in the Bioterium located at the *Centro Universitario de Ciencias de la Salud* (CUCS), from the *Universidad de Guadalajara*. The first group of hamsters (10 animals) was fed a standard diet (SD), consisting (as a percentage of total kcal) of 12% fat, 60% carbohydrates, and 28% protein. The second group (10 animals) was fed a hypercaloric diet (HCD), which consisted of the SD enriched with 1% cholesterol, 10% coconut oil, and 15% sugar. All animals (SD and HCD groups) were fed for one month. All of the subjects were previously induced to diabetes by an intraperitoneal injection with streptozotocin (STZ, Sigma-Aldrich) in sodium-citrate buffer (0.2M, pH 4.5), once a day for 3 con-

secutive days at a dose of 50, 40 and 40 mg/kg, respectively (0.3 mg/g approximately). Hamsters had free access to food, water and continued on their original diets for the duration of the study. A group HCD fed (without STZ-treatment) was included as control. After four months, the animals were anesthetized with Ketamine (65 mg/kg) and Xylazine (7 mg/kg) in order to then be sacrificed for biological sample obtaining (Figure 1). All experiments were conducted according to the internationally accepted principles for the care and use of laboratory animals, and the guidelines of the Animal Research Reporting of in vivo Experiments (ARRIVE). It is worth mentioning that the researchers have experience handling laboratory animals and were trained through Internal Committee for the Care and Use of Laboratory Animals (CICUAL) courses and respecting the Official Standard of Technical Specifications for the Production, Care and Use of Laboratory Animals (NOM-062-ZOO-1999).

Urine and blood examination

The blood glucose levels were monitored using the One-touch Ultra glucose monitoring system (Lifescan *Johnson & Johnson* Company), and hamsters were considered to be diabetic when three consecutive blood glucose determinations resulted in 200 mg/dL or above. To perform a glucose curve, glucose measurements were taken on days 3, 8, 16, 22, 29, 49, 58 and 73 after the last STZ-administration.

Urine samples were collected twenty-four hours before death from each animal (in order to determine 24-h urinary volumes) by placing the hamsters in a previously cleaned metabolic cage. In addition, approximately 10 mL of blood were obtained from cardiac puncture. Uric acid, total proteins, creatinine, albumin, urea and sodium (Na), potassium (K), and chlorine (Cl) electrolytes in blood and/or urine, were measured following standard methods in an instrument of analysis (Shyncron CX9). The GFR was determined following the equation: $GFR = (uCr \cdot uV) / (sCr) [13, 14]$.

Histopathologic analysis

Kidneys were quickly removed, set in 10 % phosphate-buffered formalin solution and then embedded in paraffin. Kidneys were cut into semithin sections of 4 - 5 μ m. The sections were stained with hematoxylin and eosin (H&E) and

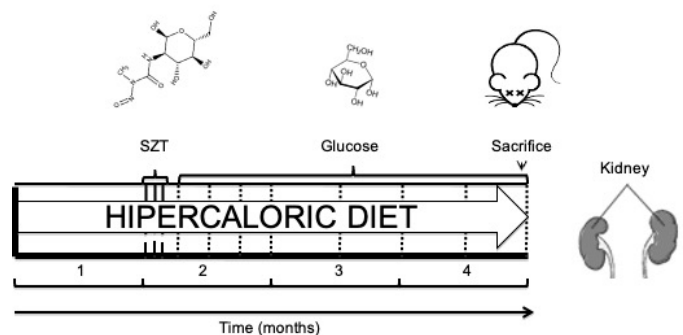


Figura 1. Diseño experimental.

Figure 1. Experimental design.

Masson's trichrome stain (HYCEL SA of CV, México). A single pathologist, unaware of the experimental protocol, analyzed all of the kidney's slides using a light microscope.

