

Restoration of DNA Damage/Repair Balance by Caloric Restriction Protects Against Diabetic Hydronephrosis

Rania Abdel-latif

Minia University

Paula-Anahi Arias-Loza

University Hospital of Wuerzburg

Helga Stopper

University of Wuerzburg

Eman M. Othman

`eman.sholkamy@uni-wuerzburg.de`

University of Wuerzburg

Article

Keywords: Calorie restriction, Hydronephrosis, renal Injury, Diabetes mellitus, ZDF rats

Posted Date: June 12th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9762633/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

Diabetic kidney disease (DKD) is a leading cause of chronic kidney failure, yet the mechanisms underlying structural abnormalities such as hydronephrosis remain poorly defined. Emerging evidence implicates DNA damage and defective repair pathways in renal injury progression.

Objective

This study aimed to investigate the role of DNA damage, repair dysregulation, and apoptosis in diabetic hydronephrosis, and to determine whether caloric restriction (CR) modulates these pathways.

Methods

Male Zucker Diabetic Fatty (ZDF) rats were subjected to ad libitum feeding or caloric restriction, with lean littermates as controls. Renal morphology was assessed histologically. DNA damage and repair were evaluated using γ -H2AX and proliferating cell nuclear antigen (PCNA), respectively, while apoptosis was assessed by caspase-3 expression. Fibrosis was quantified by histological and morphometric analyses.

Results

Diabetic rats developed marked hydronephrosis, tubular injury, and interstitial fibrosis, accompanied by increased γ -H2AX expression, altered PCNA levels, and enhanced caspase-3 activation. These findings indicate increased DNA double-strand breaks, dysregulated repair responses, and elevated apoptosis. Caloric restriction significantly attenuated hydronephrosis severity, reduced DNA damage, partially normalized PCNA expression, and suppressed apoptosis and fibrosis.

Conclusion

These findings support a mechanistic model in which DNA damage and defective repair drive apoptosis and fibrosis, contributing to diabetic hydronephrosis. Caloric restriction mitigates renal injury by modulating this DNA damage-response axis, highlighting a potential non-pharmacological strategy targeting genome stability pathways in DKD.

1. Introduction

Diabetes mellitus is among the most prevalent diseases in the adult population worldwide, characterized by hyperglycemia and associated complications in the long term [1]. Diabetic kidney disease (DKD) is a leading cause of chronic kidney failure worldwide and represents a major clinical burden associated with metabolic disorders such as obesity and type 2 diabetes. CKD and renal damage are estimated to affect 8–16% of the world population [2, 3]. Thus, screening for the development of CKD as well as searching for prevention or amelioration strategies for CKD are important requirements to enhance the life quality of patients with diabetes. While glomerular injury has been extensively studied, increasing attention is being directed toward tubular and interstitial alterations that contribute significantly to disease progression. Among these, hydronephrosis; dilation of the renal pelvis and calyces which is often observed in diabetic conditions, yet its underlying molecular mechanisms remain poorly defined [2].

Traditionally, hydronephrosis has been attributed primarily to mechanical or obstructive causes. However, emerging evidence suggests that metabolic stress may directly contribute to structural and functional renal alterations independent of physical obstruction. [4]. Chronic hyperglycemia and associated metabolic disturbances can induce oxidative stress, inflammation, and cellular injury within renal tissues. One critical but underexplored consequence of these processes is DNA damage, which has been increasingly recognized as a driver of tissue dysfunction in chronic diseases.[5, 6]. Importantly, individuals with obesity and diabetes are more prone to experiencing hydronephrosis due to the development of diabetic nephropathy [7]. Studies have shown that diabetes-related renal complications, including diabetic nephropathy which affects 30–40% of patients with type 2 diabetes, can exacerbate the likelihood of hydronephrosis, as renal function declines [8]. While the exact prevalence of hydronephrosis in diabetic patients remains unclear, it is widely acknowledged as a significant concern, particularly in those with diabetic nephropathy and urinary tract infections, who are at an elevated risk [9]. It is

well known that diabetes and obesity induce a clearly structural change in the urothelium and kidney which can lead to diabetic nephropathy and hydronephrosis, and eventually kidney failure [10]. The condition of hydronephrosis is considerably significant, yet there are few studies to determine what factors affect DNA damage and repair, which might be responsible for renal parenchymal damage [11]. Indeed, previous studies have suggested that DNA double strand breaks (DSBs) may be a major mechanism underlying hydronephrosis [11, 12]. As the level of DNA damage increases, affected cells may switch to apoptosis or cell cycle arrest, leading to cell death [13]. In the context of the hydronephrosis course, it has been reported that the expression of the DSB marker p-histone H2A family member X (γ -H2AX) increased with the level of P53 pro-apoptotic factor. Thus, we hypothesized that a more effective DNA repair system in hydronephrosis could potentially lead to reduced apoptotic damage in the kidney.

DNA damage activates complex cellular responses, including repair mechanisms and, when damage is excessive, the initiation of apoptosis. The phosphorylated histone variant γ -H2AX serves as a sensitive marker of DNA double-strand breaks [14], while proliferating cell nuclear antigen (PCNA) plays a key role in DNA replication and repair [15]. Dysregulation of these pathways can lead to impaired cellular homeostasis and contribute to progressive renal injury [16]. In parallel, activation of apoptotic pathways, such as those mediated by caspase-3, promotes loss of functional renal cells and exacerbates tissue damage [17].

Caloric restriction (CR), defined as a reduction in caloric intake without malnutrition, has been widely studied for its beneficial effects on metabolic health and longevity. In experimental models, CR has been shown to improve insulin sensitivity, reduce oxidative stress, and attenuate inflammation. Importantly, CR has also been implicated in the modulation of DNA damage response pathways and apoptosis, suggesting a potential mechanism by which it may protect against organ damage in metabolic diseases. Different reports highlighted an adaptation to a healthier lifestyle including calorie restriction (CR) diet based on its beneficial effects on the life extension of laboratory rats as well as the delay of the onset and progression of CKD and cardiovascular disease, especially in subjects with diabetes [18–20]. The CR diet was reported to suppress the inflammatory response and regulate both genes involved in cellular proliferation and factors associated with apoptosis in renal cells [21, 22]. In this regard, there has been considerable research showing that CR has beneficial multisystemic effects via regulating stress response which leads to protecting cells and tissues from injury [21, 23]. Despite these insights, the relationship between DNA damage, apoptosis, and hydronephrosis in diabetic conditions remains unclear. Furthermore, it is not known whether caloric restriction can mitigate these molecular processes in the context of diabetic renal structural abnormalities.

Therefore, the present study aimed to (1) investigate the involvement of DNA damage and apoptotic pathways in the development of hydronephrosis in Zucker Diabetic Fatty (ZDF) rats, and (2) evaluate the effect of caloric restriction on these molecular mechanisms. By linking metabolic intervention to DNA damage response and cell death pathways, this study seeks to provide new insight into the pathogenesis of diabetic hydronephrosis and identify potential targets for therapeutic intervention.

2. Materials and methods

2.1. Chemicals and reagents

If not mentioned, all the laboratory chemicals were purchased from Sigma-Aldrich (Munich, Germany).

