The present study was performed on 3-, 5-, 7- and 9-week-old HFD female rats compared with the appropriate gender and age-matched animals. We evaluated the kidney expression of angiotensin type II receptor and fibrotic and epithelial-to-mesenchymal transition (EMT) markers, by immunoblotting and immunohistochemical and histological techniques, in parallel with kidney function.

Results. In the current study, the time-course HFD-treated group showed, by immunoblotting and immunohistochemical analysis, an early time-course increase in the expression of transforming growth factor β-1 (TGFβ-1) in the entire kidney of HFD-treated rats, compared with that observed in the control group. Simultaneously, the study shows a transient increase in the expression of ZEB2 in the HFD whole kidney accompanied by a fall in the E-cadherin expression and increased collagen and fibronectin deposition. A pronounced decrease in fractional urinary sodium excretion was also demonstrated in the long-term HFD-treated rats. The decreased \( \text{FEN}_\text{Na}^- \) was accompanied by a fall in \( \text{FEP}_\text{Na}^- \) and \( \text{FEPP}_\text{Na}^- \), which occurred in association with significantly decreased \( C\text{Cr} \) and, certainly on the sodium-filtered load. The reduction in the glomerular filtration rate (GFR) occurred in parallel to proteinuria and glomerular desmin overexpression.

Conclusions. The results of the current study suggest that podocyte injury in parallel with observed proteinuria and evidence of EMT transformation are associated with long-term loss of kidney function and renal sodium and water retention.

INTRODUCTION

A worldwide epidemiological study recently showed that nearly 1460 million adults are overweight [body mass index (BMI) >25] and 502 million were considered obese (BMI >30) [1]. Obesity, a public health problem of the first order for industrialized and non-industrialized countries, dramatically causes a reduction in overall life expectancy [2]. The prevalence of obesity in different populations is mainly related to environmental factors, such as reduced physical activity and diet. Nowadays, with growing industrialization and modern lifestyle, the access to high fat and carbohydrate diets has changed the eating habits of the population. These aspects interact with genetic factors, which may explain the excessive body fat throughout the world [3]. The obesity and high-fat diet (HFD) intake are related to an increased prevalence of cardiovascular disease, dyslipidaemia, type 2 diabetes mellitus,
Kidney disorders and HFD intake

Alzheimer’s disease, chronic kidney disease and certain cancers [4–6]. Wang et al. [7] correlated, for the first time, the consumption of a HFD with decreased glomerular filtration rate (GFR), increased blood pressure, increased concentrations of plasma creatinine and enhanced tubular reabsorption of sodium. An early hypothesis to delineate the lipid nephrotoxicity was made by Moorhead et al. [8], proposing that dyslipidaemia may contribute to the progression of renal disease. Recently, an animal study using normotensive and spontaneously hypertensive rats showed that a HFD causes changes in kidney function, beginning with an inflammatory response and followed by endothelial dysfunction, rupture of the renal filtration barrier [9] and proteinuria [10].

However, most of the pathophysiology mechanisms andhumoral factors involved in excessive fat ingestion and obesity in kidney disorders remain not well known. Transforming growth factor-β (TGF-β) is a key regulator of extracellular matrix (ECM) protein deposition in renal tissue [11]. Increased expression of TGF-β mRNA in podocytes and glomeruli ECM protein deposition has been associated with focal segmental glomerulosclerosis (FSGS) [12], membranous nephropathy [13] and diabetic nephropathy [14]. Renal TGF-β1 expression is implicated in the pathogenesis of fibrosis in both glomerular and interstitial compartments [15]. Additionally, TGFβ-1 inhibits the expression of ZEB1/2, which are widely recognized E-box repressors of genes such as E-cadherin [16–18]. It is clear that a well-described phenomenon of epithelial-to-mesenchymal transition (EMT) type II plays a pivotal role in the progression of organ fibrosis [19]. EMT describes phenotypic changes in epithelial cells that lose their defined cell-cell and cell–basement membrane contacts and their structural polarity to become spindle-shaped and morphologically similar to mesenchymal/myofibroblast cells [20, 21].