The extent of renal injury was assessed by a histopathologic analysis of the glomerular sclerosis and interstitial alterations (tubular and renal atrophy replaced by fibrous connective tissue), in representative renal tissue of each experimental group. The glomerulosclerosis, renal tubule damage (tubular atrophy), and interstitial fibrosis are cataloged in five groups, between 0 and 4. Glomerulosclerosis, renal tubule damage (tubular atrophy), and interstitial fibrosis are categorized into five groups, from 0 to 4. A score of 0 indicates the absence of damage; a score of 1 represents less than 25 % of minor damage; a score of 2 corresponds to approximately 25 % to less than 50 % of damage, around the median. A score of 3 denotes about 50 % to 75 % of damage (approximately two-thirds of the area), and a score of 4 indicates damage present throughout the entire area (> 75 %) (Vázquez-Méndez *et al.*, 2020).

Fibrosis determination

Masson's trichrome-stained renal slides from each animal were analyzed, in particular measuring the area, the diameter and the fibrosis of glomeruli. The damage of renal tissue was evaluated in a computational system of image analysis, using an Olympus BX51 microscope equipped with a DP71 camera, and Image-ProPlus 6.3 software from Media Cybernetics. From each group of animals ($n = 4$), 50 glomeruli were analyzed in 20 random fields (magnification 40X) of tissue sections.

Total collagen was tested by measuring the concentration of hydroxyproline as described by Rojkind *et al.* (1974). Briefly, renal tissue was hydrolyzed with 6N HCl at 110°C for 24 hours. Samples were incubated with a chloramine-T buffer for 10 m at room temperature. Ehrlich's reagent was added, and the samples were again incubated for 45 minutes at 65 °C. Absorbance of each sample was measured at 560 nm using a spectrophotometer (Thermo Scientific, Biomate 3, USA). Collagen accumulation was expressed as percent of collagen in each experimental group.

Statistical analysis

The data are expressed as mean \pm SD. Analysis through the t-Student test was used to compare the differences between groups. Values of $P < 0.05$ were considered significant.

RESULTS

Hyperglycemia

Approximately 95 % of hamsters that receive HCD+STZ, manifested severe hyperglycemia (plasma glucose level of 250-350 mg/dL throughout all of the experiment) as shown in figure 2A. Approximately 30 % of the animals, which exhibited significant weight loss (40 g) and dehydration, as shown in Figure 2B, were humanely euthanized by cervical dislocation, performed by trained personnel in accordance with ethical guidelines to prevent further suffering. Death was confirmed

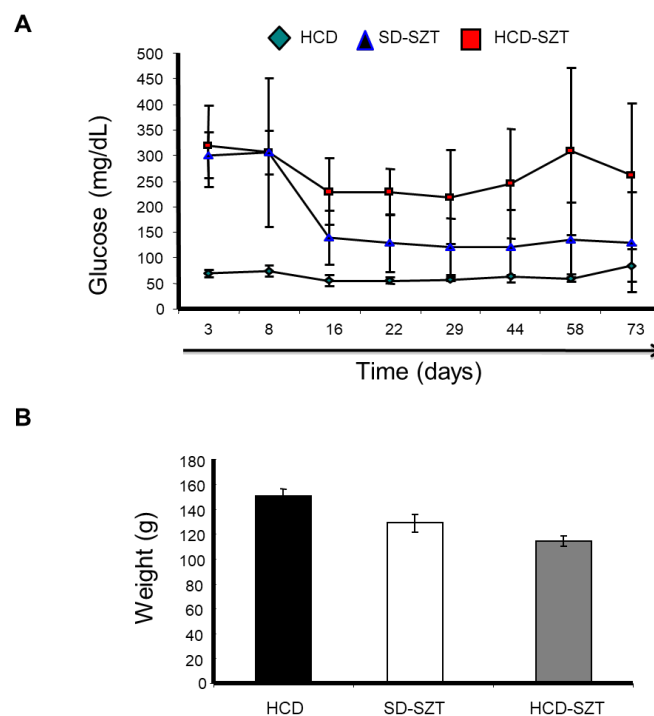


Figure 2. Determinaciones de glucosa y peso.