Antibodies: Anti- β -actin (T6199) and actin-alpha Sr (A2547 (T6199) were from Sigma-Aldrich (Taufkirchen, Germany). The anti-cleaved caspase-3 antibody (9665) and p-Histone H2A.X (S139) (20E3) were obtained from Cell Signaling Technology Inc (Beverly, USA). Anti PCNA and the secondary antibodies (sc-2004, sc-2005, sc-2020) were from Santa Cruz Biotechnology (Heidelberg, Germany).

2.2. Animal model

The ZDF is a rat model of adult- early onset type II diabetes that exhibited insulin resistance, hyperinsulinemia, and hyperglycemia as a result of its inbred leptin receptor deficiency [24]. Unlike obese Zucker rats (fa/fa), which share the leptin receptor mutation and develop obesity and metabolic syndrome without spontaneously progressing to diabetes, ZDF rats exhibit a diabetic phenotype under standard dietary conditions [25]. Our study focuses on the diabetic ZDF (fa/fa) model to evaluate the effects of CR on kidney structural damage, with particular emphasis on hydronephrosis, a feature commonly associated with diabetes.

For this study, male obese ZDF (fa/fa) and Zucker lean controls (fa/+) (ZDF-LC) rats were obtained at 11 weeks of age from Charles River Laboratories (Wilmington, MA). Animals were kept in an environmentally controlled room with 12 hrs of light/dark cycles, and free tap water access. The rats were housed individually in polycarbonate cages with corncob bedding, which was replaced twice weekly to ensure a clean environment. All Animal Use Protocols were approved by the Local Ethics Committee, University of Würzburg, Germany under ethical approval number (55.2-2531.01-120/13). This study was conducted in accordance with Institutional Animal Ethics Committee

guidelines for the care and use of laboratory animals. In accordance with the ARRIVE guidelines [26], we have systematically reported key details related to the design, conduct, and analysis of our in vivo experiments.

2.3. Experimental design

2.3.1. Study group and dietary management

After confirming hyperglycemia via tail vein blood samples, total number of animals of 20 were divided into three experimental groups: (1) lean control rats (fa/+) (ZDF-LC, n = 7), (2) ZDF fa/fa normal diet (ZDF-ND) (n = 8), and (3) ZDF fa/fa caloric restriction (ZDF-CR) (n = 5). lean rats were used as a fixed control group, while random allocation was applied to the ZDF and ZDF-CR groups to minimize bias in the study design. Diet intervention consisted of 4 weeks of daily feeding with either Purina 5008 food (protein 23%, fat 6.5%, carbohydrates 58.5%, fiber 4% and ash 8%) ad libitum for the ZDF-LC and ZDF-ND groups or CR Purina 5008 for the ZDF fa/fa CR group. Food intakes were measured daily throughout 28 days (Fig. 1), food amount of 34.03 ± 0.75 g was recorded to be consumed by ZDF-ND. For the calorie restriction group, the food amount was set at 20 g per day, based on the observed average daily intake of lean rats of the same age (20.33 ± 0.40 g). This baseline intake was measured prior to the experiment as part of preliminary observations of the lean rats' feeding behavior. Experimental design and animal allocation flow chart is provided in Supplementary Material S1

2.3.2. Feeding protocol

Using a specific feeding protocol, food is provided once a day at a consistent time to establish a routine this allows accurate monitoring of food consumption. Each day, the food provided for each cage is weighed using a precision balance. After 24 hr, the remaining food is collected and weighed to determine the actual intake. Figure 1 depicts the food consumption rate during experiments period.

For each cage, the amount of food provided, and the leftovers are recorded daily. This helps calculate the exact amount of food each rat consumes. Rats are weighed weekly to monitor their health and the impact of the calorie-restricted diet. In this study, we implemented a CR of 20 g/day as a mild CR, representing approximately a 40% reduction compared to the usual intake. This level of restriction was chosen based on established literature, where reductions of 20–40% are classified as mild to moderate CR [27]. The selected reduction aimed to distinguish it from severe caloric restriction protocols, which typically involve a reduction of over 50% and are associated with potential adverse effects, such as malnutrition or compromised health [28]. This approach ensured that the CR protocol was sufficient to evaluate its impact while maintaining the overall health and well-being of the animals throughout the study.

2.3.3. Sample size calculation

The sample size for this study was determined employing the resource equation method [29]. The calculation utilized the formula $E = \text{Total number of animals} - \text{Total number of groups}$, with the criterion that the value of E falls within the range of 10 to 20, which is deemed sufficient. Randomization was used to allocate experimental animals to control and treatment groups. An independent investigator was responsible for group allocation.

2.3.4. Experimental process

At 16 weeks of age, animals were sacrificed by decapitation under isofluran anesthesia and kidneys were collected and immediately frozen. Before organ collection, the rats were fasted for 14 hrs to standardize their metabolic state with free access to water. Fasting blood glucose was measured from tail vein blood using a glucometer, and serum insulin levels were quantified using the ELISA method.

2.4. Histopathological examination and assessment of renal fibrosis

By the end of the experiment, all animals were sacrificed, and their kidneys were collected. Kidneys were cut along the transverse axis through the hilar region and fixed in neutral buffered formalin 10%. The excised organs were routinely processed dehydrated in ascending grades of ethanol and embedded in Paraffin wax then the obtained blocks were sectioned at 4 to 5 μm thickness. The obtained tissue sections were collected on clean and dry glass slides, deparaffinized, and stained with Hematoxylin and Eosin (H&E) stain for the determination of pathological and morphological alterations using different magnification power (100x and 10x respectively) [30] or with picrosirius red (PSR) staining for detection of collagen disposition in the interstitial area of renal cortex.

Collagen deposition was assessed under polarized light and quantitated by NIH image (ImageJ) analysis as described previously [31]. In brief, the collagen content was determined by measuring the area of collagen fibers stained with PSR, excluding non-tissue components. The analysis focused on the cortical regions of the kidney. A 6 fields per sample, typically between 0.5-1 mm^2 in size, were analyzed to ensure consistency and minimize bias. All sections were examined and observed under the light microscope (Leika DMRBE, Germany).

Glomerular damage was assessed based on morphological changes, with "normal" glomeruli showing intact structure, and "damaged" glomeruli exhibiting features like thickening of the basement membrane, mesangial expansion, or sclerosis [32]. A total of 20 randomly selected glomeruli per animal were evaluated using systematic random sampling from multiple microscopic fields. Glomerular density was quantified in three randomly selected cortical regions using ImageJ software, with results expressed as glomeruli per mm²

2.5. Western Blot analysis

A part of the kidney samples with adequate size (around 50–100 mg) was crushed mechanically in a tissue homogenizer together with 200 µL homogenization buffer (0.2 M mannitol, 50 mM saccharose, and 10 mM HEPES [pH 7.5]). The homogenate was then centrifuged at 14,000 rpm for 30 min at 4°C. The protein-containing supernatant was transferred to a clean tube and the concentration of protein was determined using Bradford's method. Generally, 30 µg of protein per sample was loaded on a discontinuous acrylamide gel. After electrophoresis, the gel was blotted on the PVDF membranes.