Also, the classical vasoactive view of angiotensin II (Ang II) action has been extended due to its properties as cytokine that actively participates in the renal physiopathology [22–24]. In this manner, it has been demonstrated that Ang II stimulates the proliferation of kidney fibroblasts in culture and increases the expression of mRNA encoding TGFβ, fibronectin and collagen type I (3225). Subsequently, TGFβ may stimulate the accumulation of matrix and inflammation [24–28]. In the current study, we hypothesized that long-term HFD treatment of female rats could induce in a time-dependent fashion molecular pathway interactions involved in the kidney disorder process that may result in the loss of organ function and early occurrence of chronic kidney disease. Thus, the present study was performed in 3-, 5-, 7- and 9-week-old HFD female rats compared with gender and age-matched animals evaluating the kidney expression of Ang II receptor type I (AT1), fibrotic and EMT markers in parallel with kidney function.

Materials and Methods

Animals and diets

The experiments were approved by the Ethical Committee for Experimental Research at the Institute of Biosciences (371-CEUA) at São Paulo State University. The general guidelines established by the Brazilian College of Animal Experimentation were followed throughout the investigation. The assays were conducted on age-matched, female offspring of sibling-mated Wistar Hannover rats (250–300 g), which were allowed free access to water and standard rodent chow (Nuvital, Curitiba, PR, Brazil). Our local colonies originated from a breeding stock supplied by CEMIB/UNICAMP, Campinas, SP, Brazil. Immediately after weaning at 3 weeks of age, the animals were maintained under controlled temperature (25°C) and lighting conditions (07:00 h–19:00 h), with free access to tap water and standard rodent laboratory chow, and followed up to 8 weeks of age. The dams were maintained on a pelleted standard rodent laboratory chow (Co, standard diet from Nuvital, Curitiba, PR, Brazil) or long-term HFD for 3 (Co3, n = 5 and HFD3, n = 5), 5 (Co5, n = 5 and HFD5, n = 5), 7 (Co7, n = 5 and HFD7, n = 5) and 9 weeks (Co9, n = 5 and HFD9, n = 5) of age. The standard chow diet contained 11.9% fat and a total of 2.9 kcal/g. The HFD contained 58.3% fat and a total of 5.44 kcal/g (Table 1). Daily, 30 g of chow were offered and the residual amount was weighed to calculate the consumption. The weight of the rats were assessed three times for a week. One group of 9-week-old HFD-treated rats, compared with age-matched control rats, was subjected to a renal function test. The rats were euthanized at the end of 3, 5, 7 and 9 weeks of diet, and pelvic, gonadal and retroperitoneal adipose tissue was removed and weighed. The adiposity index was calculated by ratio of total adipose tissue weight to body weight. The kidneys were weighed and tissue samples were collected for histology, immunohistochemistry and western blot.

Renal function evaluation

The renal function tests were performed and estimated by creatinine and lithium clearance in unanaesthetized, unrestrained 9-week-old control and HFD-treated rats. Briefly, 14 h before the renal test, 60 μmol LiCl 100 g⁻¹ body weight was given by gavage. After an overnight fast, each animal received a load of tap water by gavage (5% of the body weight), followed by a second load of the same volume, 1 h later, and spontaneously voided urine was collected over a 120-min period into a graduated centrifuge tube. At the end of the experiment, blood samples were drawn through cardiac puncture in anaesthetized rats, and urine and plasma samples were collected for analysis [29–31]. Plasma and urine sodium, potassium and lithium concentrations were obtained at 8 weeks of treatment and measured by flame photometry (Micronal, B262, São Paulo, Brazil), while creatinine concentrations were determined spectrophotometrically (Instruments Laboratory, Genesys V, USA).

Measurement of proteinuria

Nine-week-old female rats from control and HFD groups received a load of tap water by gavage (5% of the body weight) and after 20 min they were housed individually in metabolic cages, and spontaneously voided urine was collected over a 2 h period and immediately stored at −20°C until processing. The proteinuria was detected using the Sensiprot kit (Labtest).
Histology and immunohistochemistry

Kidneys were removed and placed in the fixative (paraformaldehyde 4% in 0.1 M phosphate buffer, pH 7.4) for 15 h, followed by 70% alcohol until being processed for paraffin inclusion. The paraffin blocks were cut into 5-μm-thick sections. Picrosirius staining evaluated the density of collagen. Ten cortical and 10 medullary fields of histological sections for each experimental (Co, n = 20) or control (HFD, n = 20) animal were analysed, and the average of collagen density readings were determined. Images were captured with a photomicroscope and analysed by the Leica Qwin 3.1 for windows. For immunohistochemistry, parafﬁn sections were revealed with 3,3′-diaminobenzidine, counterstained with Mayer’s haematoxylin, dehydrated and mounted. No immunoreactivity was seen in control experiments in which one of the primary antibodies was omitted.