Figure 2. Glucose and weight determinations. A) shows a constant elevation of glucose concentration (mg/dL) observed in HCD-STZ group (line with red-box). B) an important decrement in weight (g) of animals HCD fed plus STZ treated. The results are presented as means \pm SD, with $p < 0.05$ indicating statistical significance.

by the absence of reflexes and cardiac activity. The hamsters that received SD+STZ showed only mild hyperglycemia (140-160 mg/dL). The undernourishment and dehydration were not as evident, but said animals still presented moderate weight loss (18 g) when compared to the former group. The remaining hamsters that were only HCD fed did not present hyperglycemia, undernourishment nor dehydration.

Biochemical analysis

The biochemical analysis demonstrated that the serum uric acid increased 28.7 % and 73 % respectively in both animal groups, the SD group, and the HCD that was treated with STZ. In urine, uric acid only decreased (61%) in diabetic animals induced by STZ that were fed with HCD. Urea in serum is normal in all 3 groups, but urine increased significantly (138 %) in diabetic animals induced with STZ plus HCD (in STZ plus SD, the group increase was 27 %). Serum creatinine (Cr) concentrations increased slightly at the end of experiment in both animal groups that were treated with STZ. Moreover, uric Cr significantly decreased (78%) in the experimental group treated with STZ plus HCD, while in the STZ + SD group, it decreased 31%. All of this data indicates a tendency consistent with chronic and progressive renal failure. Total proteins experienced no changes (urine or serum) in the 3 groups. Serum albumin decreased significantly (8%) in the animals which received STZ + HCD and increased (31%) in urine (Table 1). In the electrolytes analysis related to urine,

Table 1. Análisis bioquímico en cada grupo experimental.

Table 1. Biochemical analysis in each experimental group. Table 1A show concentration (mg/dL) of uric acid, total proteins, albumin, urea and creatinine (urine and serum) in each experimental group. Table B, show the Na, K and Cl electrolytes levels (mM/L) in serum and urine in each treatment. The results are presented as means \pm standard deviation (SD). Differences are considered statistically significant when $p < 0.05$. Statistically significant results are marked with an asterisk (*).

Table A

Group	Uric Acid (mg/dL)		Total Proteins (g/dL)		Albumin (mg/dL)		Urea (mg/dL)		Creatinine (mg/dL)	
	Serum	Urine	Serum	Urine	Serum	Urine	Serum	Urine	Serum	Urine
HCD	1.5 \pm 0.61*	10.8 \pm 2.42*	5.1 \pm 0.59	0.1 \pm 0.08	2.2 \pm 0.28	156.5 \pm 106.4	32.7 \pm 3.2	261.2 \pm 111	0.45 \pm 0.06	143.33 \pm 28.8
SD + STZ	1.9 \pm 0.54	11.2 \pm 4.42*	5.33 \pm 0.33	0.1 \pm 0.14	2.3 \pm 0.10*	152.2 \pm 33.19	35 \pm 1.2	333.7 \pm 169	0.46 \pm 0.13	103.26 \pm 46.4
HCD + STZ	2.6 \pm 0.71*	4.2 \pm 4.8*	5.2 \pm 0.57	0.1 \pm 0.10	2.1 \pm 0.07*	205.6 \pm 60.6	35.5 \pm 4.9	621 \pm 313.6*	0.61 \pm 0.36	32.87 \pm 22.2*