The membranes were blocked overnight in 5% (w/v) nonfat milk powder in Tris-buffered saline with Tween 20 (TBS-T) (5 mM TRIS, 150 mM NaCl, 0.05% (v/v) Tween-20), then incubated with primary antibody (Anti caspase-3 (1:500), anti PCNA (1:50), Anti actin alpha Sr (1:500) and anti β-actin (1:5000)) overnight at 4°C. After washing the excess of the primary antibody, membranes were incubated for 1 h with horse radish peroxidase (HRP)-conjugated secondary antibody, followed by washing. After incubation with HRP substrate (Luminol-solution) for 5 min, the membrane was exposed to a light-sensitive film, and the film was developed afterward.

All the samples were measured with coded protocol.

2.6. Detection of phosphorylated H2AX sites

We followed the methods of Bankoglu et al., (2016) [33] in the detection of the phosphorylated H2AX (a DSB marker). Antibody staining against phosphorylated Ser-139 at the C-terminus of histone H2AX was performed on paraffin sections of the kidney samples to detect the DSBs. The paraffin sections (5 µm) were prepared with a microtome (LEICA RM 2165, Wetzlar, Germany) and mounted on positively charged slides. The sections were heated at 60°C for an hour, deparaffinized (3 x 4 min Roti-Histol, 2 x 3 min 100% EtOH, 1 x 2 min 70% EtOH), and washed with PBS. Antigen retrieval was achieved by cooking the slides for 4 min in citrate buffer (10 mM sodium citrate, pH 6) on the highest level in a pressure cooker. To cool down, the slides were washed with PBS. The sections were blocked in 5% donkey serum for an hour at room temperature in dark. To reduce background staining, endogenous peroxidase activity was depleted by incubation with 3% of H₂O₂ for 15 min at room temperature in dark.

Prior to the incubation of the biotinylated antibody, slides were incubated successively with 0.001% avidin and biotin for 15 min at room temperature in the dark. Later on, primary antibody (γ H2AX (Ser139, clone 20E3) rabbit mAb; 1:200) incubation was performed overnight at 4°C in the dark. Following the washing steps, biotin-conjugated secondary antibody (donkey anti-rabbit IgGB, sc2089, Santa Cruz Biotechnology; 1:200) incubation was performed for 45 min, at room temperature in dark. After washing the sections with PBS, the sections were incubated with the avidin biotin complex (ABC) reagent (Vectastin-Elite ABC reagent: two drops of reagent A and reagent B mixed with 10 mM sodium phosphate, 0.9% NaCl, pH 7.5) for 30 min at room temperature in dark and washed again in PBS. Sections were incubated with diaminobenzidine (DAB) chromogen (Vector Laboratories) for 10 min at room temperature. DAB reacts with HRP in the presence of peroxide to yield an insoluble, brown-colored product at locations where peroxidase-conjugated antibodies are bound to samples. Counterstaining was done with Ehrlich's hematoxylin (1 g hematoxylin, 48 mL 99.8% isopropanol, 51.9 mL H₂O d, 50 mL glycerol, 1.5 g potassium alum, 5 mL acetic acid, 0.2 g potassium iodate) for three min and then the sections were washed for 10 min in pure water. Before mounting with Eukitt (Fluka), the sections were dehydrated (1 x 1 min 70% EtOH, 4 x 2 min 100% EtOH, 2 x 3 min Roti-Histol). Pictures were taken under an Eclipse 55i microscope (Nikon GmbH, Düsseldorf, Germany) and a Fluoro Pro MP 5000 camera (Intas Science Imaging Instruments GmbH, Göttingen, Germany) at 200-fold magnification. The percentage of positive cells was assessed by scoring 100 tubuli per sample [34].

2.7. Statistical analysis

All data are collected in a coded manner and are presented as mean ± SEM or Mean ± SD after the decoding. Statistical analysis was performed with GraphPad® Prism (version 8.0.2). The statistical significance was assessed using a one-way analysis of variance (ANOVA) followed by Tukey–Kramer post-analysis test for comparison between groups with normal distribution. A Kruskal-Wallis test was used for the abnormal distribution of data then Dunn's Multiple Comparison test was applied. p<0.05 was considered statistically significant.

3. Results

1. Metabolic phenotype and general characteristics

Diabetic animals exhibited the expected metabolic phenotype, including increased body weight, hyperglycemia, and insulin resistance compared with lean controls. Caloric restriction (CR) significantly improved these parameters, as evidenced by reduced body weight gain and improved glycaemic control relative to ad libitum-fed diabetic rats (Table 1).

At baseline, ZDF-ND and ZDF-CR groups exhibited significantly higher body weights compared to the ZDF-LC group ($P < 0.05$). At the time of organ collection, both ZDF-ND and ZDF-CR groups maintained significantly greater body weights than the ZDF-LC group ($P < 0.05$).

Absolute kidney weight was significantly increased in the ZDF-ND group (1719.37 ± 148.33 mg) compared to the ZDF-LC group (985.14 ± 79.73 mg, $P < 0.05$). The ZDF-CR group demonstrated a kidney weight (1272.6 ± 53.17 mg) that was significantly lower than the ZDF-ND group but remained higher than the ZDF-LC group ($P < 0.05$). Relative kidney weight was highest in the ZDF-ND group (4.56%) compared to the ZDF-LC (3.35%) and ZDF-CR groups (3.59%) (Table 1)

Table 1 shows that fasting blood glucose levels were significantly elevated in the ZDF-ND group (13.41 ± 4.07 mmol/L) compared to the ZDF-LC group (6.22 ± 0.86 mmol/L, $P < 0.05$). The ZDF-CR group also exhibited significantly higher fasting glucose levels (12.09 ± 6.0 mmol/L) compared to the ZDF-LC group ($P < 0.05$), but no significant difference was observed between ZDF-ND and ZDF-CR groups.

Fasting serum insulin levels were significantly higher in the ZDF-ND group (0.30 ± 0.40 nmol/L) compared to the ZDF-LC group (0.10 ± 0.04 nmol/L, $P < 0.05$). The ZDF-CR group exhibited a moderate increase in fasting insulin (0.125 ± 0.01 nmol/L), which was significantly lower than the ZDF-ND group ($P < 0.05$) but remained elevated compared to the ZDF-LC group ($P < 0.05$).

These findings confirm the metabolic efficacy of the intervention and provide the physiological context for subsequent renal analyses.

Table (1)

Body weight at beginning and end of experiment, Absolute and relative kidney weight, fasting glucose and insulin levels in ZDF lean, and obese ZDF rats (normal and caloric restriction diet)

Groups	N	Body wt. at beginning (g)		Body wt. at organ collection (g)		Absolute Kidney wt. (mg)		Relative kidney wt. (mg/g%)	Fasting Blood glucose (mmole/L)		Fasting serum insulin nmol/L	
		Mean $\pm$ SD	Median (IQR)	Mean $\pm$ SD	Median (IQR)	Mean $\pm$ SD	Median (IQR)		Mean $\pm$ SD	Median (IQR)	Mean $\pm$ SD	Median (IQR)
ZDF-LC	7	267.40 $\pm$ 18.80	265 (279 – 250)	293.70 $\pm$ 16.90	295 (308 – 282)	985.14 $\pm$ 79.73)	976 (1040 – 935)	3.35%	6.22 $\pm$ 0.86	6.16 (6.77–5.60)	0.10 $\pm$ 0.04	0.084 (0.076–0.161)
ZDF-ND	8	357.00 $\pm$ 30.50*	352(383 – 333)	377.40 $\pm$ 48.20*	365.5 (411.5–345)	1719.37 $\pm$ 148.33*	1689 (1858–1606.5)	4.56%	13.41 $\pm$ 4.07*	13.47 (15.64–10.48)	0.30 $\pm$ 0.40 *	0.104 (0.365 – 0.08)
ZDF-CR	5	375.40 $\pm$ 43.80*	384 (400 – 384)	354.40 $\pm$ 50.30*	321 (400 – 319)	1272.6 $\pm$ 53.17 #	1271 (1323 – 1246)	3.59%	12.09 $\pm$ 6.0 *	10.43 (10.54–10.31)	0.125 $\pm$ 0.01 #	0.125 (0.126 – 0.122)