Western blot

Whole kidneys were obtained from the Co and HFD groups. The tissue was minced coarsely and homogenized immediately in 10 volumes of solubilization buffer [10 mM/L Triton-X 100, 100 mM Tris (hydroxymethyl) aminomethane (Tris) pH 7.4, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM ethylenediaminetetraacetic acid, 10 mM sodium vanadate, 2 mM phenylmethylsulfonyl fluoride and 0.1 mg/mL aprotinin at 4°C, using a polytron PTA 20S generator (model PT 10/35, Brinkmann Instruments, Westbury, NY) operated at maximum speed for 20 s. The tissue extracts were centrifuged at 11 000 rpm at 4°C for 40 min, and the supernatants were used as a sample. Protein quantification was performed using the Bradford method. For quantification, both tissue and total extract samples (250 μg of protein) were subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). After electrophoretic separation, proteins were transferred to nitrocellulose membranes and then blotted with a specific antibody. The samples were treated with Laemml buffer containing 100 mM dithiothreitol, heated in a boiling water bath for 4 min and subjected to 8% SDS-PAGE in a Bio-Rad minigel apparatus (Mini-Protein, Bio-Rad). Electrophoretic transfer of proteins from the gel to the nitrocellulose membranes was performed for 90 min at 120 V (constant) in a Bio-Rad mini transfer apparatus (Mini-Protean). The non-specific protein binding to the nitrocellulose was reduced by pre-incubating the filter for 2 h at 22°C in a blocking buffer (5% non-fat dry milk, 10 mM Tris, 150 mM NaCl and 0.02% Tween 20). Nitrocellulose blots were then incubated at 4°C overnight with primary antibodies (TGFβ-1, AT1R, ZEB-2 and E-cadherin) diluted in blocking buffer (3% non-fat dry milk, 10 mM Tris, 150 mM NaCl and 0.02% Tween 20). The bands’ immunoreactivity was detected using the enhanced chemiluminescence method (RPN 2108 ECL Western blotting analysis system; Amersham Biosciences). Images of the developed radiographs were scanned (Epson Stylus 3500) and band intensities were quantified by optical densitometry (Scion Image Corporation). To ensure equal loading, membranes were stained with reversible Ponceau to discard possible inequalities in protein loading and/or transfer, in western blots [32]. Only homogeneously stained membranes were employed in the study.

### Data presentation and statistical analysis

Data obtained from this study are expressed as the mean ± SEM or median and quartile deviation as appropriate. Creatinine clearance was used to estimate GFR, and lithium clearance (C_{Li}) was used to assess the proximal tubule output [29–31]. Fractional sodium excretion (FE_{Na}) was calculated as \( \frac{C_{Na}}{C_{Cr}} \times 100 \), where \( C_{Na} \) is the sodium clearance and \( C_{Cr} \) is the creatinine clearance. The fractional proximal (FE_{Na}) and post-proximal (FE_{PNa}) sodium excretion was calculated as \( \frac{C_{Na}}{C_{Cr}} \times 100 \) and \( \frac{C_{Na}}{C_{Li}} \times 100 \), respectively. Statistical analyses were performed using Kruskal–Wallis, one-way analysis of variance (ANOVA) or Student’s t-test, as appropriate, from GraphPad Prism 5.01 (GraphPad Software, Inc.). Post hoc comparisons between the selected means were performed using Tukey’s multiple comparisons test. The energy content of each diet is listed in Table 1.