Table B

Group	Na (mM/L)		K (mM/L)		Cl (mM/L)	
	Serum	Urine	Serum	Urine	Serum	Urine
HCD	132.5 \pm 14.7	137.8 \pm 50.6	6.4 \pm 0.5*	241.6 \pm 9.9*	91.8 \pm 9.6	114.1 \pm 83.9
SD + STZ	145.4 \pm 0.94	107 \pm 60.6	6.2 \pm 0.4*	190.7 \pm 56.7*	101.2 \pm 1.2	58.24 \pm 46.5
HCD + STZ	140.4 \pm 7.2	107.5 \pm 47.2	7.7 \pm 0.6*	83.2 \pm 39.7*	100.9 \pm 2.8*	0 \pm 0

sodium decreased in both STZ-treated groups, while in serum no significant changes were present. In serum, potassium showed an increase of 20 % exclusively in the animals that received STZ + SD. While in urine, the potassium decreased significantly to 66 % in this same group (the other group treated with STZ decreased as well, but in less proportion - 21 %). In serum, chlorine increased in both STZ-treated groups to 10 %. Nevertheless, in urine, these same groups significantly decreased the concentration to 50 % for STZ + SD and 100 % for STZ+HCD.

Renal function

GFR was calculated with respect to renal creatinine clearance, in 24-h urine collection (Figure 3A). A single measurement of serum creatinine was done, as indicated by the Cockcroft-Gault equation. As shown in Figure 3B, the GFR in HCD and in SD plus STZ groups was similar (0.671 and 0.672 mL/min, respectively), but in diabetic animals, HCD fed, the GFR decreased significantly, around 30 % (0.491 mL/min).

Histopathologic analysis

The glomerular tissue damage was determined to traverse histomorphometric changes of glomeruli, characterized by ECM accumulation in the intra- and extra-glomerular space. This alters the circular shape, whose evolution can progress to functional atrophies or Kimmelstiel-Wilson lesion (Islas Andrade and Revilla Monsalve, 2005), and in addition, presents extravasations even in the Bowman's space. The interstitial matrix was determined by the presence or absence of vacuolization-glycogen drops (Armani-Ebstein changes), which have been related to hyper-glucose (Islas Andrade and Revilla Monsalve, 2005). The animals of the HCD group presented enlargement of the capillary wall, mesangial-intercellular expansion, decrement of the space in the Bowman's capsule, tubule interstitial vacuolization, loss of luminal area, initial

changes in the presence of ECM proteins, and an increment of inter-tubular space, as shown in Table 2. Animals of the SD-STZ group showed similar characteristics, though more prominent, alongside glomerular atrophy. Notably, animals that received SD plus STZ demonstrated an exacerbation of significantly aggressive features and atrophy with extrusion into Bowman's space. Furthermore, significant evidence of glomerulosclerosis, tubular atrophy, and interstitial fibrosis was observed after feeding animals with HCD-STZ, as shown in Table 3. At the end of the experiments, the score of glomerulosclerosis, tubular atrophy, and interstitial fibrosis was 1 in

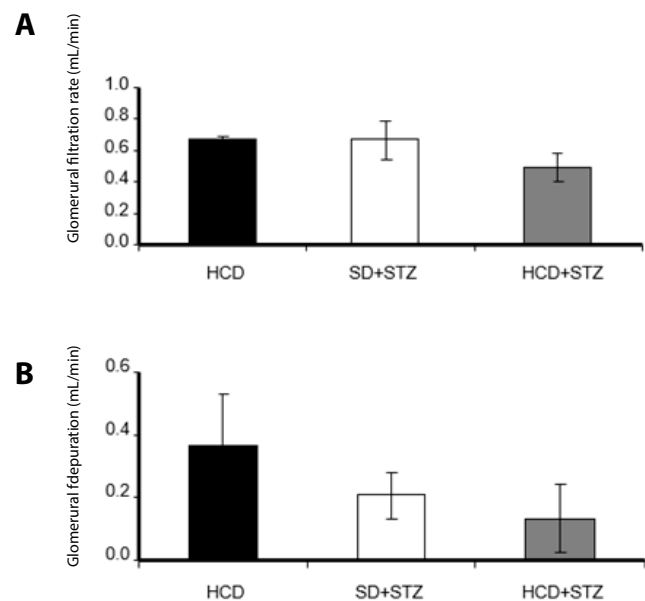
**Figure 3.** Análisis de función renal.