IQR; Interquartile Range (75%-25%), SD; standard deviation. ZDF-LC; lean normal group, ZDF-ND, normal diet, and ZDF-CR calorie restriction diet. * Significant in comparison to Lean control (LC), # significant in comparison to normal diet (ND) group, $p \leq 0.05$

2. Type 2 diabetes induces hydronephrosis and severe renal structural remodeling, which is attenuated by caloric restriction

Hydronephrosis was quantified according to the criteria defined by Vora and colleagues [35]: (0) Normal, either no or minimal space between papilla and calyx. (1) Mild, narrow but definable calyceal space with normal papillary shape; (2) Moderate, dilation of the calyx, compression of the papilla but preserved papillary shape; (3) Severe, gross distension of the calyx, severe compression of the lateral cortex and marked distortion of papilla.

Histological examination revealed pronounced hydronephrosis in diabetic animals, characterized by dilation of the renal pelvis and calyces. This was accompanied by tubular injury, including epithelial degeneration and tubular dilation, as well as expansion of the interstitial compartment (Fig. 2 & Table.2).

Across the groups, the kidney morphology for most ZDF-LC rats appeared normal (85.7%), while only 12.5% of ZDF-ND had normal kidney morphology. Mild hydronephrosis was more prevalent in the ZDF-CR group (60%) compared to lean controls (14.3%). Severe hydronephrosis was present in 50% of ZDF-ND rats but absent in both lean controls and ZDF-CR rats. (Fig S2. Additional histological images demonstrating morphological legions)

Table (2)

Scoring and distribution of hydronephrosis in lean control and ZDF rats (normal and caloric restriction diet)

Normal	Lean (N.=7)	ND (N. = 8)	CR (N. = 5)
	6 (85.7%)	1 (12.5%)	3 (42.85%)
Mild	1 (14.30%)	2 (25%)	4 (57.14%)
Moderate	0 (0%)	1 (12.5%)	0 (0%)
Sever	0 (0%)	4 (50%)	0 (0%)

Values are presented as frequency with percentage of total animals in parentheses, where Lean; lean normal group, ND; ZDF normal diet and CR; ZDF calorie restriction diet.

In addition, Histopathological examination of the kidneys showed a difference in the degree of glomerular damage between the three study groups. A significant increase in the number of damaged glomeruli was observed in the ZDF-ND group in comparison to the ZDF-LC control and ZDF-CR group (Fig. 3A), which likely contributes to the observed reduction in the glomeruli density in the same group in comparison with the other two groups (Fig. 3B). (Fig S3, S4. Additional histological images demonstrating histopathological legions)

3- Caloric restriction suppresses diabetes-induced renal fibrosis

The quantitative analysis confirmed a significant increase in fibrosis in diabetic kidneys compared with controls (Fig. 4). CR markedly attenuated these structural abnormalities. Treated animals exhibited reduced pelvic dilation and preservation of tubular architecture, along with a significant reduction in fibrotic area (Fig. 4).

The development of fibrosis in the hydronephrotic kidney is characterized by the deposition of collagen in the renal interstitium. We assessed renal collagen deposition after PRS staining, representative histological images (Fig. 4 panel [I]) reveal marked collagen accumulation in the ZDF-ND group, which is reduced in the ZDF-CR group. Image processing analyses (Fig. 4 Panel [I]) further highlight differences in collagen deposition between groups showing a significant increase ($P < 0.05$) in collagen deposition in ZDF-ND compared to ZDF-LC. Additionally caloric restriction significantly reduced collagen accumulation in ZDF-CR compared to ZDF-ND ($P < 0.05$). (Fig.S5 Additional histological images illustrating collagen deposition after PRS staining)

Activation of the fibrogenesis process was investigated by measuring the upregulation of the actin alpha level. We observed that the protein expression in the renal tissues of the ZDF-ND rats' group, showed a significant increase when compared to ZDF-LC ($P < 0.05$), suggesting enhanced fibrosis. ZDF-CR significantly reduced alpha-actin expression compared to ZDF-ND ($P < 0.05$), indicating a protective effect against fibrosis (Fig. 5).

6. Dysregulation of DNA repair pathways in diabetes nephropathy is restored by caloric restriction

To evaluate genomic instability, DNA damage was assessed by γ -H2AX expression. Diabetic kidneys showed a significant increase in γ -H2AX-positive cells compared with controls (Fig. 6), indicating elevated DNA double-strand breaks. While CR significantly reduced γ -H2AX expression, suggesting a lower DNA damage burden in treated animals, these structural alterations coincide with increased γ -H2AX expression, suggesting that DNA damage may underlie the observed tissue remodelling.

5. Dysregulation of DNA repair is associated with diabetic renal injury

PCNA expression was significantly altered in diabetic animals compared with controls (Fig. 7A), consistent with disruption of DNA replication and repair processes. CR partially normalized PCNA expression (Fig. 7A), indicating improved regulation of DNA repair

pathways.

6. Diabetes promotes renal apoptosis, which is suppressed by caloric restriction

Apoptotic activity, assessed by caspase-3 expression, was significantly increased in diabetic kidneys (Fig. 7B). This indicates enhanced apoptotic signalling in response to metabolic stress. CR significantly reduced caspase-3 activation, consistent with decreased apoptotic cell death.

4. Discussion

This study identifies DNA damage and its downstream consequences as central contributors to the development of diabetic hydronephrosis and provides evidence that caloric restriction (CR) exerts renoprotective effects through coordinated modulation of these pathways. Rather than representing a purely structural or obstructive phenomenon, our findings support a model in which hydronephrosis in the diabetic kidney is driven, at least in part, by intrinsic cellular injury mechanisms involving genomic instability, impaired repair responses, and apoptosis.

A key finding is the robust elevation of γ -H2AX in diabetic kidneys, indicating increased DNA double-strand breaks. This aligns with growing evidence that hyperglycemia-driven oxidative stress and mitochondrial dysfunction induce genomic instability in renal cells, contributing to the progression of diabetic kidney disease (DKD)[36, 37]. While DNA damage has been increasingly recognized in glomerular injury, its role in structural abnormalities such as hydronephrosis has remained largely unexplored. Our data extend this framework, suggesting that DNA damage is not only a marker of injury but may actively contribute to renal architectural remodelling.