### Table 1. Composition of standard rodent laboratory diet (standard diet, 11.3%) and long-term HFD (58.3%, AIN 93G modified)

<table>
<thead>
<tr>
<th>Diet composition</th>
<th>Standard diet 11.3% (in g kg(^{-1}) of chow)</th>
<th>HF diet 58.3% (in g kg(^{-1}) of chow)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amido</td>
<td>397.5</td>
<td>52</td>
</tr>
<tr>
<td>Cornstarch dextrinidate</td>
<td>132</td>
<td>143</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>66</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>629.5</td>
<td>261</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>271</td>
</tr>
<tr>
<td>L-cysteine</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cholin bitartrate</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Protein</td>
<td>205.5</td>
<td>276.5</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>70</td>
<td>34</td>
</tr>
<tr>
<td>Lard (saturated fat)</td>
<td>—</td>
<td>333</td>
</tr>
<tr>
<td>Total fats</td>
<td>70</td>
<td>367</td>
</tr>
<tr>
<td>Cellulose microfine (fibre)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Fibre</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Energy content</td>
<td>2.93 kcal g(^{-1}) of chow</td>
<td>5.44 kcal g(^{-1}) of chow</td>
</tr>
</tbody>
</table>

The standard chow diet contained 11.3% fat and 2.93 kcal g\(^{-1}\) of chow, while the HFD contained 58.3% fat and a total of 5.44 kcal g\(^{-1}\) of chow.
performed with the Neuman–Keuls–Student contrast test, when initial ANOVA indicated statistical differences between experimental groups. Comparisons involving only two means within or between groups were carried out using a Student’s \( t \)-test. The level of significance was set at \( P \leq 0.05 \).

**RESULTS**

The HFD group presented significant reduction of the food intake from 3 to 9 weeks of age (Figure 1A) despite enhanced caloric intake (Figure 1B). The body weight was increased from the seventh week in the HFD intake group (Figure 2A), and the adiposity index was elevated beyond the fifth week (Figure 2B) when compared with control age-matched rats. For 3, 5 and 7-week-old HFD rats, we do not verify any change in urinary protein excretion; however, in HFD-treated rats a significantly increased urinary protein excretion was observed when compared with the control group (Figure 3).

**Renal function data**

The data on renal function for 9-week-old rats from both groups (Co and long-term HFD) are summarized in Figure 4.

![Figure 1: Representation of food (A) and caloric (B) intake after 3, 5, 7 and 9 weeks of standard (Co) and HFD. Data are expressed as mean ± SEM (n = 5). *P ≤ 0.05 or **P ≤ 0.01 versus control (ANOVA; post hoc Neuman–Keuls–Student contrast test).](https://academic.oup.com/ndt/article-abstract/28/10/2464/1809562)

![Figure 2: In (A) we have the representation of body weight and in (B) the adiposity index after 3, 5, 7 and 9 weeks of standard (Co) and HFD. Results are expressed as mean ± SEM (n = 5). Data are expressed as mean ± SEM (n = 5). *P ≤ 0.05 or **P ≤ 0.01 versus control (ANOVA; post hoc Neuman–Keuls–Student contrast test).](https://academic.oup.com/ndt/article-abstract/28/10/2464/1809562)

The urinary flow rates did not significantly differ among the groups during the renal tubule sodium handling studies. The GFR, estimated by \( C_{Cr} \), after oral water load and fractional urinary sodium excretion (\( \text{FE}_{\text{Na}} \)), was significantly lower in HFD intake rats >9 weeks old, when compared with the standard diet intake age-matched group, as follows: HFD 9 weeks: 396.6 ± 37.6 mL/min/100 g bw versus Co 9 weeks: 539.3 ± 42.6 mL/min/100 g bw (\( P = 0.0366 \)) and HFD 9 weeks: 0.19 ± 0.043% versus Co 9-weeks: 0.33 ± 0.043% (\( P = 0.0335 \)), respectively. The decreased \( \text{FE}_{\text{Na}} \) in HFD-intake rats was accompanied by a significant decrease in proximal (\( \text{FE}_{\text{P}_{\text{Na}}} \)) and post-proximal sodium excretion, compared with the standard diet intake age-paired control group (Figure 4). This consistent fall in \( \text{FE}_{\text{Na}} \), \( \text{FE}_{\text{P}_{\text{Na}}} \) and in \( \text{FE}_{\text{P}_{\text{Na}}} \) produced by long-term HFD intake was followed by decreased kaliuresis in the entire experimental group of the present investigation.