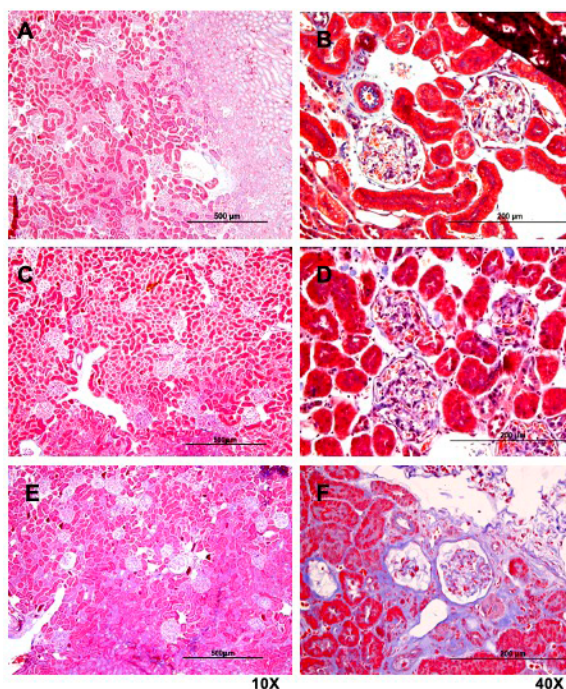
Figure 3. Renal function analysis. A) represent creatinine depuration in HCD, standard diet plus streptozotocin (SD-STZ) and hypercaloric diet plus streptozotocin (HCD-STZ) animals; B) indicate the glomerular filtration rate in each experimental group. The results are represented as mean \pm SD, in mL/min.



CHARACTERISTICS	HCD	SD-STZ	HCD-STZ
Thickening of capillary wall	✓	✓	✓
Intercellular-mesangial expansion	✓	✓	✓
Decrease in space in Bowman's capsule	✓	✓	✓
Tubulo-interstitial vacuolization	✓	✓	✓
Loss of the luminal area	✓	✓	✓
Increment of ECM proteins	✓	✓	✓
Increase in inter-tubular spaces	✓	✓	✓
Glomerular atrophy		✓	✓
Atrophy with extrusion into Bowman's space			✓

Tabla 2. Análisis histopatológico.

Table 2. Histopathological evaluation. The renal characteristics in each experimental group are indicated by the ✓ presence and the damage intensity it represented by its size.



Characteristic	HCD	SD-STZ	HCD-STZ
Glomerular sclerosis	1	2	3
Tubular atrophy	1	1	1
Interstitial fibrosis	1	1	2
Total	3	4	6

Tabla 3. Índice de daño renal.

Table 3. Kidney damage rate. The group of HCD-STZ animals displayed an exacerbated damage assessed by increments of glomerular sclerosis, interstitial fibrosis, and tubular atrophy. A total of 4 cortical fields per animal were analyzed.

the HCD group; glomerulosclerosis (2), tubular atrophy, and interstitial fibrosis (1) in the SD group. This suggests that, by the end of the experiments, renal scarring was moderate to severe in both groups. Nevertheless, in the HCD-STZ group, glomerulosclerosis and interstitial fibrosis (3 and 2 scores, respectively) were exacerbated.

Fibrosis determinations

Renal fibrosis evaluated by a morphometric analysis and hydroxyproline biochemical determinations, demonstrated a significant increase only in the animals that receive HCD+STZ, as much in glomeruli, interstice, and crust (36.3, 75.2 and 70.7 %, respectively) (Figure 4 E, F). The SD+STZ group (Figure 4 C, D) presented a slight increase of glomerular, interstitial, and cortical extracellular matrix (1.7, 12.5 and 11.2 %, respectively) with respect to HCD group (Figure 4 A, B). The progression of collagen deposition in kidney tissue was also confirmed by the measurement of tissue hydroxyproline content, that progressively increased in the HCD+STZ group. Results obtained with histological analysis and hydroxyproline determinations showed structural changes in renal tissue during the development of kidney fibrosis.