Concomitant alterations in PCNA expression further support disruption of DNA repair processes. PCNA is essential for replication fork stability and DNA repair, and its dysregulation has been linked to maladaptive repair and cellular senescence in chronic kidney injury[38]. In the present study, altered PCNA levels in diabetic animals likely reflect an insufficient or dysregulated repair response to persistent genomic insult. This imbalance between damage and repair provides a mechanistic basis for the observed increase in apoptosis, as evidenced by elevated caspase-3 activity. Apoptotic loss of tubular epithelial cells is a well-established driver of tubular atrophy and interstitial fibrosis in DKD[39], both of which were prominent in our model.

Importantly, Mechanistically, the protective effects of CR are likely multifactorial. Previous studies have demonstrated that CR reduces oxidative stress, enhances mitochondrial efficiency, and activates cytoprotective signaling pathways such as AMPK and sirtuins, all of which contribute to improved genomic stability and cell survival [40, 41]. Although these upstream regulators were not directly assessed in the present study, the observed reductions in DNA damage and apoptosis are consistent with these established effects and provide a functional link between metabolic intervention and preservation of renal integrity.

The present findings also challenge the traditional view of hydronephrosis as a predominantly mechanical condition. While obstruction and altered urine flow remain important contributors, our results support a complementary mechanism in which intrinsic cellular injury-driven by DNA damage and apoptosis facilitates structural deterioration. This concept is consistent with emerging perspectives that emphasize the role of metabolic and inflammatory stress in shaping renal architecture independently of overt obstruction [42, 43]. Thus, hydronephrosis in the diabetic context may reflect a convergence of mechanical and molecular insults.

Several limitations should be acknowledged. First, the use of a single animal model limits direct clinical translation, although the Zucker Diabetic Fatty rat is well established for studying metabolic kidney disease. Second, while strong associations between DNA damage, apoptosis, and renal pathology were observed, causality cannot be definitively established. Third, upstream regulators of the DNA damage response, including oxidative stress markers and signaling intermediates such as AMPK or SIRT1, were not directly assessed. Future studies integrating targeted molecular interventions will be required to delineate these pathways more precisely.

In conclusion, our findings identify DNA damage and apoptosis as central drivers of diabetic hydronephrosis and demonstrate that CR mitigates these processes through modulation of the DNA damage response. These results provide a mechanistic framework linking metabolic stress to renal structural injury and support the concept that targeting genome stability pathways may represent a viable strategy for slowing the progression of diabetic kidney disease.

Conclusion

This study is among the first to link DNA damage signaling to hydronephrosis in a metabolic disease context, and we propose that a DNA damage-apoptosis-fibrosis axis drive hydronephrosis in diabetes mellitus. In addition, non-pharmacological CR diet is associated with mitigation and protection against hydronephrosis and renal damage in the ZDF rat model. Our findings demonstrate that CR diet can mitigate apoptosis level and DNA damage targetable pathway in renal tissues. While a causal relationship between dietary changes and improvements in kidney status cannot be established within the scope of this study, hyperinsulinemia may be one contributing factor to the observed protective effects. Further research is needed to fully understand the underlying mechanisms driving these findings.

Abbreviations

CKD: Chronic kidney disease

CR: Calorie restriction

DAB: Diaminobenzidine

DSBs: DNA double strand breaks

H&E: Hematoxylin and Eosin

HRP: Horse radish peroxidase

LC: Lean control

ND: Normal diet

PCNA: Proliferating cell nuclear antigen

γ-H2AX: p-histone H2A family member X

PSR: Picrosirius red

TBS-T: Tris-buffered saline with Tween 20

ZDF: Zucker diabetic fatty rats

Declarations

Ethics approval and consent to participate

All Animal Use Protocols were approved by the Local Ethics Committee, University of Würzburg, Germany under ethical approval number (55.2-2531.01-120/13)

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that there is no conflict of interest.

Funding

This work is partly supported by the Competence Network of Heart Failure funded by the Integrated Research and Treatment Center (IFB) of the Federal Ministry of Education and Research (BMBF) number 01E01504, and Faculty of Pharmacy-Minia University, Egypt.

Authors' contributions

Paula-Anahi Arias-Loza, Eman M. Othman, and Helga Stopper: Conceptualization, Methodology, Experimental conduction.

Rania Abdel-latif: Data curation, Writing- Original draft preparation.

Rania Abdel-Atif, Paula-Anahi Arias-Loza and Eman M. Othman: Visualization, Investigation.

Eman M. Othman and Helga Stopper: Supervision.

Paula-Anahi Arias-Loza and Eman M. Othman Validation:

Rania Abdel-Atif, Paula-Anahi Arias-Loza, Helga Stopper, and Eman M. Othman: Writing- Reviewing and Editing

Acknowledgements

Non applicable

References

1. Alam, S. et al. Diabetes Mellitus: insights from epidemiology, biochemistry, risk factors, diagnosis, complications and comprehensive management. *Diabetology* **2** (2), 36–50 (2021).
2. Jha, V. et al. Chronic kidney disease: global dimension and perspectives. *Lancet* **382** (9888), 260–272 (2013).
3. Couser, W. G. et al. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. *Kidney Int.* **80** (12), 1258–1270 (2011).
4. Darrad, M., Gupta, A. & Rukin, N. *Hydronephrosis*. Blandy's Urology, : pp. 165–188. (2019).
5. Frassetto, L. & Kohlstadt, I. Treatment and prevention of kidney stones: an update. *Am. Family Phys.* **84** (11), 1234–1242 (2011).
6. Marsh, S. A. et al. Cardiovascular dysfunction in Zucker obese and Zucker diabetic fatty rats: role of hydronephrosis. *Am. J. Physiol. Heart Circ. Physiol.* **293** (1), H292–H298 (2007).
7. Bilan, V. P. et al. Diabetic nephropathy and long-term treatment effects of rosiglitazone and enalapril in obese ZSF1 rats. *J. Endocrinol.* **210** (3), 293 (2011).
8. Schernthaner, G. & Schernthaner, G. H. Diabetic nephropathy: new approaches for improving glycemic control and reducing risk. *J. Nephrol.* **26** (6), 975–985 (2013).
9. Abdelmaboud, S. O. et al. Sonographic evaluation of hydronephrosis and determination of the main causes among adults. *Int. J. Med. Imaging.* **3** (1), 1–5 (2015).
10. Pugsley, M. K. et al. Use of the ZDF rat to model dietary fat induced hypercoagulability is limited by progressive and fatal nephropathy. *J. Pharmacol. Toxicol. Methods.* **107**, 106933 (2021).
11. Moon, C., Moon, H. Y. & Kim, C. S. The Expressions of p53, γ -H2AX and Ku70/Ku80 That was Caused by Hydronephrosis in the Kidney of Rats. *Korean J. Urol.* **48** (9), 976–983 (2007).
12. Chang, I. Y. et al. Repression of apurinic/apyrimidinic endonuclease by p53-dependent apoptosis in hydronephrosis-induced rat kidney. *Free Radic. Res.* **45** (6), 728–734 (2011).
13. Bernstein, C. et al. DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. *Mutat. Research/Reviews Mutat. Res.* **511** (2), 145–178 (2002).
14. Yuan, J., Adamski, R. & Chen, J. Focus on histone variant H2AX: to be or not to be. *FEBS Lett.* **584** (17), 3717–3724 (2010).
15. Strzalka, W. & Ziemienowicz, A. Proliferating cell nuclear antigen (PCNA): a key factor in DNA replication and cell cycle regulation. *Ann. Bot.* **107** (7), 1127–1140 (2011).
16. Li, J. F. et al. 1.25(OH)2D3 decreases PCNA and mTOR expression and alleviates renal injury in Thy-1 nephritis rat model. *PLoS One.* **19** (12), e0311000 (2024).
17. Yang, B. et al. Caspase-3 and apoptosis in experimental chronic renal scarring. *Kidney Int.* **60** (5), 1765–1776 (2001).
18. Le Bourg, E. Predicting whether dietary restriction would increase longevity in species not tested so far. *Ageing Res. Rev.* **9** (3), 289–297 (2010).
19. Phelan, J. P. & Rose, M. R. Why dietary restriction substantially increases longevity in animal models but won't in humans. *Ageing Res. Rev.* **4** (3), 339–350 (2005).
20. Piper, M. D. et al. Dietary restriction and aging: a unifying perspective. *Cell. Metab.* **14** (2), 154–160 (2011).
21. Mahani, F. D. et al. Effects of caloric and time restriction diets on kidney health in rat model of postmenopausal acute kidney injury: An apoptosis and histopathological study. *Iran. J. Basic. Med. Sci.* **25** (3), 390 (2022).