**Histology and immunohistochemistry**

The density of collagen evaluated by Picrosirius staining was semi-quantitative and significantly enhanced from 5 to 9 weeks of diet intake in the cortical zone of HFD group kidneys compared with control (Figure 5). In the medullar zone, the elevated collagen content was observed beyond 3 weeks of
The immunohistochemical analysis showed that TGFβ-1 is located primarily in both peritubular and mesangial ECM in control animals (Figure 6A, C, E and G). However, after 3 weeks of HFD intake, TGFβ-1 immunoreactivity was increased throughout the entire kidney tissue, in both the cortex and medulla (Figure 6A and B), decreasing progressively beyond 5 weeks of high-fat diet ingestion (Figure 6C–F). Only for the 9-week-old HFD rats, did we again observe increased TGFβ-1 expression in the renal cortical zone (Figure 6G and H). The immunostaining for the AT1 receptor was verified in the Co3 renal cortical portion with a more intensive marker in glomerular cells, brush border of the tubule proximal cells and in the renal vascular walls (Figure 7A). The same pattern of immunostaining distribution was also observed in the HFD3 group; however, this staining was more accentuated (Figure 7B). This increased intensity of immunostaining was also found in the HFD medullary portion when compared with the age-matched Co3 group (Figure 7A and B insets). In the group HFD5 renal AT1 receptors are preferentially observed in the nucleus and brush border of the proximal segments of the nephron (Figure 7C and D). We verified enhanced expression of this receptor in the renal medulla of HFD5 (Figure 7C and D insets). After 7 weeks of consumption of HFD, there was an increase in the immunoreactivity for AT1 receptor in post-proximal segments of the nephron, in both the cortex and medulla, with a reduction in the glomeruli (Figure 7E and F). Beyond week 9 of HFD intake, there was an increase of AT1 receptors throughout the kidney tissue (Figure 7G and H). By kidney immunostaining of the transcription factor ZEB2, we verified predominant glomeruli, post-proximal nephron and collector.
**FIGURE 5:** Graphical representation of cortical (on left) and medullary (on right) renal collagen content after 3, 5, 7 and 9 weeks of standard (Co) and HFD. Results are expressed as mean ± SEM (n = 5). *P ≤ 0.05 or **P ≤ 0.01 versus control (ANOVA; *post hoc* Neuman–Keuls–Student contrast test).

**FIGURE 6:** Renal TGFβ-1 immunostaining in the cortex and medulla (inserts). In the left, we have control groups after 3 (A), 5 (C), 7 (E) and 9 (G) weeks of standard diet. In the right, are the groups submitted to HFD for 3 (B), 5 (D), 7 (F) and 9 (H) weeks. G: glomerulus.
cell location (Figure 8). After 3 weeks of HFD consumption, the immunoreactivity was higher to ZEB2 and can be best evidenced in the medullary region of HFD group (Figure 8A and B). This increase was more intense after 5 weeks of HFD (Figure 8C and D). The HFD7 group also showed increased expression of ZEB2 in both cytosol and nuclei of post-proximal segment cells (Figure 8E and F). In group HFD9, we could not verify overexpression of ZEB2 in glomeruli cells, rather, ZEB2 was predominantly expressed in the post-proximal and collector tubules (Figure 8G and H). After 3 and 5 weeks of HFD treatment, we did not find any qualitative difference in renal fibronectin and desmin immunostaining, but, beyond 7 weeks of HFD treatment, we found enhanced glomerular expression when compared with that observed in age-matched Co7 rats (Figure 9). This enhanced expression was greater in the HFD9 group when fibronectin appeared in the glomerular filtration membrane and the surrounding glomerular capillaries (Figure 10).
Western blot analysis

The western blot semi-quantitative analyses confirm most of the findings observed in the immunohistochemical studies as shown in Figure 11. In the HFD group, the renal TGFβ-1 expression was enhanced from 3 to 5 weeks (174 and 35%, respectively) and was reduced from 7 to 9 weeks (22 and 33%, respectively) when compared with that observed in the Co groups (Figure 11 and 12). We did not observe any significant alterations in the HFD group AT1 receptor expression (Figure 11 and 12), by immunoblotting. When compared with the control, ZEB2 expression was enhanced at all time periods studied but the significant rise (90%) occurred after 5 weeks of HFD. Conversely, E-cadherin expression was reduced in the HFD group from 3 to 9 weeks (28, 48, 45 and 50%, respectively) when compared with an appropriate age-matched control group (Figures 11 and 12).