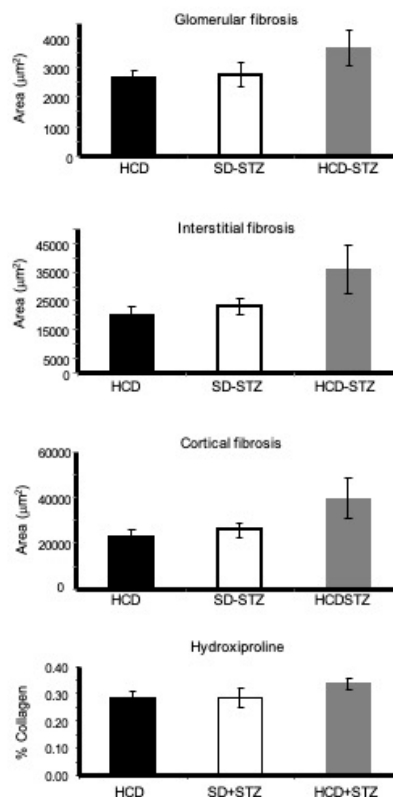


Figura 4. Determinación de fibrosis.

Figure 4. Fibrosis determination. Representative images of renal tissue Masson trichrome stained in each experimental group, where A and B correspond to HCD, C and D for SD-STZ and E and F to HCD-STZ animal groups. The panels of graphics indicate fibrosis determination (μm^2) by computational morphometry in glomerular, interstitial, and cortical regions represented as mean \pm SD in each group of animals, similarly, the percentage of collagen proteins (hydroxyproline) is shown.

DISCUSSION

End-stage renal disease is one of the most prevalent and irreversible complications of hypertension, diabetes, and inherent renal diseases; it affects a significant number of people worldwide (Al-Rajhi and Al Salmi, 2020). The incidence of ESRD is increasing substantially, which, as a result, means the impact of said condition and its treatment has widespread consequences for healthcare services, individuals, and society (Al-Rajhi and Al Salmi, 2020). ESRD causes significant biochemical abnormalities leading to symptom groups such as the uremic syndrome, which negatively impacts an individual's quality of life. In this renal disease, the progressive accumulation of ECM in the glomeruli (glomerulosclerosis) and between tubules (tubule-interstitial fibrosis) are the final manifestations of chronic kidney disease (Cho, 2010).

Streptozotocin (STZ), an antibiotic derived from *Streptomyces achromogenes*, has significant antimicrobial action for a wide spectrum of organisms. Furthermore, toxicology studies in dogs and rhesus monkeys demonstrated that STZ had a potent diabetogenic effect (Mariee *et al.*, 2009). On the other hand, rodents have been a preferred model for the study of diabetic pathophysiology because of their similarities to humans. In this regard, hamsters exhibit symptoms of diabetes that are similar to those in humans (Sweta *et al.*, 2018).

In this experimental design, it was demonstrated that the administration of HCD+STZ in Golden Sirius Hamster (*Mesocricetus auratus*) increases the blood-glucose concentration when compared to animals fed with a normal diet plus STZ administration. However, Islam *et al.* (2009) stated that long-term high fat, either alone or in combination with STZ, induces constant hyperglycemia. Tesch and Allen (2007) proposed that the severity of injury is dependent on the genetic background of the species. The HCD+STZ group also had polyuria and loss of weight, probably due to the hydroelectric imbalance of organisms.

The excess of fluid dissolved substances can lead to an increase in the amount of urine produced by the kidneys. It also depends on the filtering capacity of the kidney; when there are renal tubules, it may be unable to reabsorb the filtered blood, which determines an increase in the amount of urine produced. Other studies reported factors that have been associated with renal disease, such as serum uric acid (Hovind *et al.*, 2009). In this particular article, an increment in serum uric acid and diminution in urine is reported. Nevertheless, the role of uric acid in the development of diabetic nephropathy is not yet completely understood. More studies in rats indicated that the main injuries from uric acid increase are glomerulosclerosis, interstitial fibrosis, and arteriolar disease. The mechanism of the lesion appears to be related to the development of preglomerular arteriolar disease, which impairs the renal autoregulatory response and thereby causes glomerular hypertension (Feig *et al.*, 2008). More recently, epidemiologic studies suggest that elevated uric acid levels are an independent predictor of the development of microalbuminuria (Feig *et al.*, 2008; Lee *et al.*, 2006). Also, increased blood urea nitrogen and creatinine in diabetic rats indicate