22. Zhang, N. et al. Calorie restriction-induced SIRT6 activation delays aging by suppressing NF- κ B signaling. *Cell. Cycle*. **15** (7), 1009–1018 (2016).
23. Rohrbach, S. et al. Impact of caloric restriction on myocardial ischaemia/reperfusion injury and new therapeutic options to mimic its effects. *Br. J. Pharmacol.* **171** (12), 2964–2992 (2014).
24. Kollarova, M. et al. ZDF (fa/fa) rats show increasing heterogeneity in main parameters during ageing, as confirmed by biometrics, oxidative stress markers and MMP activity. *Exp. Physiol.* **107** (11), 1326–1338 (2022).
25. Xu, X. et al. FRI031 A Novel Strategy To Detect Leptin Receptor (Lepr, fa) Mutation In Obesity And Diabetic Animal Model (Zucker Rats). *J. Endocr. Soc.* **7** (Supplement_1), bvad114 (2023).
26. Kilkenny, C. et al. Animal research: reporting in vivo experiments: the ARRIVE guidelines. *Br. J. Pharmacol.* **160** (7), 1577 (2010).
27. Oudmaijer, C. et al. *Clinical implications of nutritional interventions reducing calories, a systematic scoping review* (Clinical nutrition ESPEN, 2024).
28. Zhang, X. et al. Caloric restriction, friend or foe: effects on metabolic status in association with the intestinal microbiome and metabolome. *J. Agric. Food Chem.* **70** (43), 14061–14072 (2022).
29. Charan, J. & Kantharia, N. How to calculate sample size in animal studies? *J. Pharmacol. Pharmacotherapeutics*. **4** (4), 303–306 (2013).
30. Bancroft, J. D. G. M, *Theory and practice of histological techniques*. 6th edition (ed. K SuvaranLCA B JDCL). Elsevier Health Sciences, London, UK., : pp. 121–134. (2008).
31. Summers, S. A. et al. Mast cell activation and degranulation promotes renal fibrosis in experimental unilateral ureteric obstruction. *Kidney Int.* **82** (6), 676–685 (2012).
32. Hodgin, J. B. et al. Quantification of glomerular structural lesions: associations with clinical outcomes and transcriptomic profiles in nephrotic syndrome. *Am. J. Kidney Dis.* **79** (6), 807–819 (2022). e1.
33. Bankoglu, E. E. et al. Impact of weight loss induced by gastric bypass or caloric restriction on oxidative stress and genomic damage in obese Zucker rats. *Free Radic. Biol. Med.* **94**, 208–217 (2016).
34. Bankoglu, E. E. et al. Role of PTEN in Oxidative Stress and DNA Damage in the Liver of Whole-Body Pten Haplodeficient Mice. *PLoS One*. **11** (11), e0166956 (2016).
35. Vora, J. P. et al. Evolution of metabolic and renal changes in the ZDF/Drt-fa rat model of type II diabetes. *J. Am. Soc. Nephrol.* **7** (1), 113–117 (1996).
36. Forbes, J. M. & Thorburn, D. R. Mitochondrial dysfunction in diabetic kidney disease. *Nat. Rev. Nephrol.* **14** (5), 291–312 (2018).
37. Cai, A. et al. Impact of stress hyperglycemia ratio on acute kidney injury and mortality in patients with cardiogenic shock: a retrospective analysis. *Front. Endocrinol. (Lausanne)*. **16**, 1606819 (2025).
38. Babaeenezhad, E. et al. *D-Limonene Alleviates Acute Kidney Injury Following Gentamicin Administration in Rats: Role of NF-kappaB Pathway, Mitochondrial Apoptosis, Oxidative Stress, and PCNA*. *Oxid Med Cell Longev*, 2021: p. 6670007. (2021).
39. Erekat, N. S. Programmed Cell Death in Diabetic Nephropathy: A Review of Apoptosis, Autophagy, and Necroptosis. *Med. Sci. Monit.* **28**, e937766 (2022).
40. Barcena, M. L. et al. Role of AMPK and Sirtuins in Aging Heart: Basic and Translational Aspects. *Aging Dis.* **16** (6), 3335–3360 (2024).
41. Shinmura, K. *Effects of caloric restriction on cardiac oxidative stress and mitochondrial bioenergetics: potential role of cardiac sirtuins*. *Oxid Med Cell Longev*, 2013: p. 528935. (2013).
42. Fougeray, S. et al. Metabolic stress promotes renal tubular inflammation by triggering the unfolded protein response. *Cell. Death Dis.* **2** (4), e143 (2011).
43. Norregaard, R. et al. Obstructive nephropathy and molecular pathophysiology of renal interstitial fibrosis. *Physiol. Rev.* **103** (4), 2827–2872 (2023).

Figures

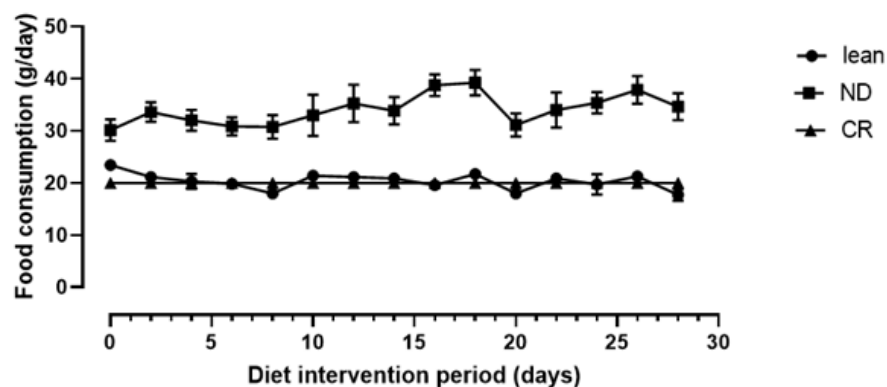


Figure 1

Mean daily food consumption during experimental period (28 days) in rat study groups. Data are represented as mean $\pm$ SEM. $n=7$ for ZDF-LC, $n=8$ for ZDF-ND and $n=5$ for ZDF-CR. ZDF-LC; lean normal group, ZDF-ND, normal diet, and ZDF-CR calorie restriction diet.