FIGURE 8: Renal ZEB2 immunostaining in the cortex and medulla (inserts). In the left, we have control groups after 3 (A), 5 (C), 7 (E) and 9 (G) weeks of standard diet. On the right are the groups submitted to HFD for 3 (B), 5 (D), 7 (F) and 9 (H) weeks. G: glomerulus, *post-proximal segments.

DISCUSSION

More and more evidence is emerging that highlights the far-reaching consequences of HFD on kidney morphology and functional disorders. Lin et al. [10] demonstrated that a diet rich in saturated fats is directly associated with the presence of albuminuria in middle-aged adults and the elderly by proposing the hypothesis that saturated fat intake may be related to elevated albuminuria due to increased inflammation markers. Here, in a long-term HFD-treated female rat model, we focus on an epithelial-to-mesenchymal transdifferentiation process as a novel mechanism that promotes renal fibrosis. Also, in the present study we investigated whether known causes of renal fibrosis angiotensin II and TGFβ-1 act through this pathway. We may state that, at least in part, the time-dependent kidney
FIGURE 9: The figures show the representative glomerular immunoreactivity pattern after 7 weeks in both of the studied groups. In (A) (Co) and (B) (HFD), we have fibronectin and in (C) (Co) and (D) (HFD) desmin immunostaining. Fibronectin immunoreactivity is accentuated in HFD glomeruli (B) as well as desmin (D). Arrows: glomerular basement membrane.

FIGURE 10: In (A) (Co) and (B) (HFD), we have fibronectin and in (C) (Co) and (D) (HFD) desmin immunostaining. The figures show the representative glomerular immunoreactivity pattern after 9 weeks in both of the studied groups. Fibronectin as well as desmin immunoreactivity is more accentuated than that observed in HFD7 glomeruli. Arrows: glomerular basement membrane.
expression of fibrotic and EMT markers are associated with later adult renal function disorder as an outcome, suggesting that the kidney is an organ in which a prolonged high-fat diet intake may underlie early loss of organ function and occurrence of chronic kidney disease.

In the current study, the time-course HFD-treated group showed decreased food ingestion, in grams, despite a greater caloric intake which in turn was associated with significant enhancement of the body weight and adipose tissue deposition, mainly, beyond 7 weeks of age when compared with age-
matched control animals. Interestingly, the increased body weight under conditions of high dietary fat availability occurred in the absence of overfeeding, suggesting that these adaptive responses were mainly driven by the macronutrient composition of the diet. Further, the semi-quantitative immunoblotting and qualitative immunohistochemical analysis in the present study demonstrated an early time-course increase in the expression of TGFβ in the entire kidney of HFD-treated rats, compared with that observed in the control group. Simultaneously, the study shows a transient increase in the expression of ZEB2 in HFD whole kidney by immunoblotting between the third and seventh week of age accompanied by a fall in the E-cadherin expression and followed beyond the seventh week by increased collagen deposition.

By immunohistochemistry, the present study verified beyond the seventh week of diet in the HFD group a striking enhance in the glomerular expression of TGFβ-1, desmin, fibronectin and collagen, intrinsically related to the fibrotic process. These results in the current study confirm the recent findings, indicating that glomerular cells, particularly the podocytes, undergo phenotypic conversion, characterized by a loss of podocyte-specific markers and a gain of transitional features, a process reminiscent of EMT [33]. In this study, we observed an enhanced expression of collagen that was accompanied by enhanced glomerular expression of desmin, and reduced E-cadherin. In immortalized mouse podocyte culture, Li et al. [33] showed that after TGFβ-1 stimulation also occurred loss of epithelial markers how ZO-1 and acquisition of mesenchymal markers how desmin, collagen I and fibronectin. The production of interstitial matrix compounds suggests that podocytes have adapted a mesenchymal phenotype after injury, which could profoundly change their functions [33]. TGFβ-1 triggers tubular EMT, and its expression are up-regulated in virtually every type of chronic kidney disease [34, 35]. From the present data, we may affirm that EMT may be an early and predominant response of renal, particularly, of podocytes in most pathophysiological conditions. In whole kidney, the TGFβ-1 expression is enhanced in a similar pattern to that observed in type I collagen. Thus, in parallel to enhanced expression of TGFβ-1, a major inducer of EMT in epithelial cells [36, 37], we found upregulation of ZEB2, an EMT-inducing transcriptional factor [37–40], which in turn downregulated E-cadherin renal expression. Additionally, the current study shows an increased expression of proteins considered mesenchymal markers, including desmin, fibronectin and collagen. Corroborating to current findings, Liu [41] speculates that transition of podocytes and other renal epithelial cells after injury may play a critical role in causing dedifferentiation and cells dysfunction, which ultimately leads to defective glomerular filtration with proteinuria and glomerulosclerosis and tubule transport disorder. The tubule cells, and particularly podocytes, are highly terminally differentiated cells that are incapable of regenerative postnatal replication, thus, the loss of podocytes represents potential starting points for irreversible glomerular injury [42]. Thus, as demonstrated in the study, the podocyte injury underlies most forms of proteinuric kidney diseases [43] and is an essential feature of progressive glomerular diseases [44].