progressive renal damage, which is taken as an index of altered GFR in diabetic nephropathy (Breyer *et al.*, 2005; Kuhad *et al.*, 2009). In the findings section of this article, the non-treated animals did not show an increase in the serum levels of urea nitrogen, in comparison with the STZ-plus treated animal where the urea significantly elevated.

Proteinuria is an indicator of glomerular damage and may be used as a measure for diabetic glomerulopathy. In diabetic nephropathy, it is strongly associated with pathological changes of diffusion and, less commonly, with the nodular form of diabetic glomerulosclerosis. The results presented in this article did not show changes in the levels of total proteins, and proteinuria is also viewed as a hemodynamic promoter of the progression of the disease in diabetic nephropathy (Williams, 2005). Urine albumin secretion is considered to be one of the most sensitive markers of renal injury. Results indicate a significant increase in albuminuria and a decrease in serum albumin in HCD+STZ. Reports by Lassila *et al.* (2004) also show that STZ-treated mice (C57BL/6) hyperlipidemic ApoE deficient, present an increase in the secretion of urine albumin (Lassila *et al.*, 2004).

Oh *et al.* (2007) suggest that these hemodynamic changes are related to altered sodium balance, which might be associated with hemodynamic changes, responsible for the progression of diabetic nephropathy. This research found decreased urinary sodium in both groups with treatment plus diet, and not diet. Mapanga *et al.* (2009) reported that rats with STZ-induced diabetic nephropathy exhibited weekly decreases in urinary sodium excretion. As such, it is suggested that the increase of GFR possibly expanded the filtered load of sodium in the tubular filtrate, hence facilitating urinary sodium excretion. Furthermore, the elevated GFR observed in diabetic patients is the result of an increase in glomerular capillary surface area, plus an additional mechanism altering one or more of the other determinants of GFR. Currently, said other mechanism is considered to be a reduced tubuloglomerular feedback signal (Mapanga *et al.*, 2009).

The diabetic kidney displays a primary increase in proximal tubular fluid and an electrolyte reabsorption, thereby presenting a reduced concentration of Na^+ , K^+ and Cl^- to the macula densa cells of the juxtaglomerular apparatus. The resultant decreased uptake of these ions by these cells elicits an increase in the GFR of that nephron via a reduction in vascular tone, predominantly of the afferent arteriole (Singer, 2007). Similarly, the K^+ and Cl^- concentrations decreased in STZ-treated groups, but they were lower in the animals that received STZ plus the special diet. In serum, a significant increment of Cl^- was observed in both STZ-treated groups, and K^+ only increased in the STZ-treated plus high-fat diet fed group.

Renal function was assessed by measuring plasma and urine levels of creatinine. It has also been observed that increased serum creatinine in diabetic rats indicates progressive renal damage (Breyer *et al.*, 2005), which is taken as an index of altered GFR in diabetic nephropathy. In this experiment, we reported a significant decrease of urinary