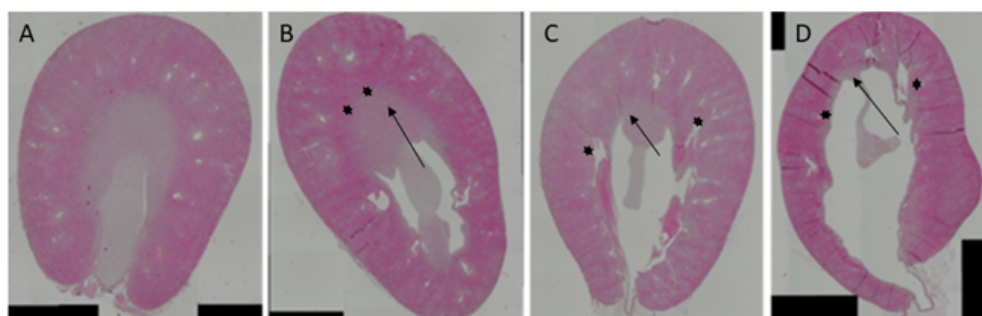


Figure 2

Representative images of morphologic changes hydronephrosis in rat study groups where A; Normal, B; Mild, indicate the calyceal space (arrow) and normal papillary shape (star). C; Moderate show dilated calyx (arrow) and compressed papilla (star). D; Severe, highlight gross calyceal distension (arrow) and distorted cortex/papilla (star). $n=7$ for ZDF-LC, $n=8$ for ZDF-ND and $n=5$ for ZDF-CR; lean normal group, ZDF-ND, normal diet, and ZDF-CR calorie restriction diet.

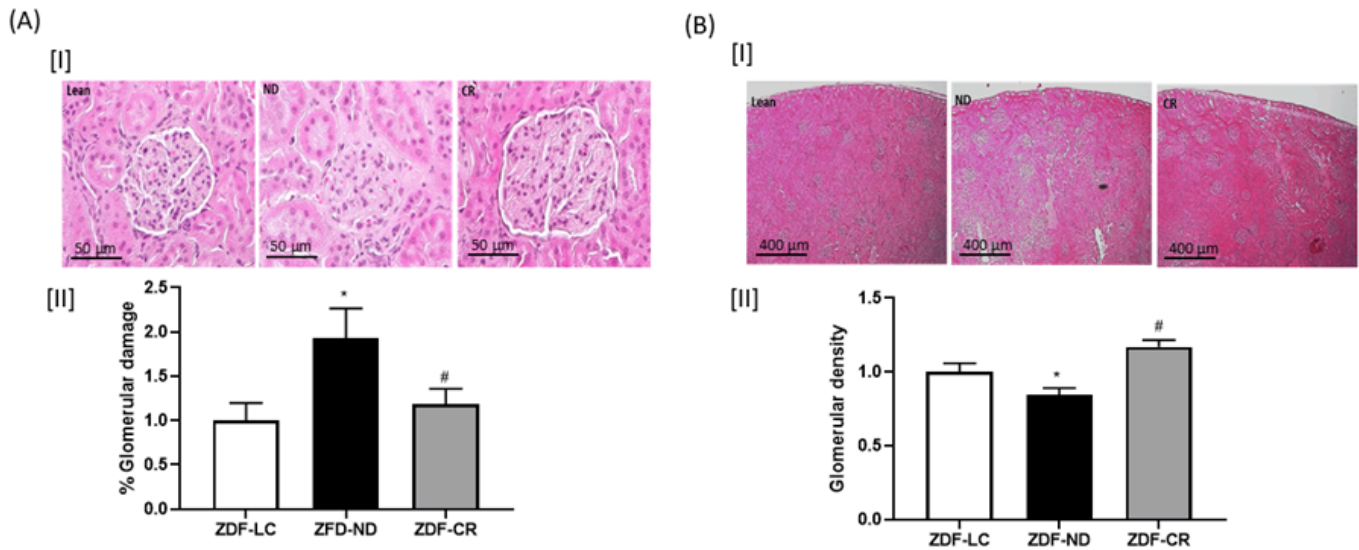


Figure 3

Examination of the kidney sections for A) Glomeruli damage, B) Glomeruli dentistry in ZDF-LC, ZDF-ND and ZDF-CR groups. Panel [I] showed the photomicroscopical images and panel [II] showed a semiquantitative analysis for histological changes. Data are represented as mean $\pm$ SEM, * Significant in comparison to ZDF-LC group, # significant in comparison to ZDF-ND group, $p \leq 0.05$. $n=7$ for ZDF-LC, $n=8$ for ZDF-ND and $n=5$ for ZDF-CR; lean normal group, ZDF-ND, normal diet, and ZDF-CR calorie restriction diet.

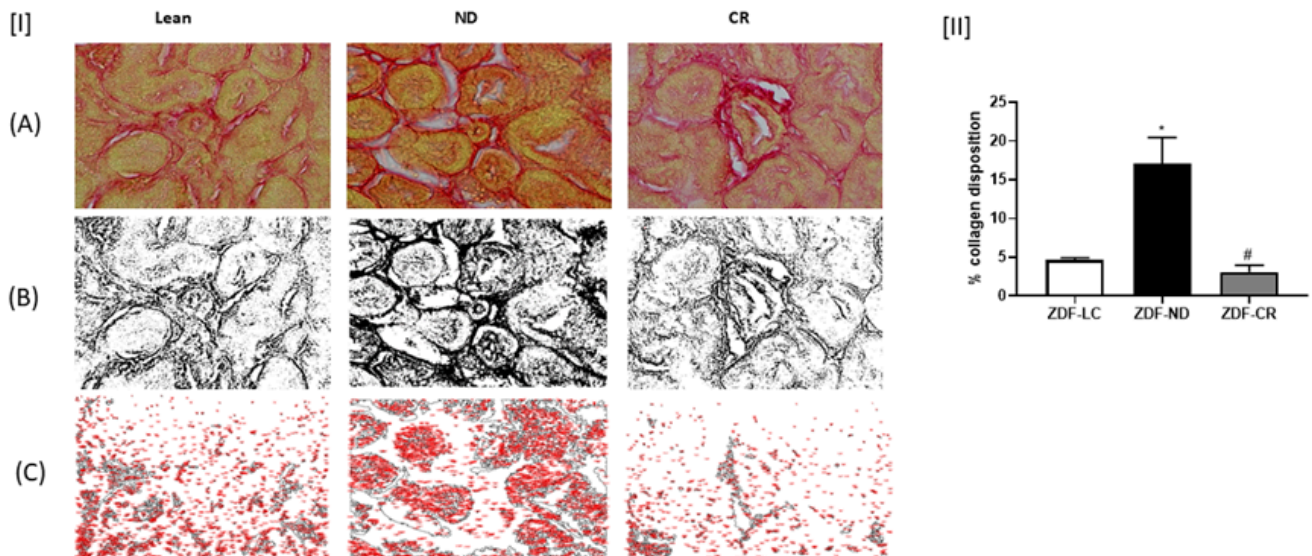


Figure 4

Photomicrographs of collagen deposition in ZDF-LC, ZDF-ND and ZDF-CR groups after picrosirius red (PRS) staining. Panel [I] showed representative kidney sections for the study groups visualized in color, viewed under polarized light, at original magnification $\times 40$ (A) in black and white (B), and after NIH image analysis (C). panel [II] showed a semiquantitative analysis for collagen disposition. Data are

represented as mean $\pm$ SEM, * Significant in comparison to ZDF-LC group, # significant in comparison to ZDF-ND group, $p \leq 0.05$. n= 7 for ZDF-LC , n=8 for ZDF-ND and n=5 for ZDF-CR; lean normal group, ZDF-ND, normal diet, and ZDF-CR calorie restriction diet.