Studies have shown that Ang II plays a key role in the progression of chronic kidney damage, contributing to renal fibrosis. Many in vitro and experimental studies have demonstrated that Ang II activates renal cells to produce profibrotic factors and ECM proteins [45, 46]. The interrelation between Ang II and TGFβ is well established. Ang II and TGFβ share many profibrotic mediators and intracellular signalling systems [47, 48]. Therefore, in this study, an increased immunohistochemical renal expression of Ang II receptors could be the driving force that initiated the profibrotic process by enhanced TGFβ-1 renal expression. The unchanged immunoblotting response to AT1 receptors in HFD-treated compared with control rats may result in the utilization of whole kidney extracts by this technique. Also, the blotting data of whole kidney extracts, at different ages, may reflect an uneven protein kidney expression, as revealed by qualitative immunohistochemistry of nephrin structures.

In rats, the HFD causes renal injury that is preceded by endothelial dysfunction and hypertension, both induced by increasing oxidative stress, powerful inflammatory response and disruption of the renal filtration barrier [9, 49]. The present investigation also demonstrated a pronounced decrease in fractional urinary sodium excretion in long-term HFD-treated rats. The decreased FE\textsubscript{Na} was accompanied by a fall in FEP\textsubscript{Na} and FEP\textsuperscript{*}Na and occurred in association with significantly decreased C\textsubscript{Cr} and certainly on the sodium-filtered load. The precise mechanism of these phenomena remains unknown. Although the precise mechanism by which renal sodium excretion declines in HFD rats remains to be elucidated, the loss of organ function, the sympathetic neural and renin–angiotensin activity and renal non-control of the fluid and electrolyte balance are thought to play a dominant role in the long-term sodium and water retention. Studies in dogs who became obese due to a HFD showed that 5 weeks after starting the diet, there was a 30% increase in glomerular filtration relative to baseline, and in the same model, 9 weeks after induction of obesity, expansion of Bowman’s capsule an increased mesangial matrix were observed [50]. This was associated with an increased expression of TGFβ-1 and glomerular increase in the concentration of glycosaminoglycans in the tubular basement membranes. Factors, such as leptin [51] and oxidative stress [52], may contribute to sympathetic drive in HFD-intake animals. Our results may suggest the participation of increasing oxidative stress and inflammation associated with renal alterations in HFD-induced rats. Although we were not able to demonstrate by immunoblotting, the tissue renin–angiotensin system may be upregulated in HFD rats as demonstrated by immunostaining; thus, increased renal angiotensin may induce ROS production by NAD(P)H oxidases [53]. In this way, confirmatory experiments are needed before this conclusion can be wholly made. In conclusion, according to our present knowledge, these are the first data showing progressive kidney dysfunction induced by HFD treatment, particularly showing a striking structural disorder associated with enhanced glomerular expression of proteins intrinsically related to the fibrotic process. The results led us to hypothesize that enhanced ZEB2, recognized E-box repressors of E-cadherin gene, would be prior induced by TGFβ-1 resulting
subsequently in the rise of fibronectin and type I collagen expression. Additionally, we suggest that presumable podocyte injury in parallel with observed proteinuria and evidence of EMT transformation are associated with long-term loss of kidney function and with renal sodium and water retention [54].

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CONFLICT OF INTEREST STATEMENT

None declared.

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