creatinine but a moderate elevation of serum creatinine in the HCD-STZ animals. The same animal group showed a significant decrement of creatinine depuration and alteration in the glomerular filtration rate. Popov *et al.* (2003) reported that hamsters with HCD presented augmented creatinine concentrations related to disturbances of the renal function. Said concentrations progress to nodular glomerulosclerosis and nephropathy after 20 weeks of diet (Popov *et al.*, 2003). Yu *et al.* (2022) indicated that a high-fat diet in mice impairs renal function and increases proteinuria and proinflammatory cytokines by Wnt/ β -catenin signaling induction. Similarly, Laurentius *et al.* (2019) stated that obesity induced by a high-fat diet in aging Long-Evans rats causes damage in renal structures due to an inflammatory microenvironment. Seikrit *et al.* (2021) reported that a hypercaloric diet induces podocyte damage and microinflammation in aged, non-diabetic rats for five months. Sugyeong *et al.* similarly observed that a high-fat diet increased protease-activating receptor 2 (PAR2) levels in the renal tubule epithelial region with increased inflammatory responses, oxidative stress, and fibrosis (Ha *et al.*, 2022). However, Elsisy *et al.* (2021) suggested that a high-fructose diet induces more severe kidney damage in rats during 4 weeks than a high-fat diet within the same timeframe.

The results of this article showed that the histopathological features of capillary wall thickening, intercellular-mesangial expansion, decreased Bowman's space, increased ECM proteins, and glomerular atrophy with extrusion into Bowman's space are more aggressive in the HCD+STZ group. Similar characteristics were reported by Popov *et al.* (2023) in Golden Syrian hamsters with a high-fat diet for 20 weeks. Some of the main attributes are that the glomerular basal membrane (GBM) was modified, and the thickness of the GBM gradually increased. In addition, after 4 weeks of a high-fat diet, the thickened GBM developed focal enlargements and nodules that apparently compress and diminish the vascular capillary lumen. After 20 weeks, the hamsters with the high-fat diet showed that the mesangial area had increased (Popov *et al.*, 2003).

The pathogenesis of chronic renal disease is characterized by a progressive decline of renal function and continuous accumulation of ECM. This in turn leads to diffuse fibrosis, probably by undergoing endothelial-myofibroblast transition in endothelial cells (Li *et al.*, 2009; Efstratiadis *et al.*, 2009). Renal scarring was assessed and blinded for this study. It is reported that HCD+STZ fed animals were cataloged in > 50 % of glomerular sclerosis, and between 20 % - 50 % of interstitial fibrosis.

Finally, it is demonstrated that all animals with HCD+STZ show an increment of glomerular, interstitial, and cortical ECM (Figure 5), when compared to the control group of only HCD. To corroborate the increment of renal fibrosis, a slight increase of collagen (hydroxyproline) was found only in the HCD+STZ group. In the pathogenesis of tubulointerstitial fibrosis, many cytokines and chemokines participated, particularly in the activation of the myofibroblast. One of said factors is the transforming growth factor- β 1 (TGF- β 1) and

platelet-derived growth factor (PDGF) (Lane *et al.*, 2002). The second part of our investigation seeks to analyze the physiopathology of the experimental model of diabetic nephropathy at the molecular level.

CONCLUSIONS

This study conclusively demonstrates that the administration of STZ in Golden Syrian hamsters, combined with HCD, induces a significantly more severe metabolic and renal profile compared to a SD. The HCD+STZ group not only exhibited severe hyperglycemia but also experienced higher mortality, pronounced elevation of uric acid, and renal deterioration evidenced by reduced glomerular filtration rate and marked histological changes such as glomerulosclerosis and interstitial fibrosis. These findings suggest that the combination of an HCD in diabetes exacerbates metabolic and renal complications, highlighting the critical importance of dietary factors in the progression of renal disease in diabetic models. This research provides significant evidence on the impact of diet in the development and worsening of diabetes-associated pathologies, which could have important implications for the clinical management of the disease in humans.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest in this work.

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Renal physiopathologic changes in diabetic Golden-Syrian hamsters (*Mesocricetus auratus*) fed with hypercaloric diet
Cambios fisiopatológicos renales en hámster sirio-dorado (*Mesocricetus auratus*) diabéticos alimentados con dieta hipercalórica

Biotecnia
vol. 26, e2420, 2024
Universidad de Sonora, División de Ciencias Biológicas y de la Salud,
ISSN-E: 1665-1456

DOI: <https://doi.org/10.18633/biotecnia.v26.2420>