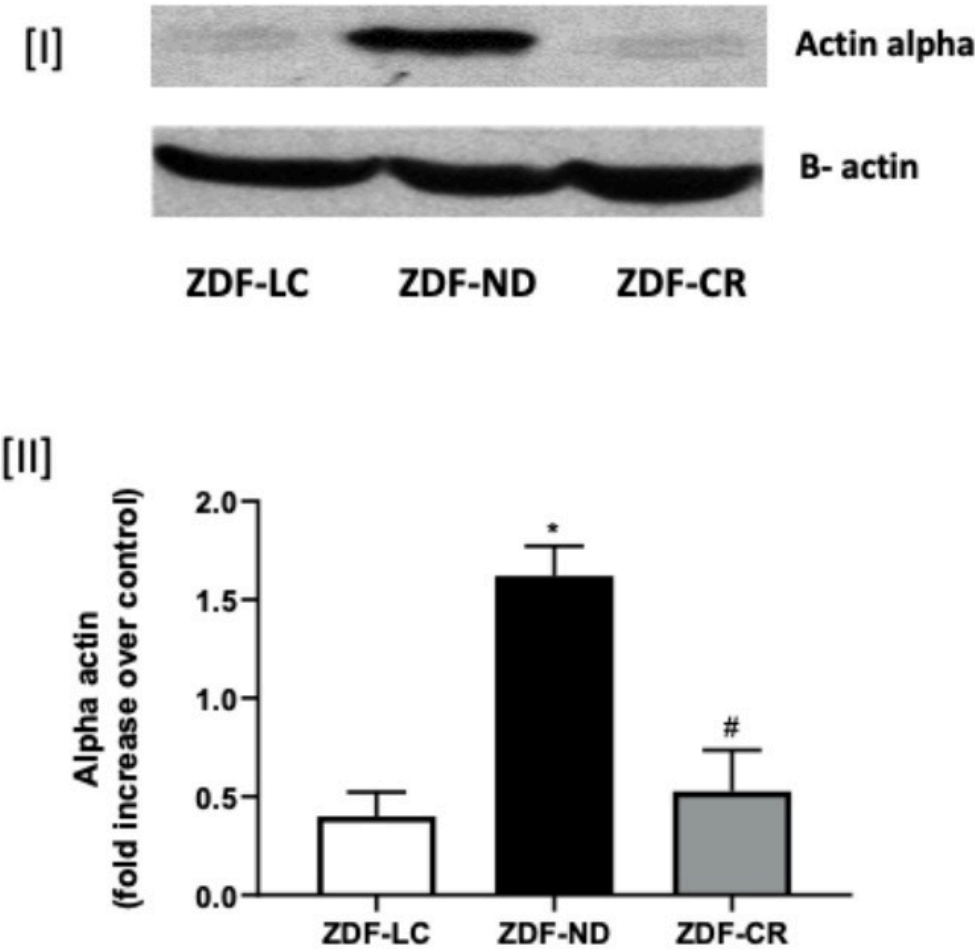
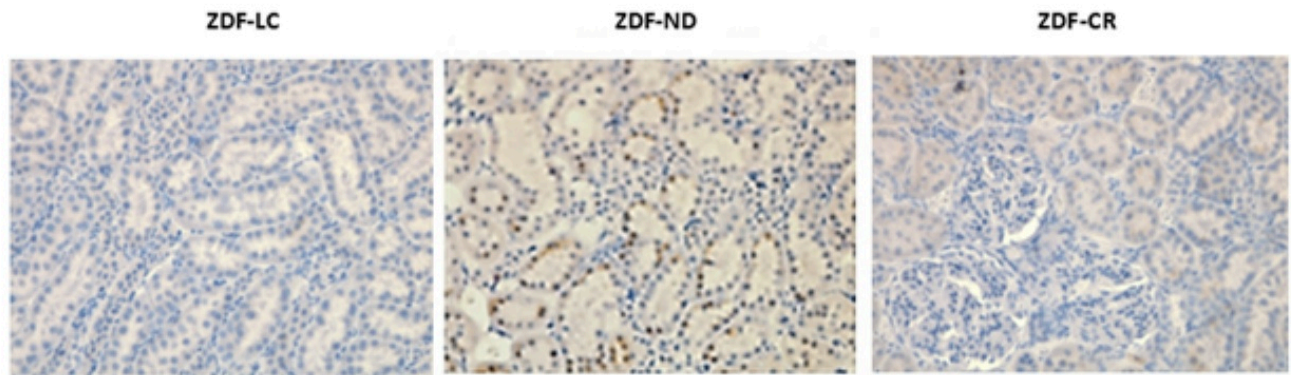


Figure 5

Panel [I] Representative western blot analysis of actin alphaprotein expression of kidney tissues, showing protein bands of each group. Panel [II] Representative graphs of densitometric analysis of protein expression of actin alpha in ZDF-LC, ZDF-ND and ZDF-CR groups. Data are represented as mean $\pm$ SEM, * Significant in comparison to ZDF-LC group, # significant in comparison to ZDF-ND group, $p \leq 0.05$. n= 7 for ZDF-L, n=8 for ZDF-ND and n=5 for ZDF-CR. ZDF-LC; lean normal group, ZDF-ND, normal diet, and ZDF-CR calorie restriction diet.

[I]



[II]

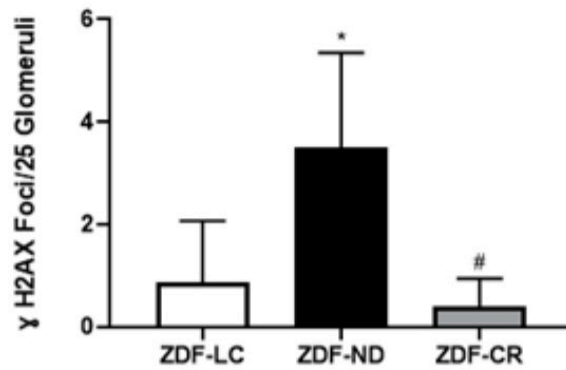


Figure 6

Immunohistochemical analysis of γ -H2AX Foci frequency in renal cortex region of ZDF-LC, ZDF-ND and ZDF-CR groups. Panel [I] showed a representative photomicroscopical image of γ -H2AX foci stain in study groups. Panel [II] showed a semiquantitative analysis for γ -H2AX Foci frequency in the study groups, and. Data are represented as mean $\pm$ SEM, * Significant in comparison to ZDF-LC group, # significant in comparison to ZDF-ND group, $p \leq 0.05$. $n = 7$ for ZDF-LC, $n = 8$ for ZDF-ND and $n = 5$ for ZDF-CR. ZDF-LC; lean normal group, ZDF-ND, normal diet, and ZDF-CR calorie restriction diet.

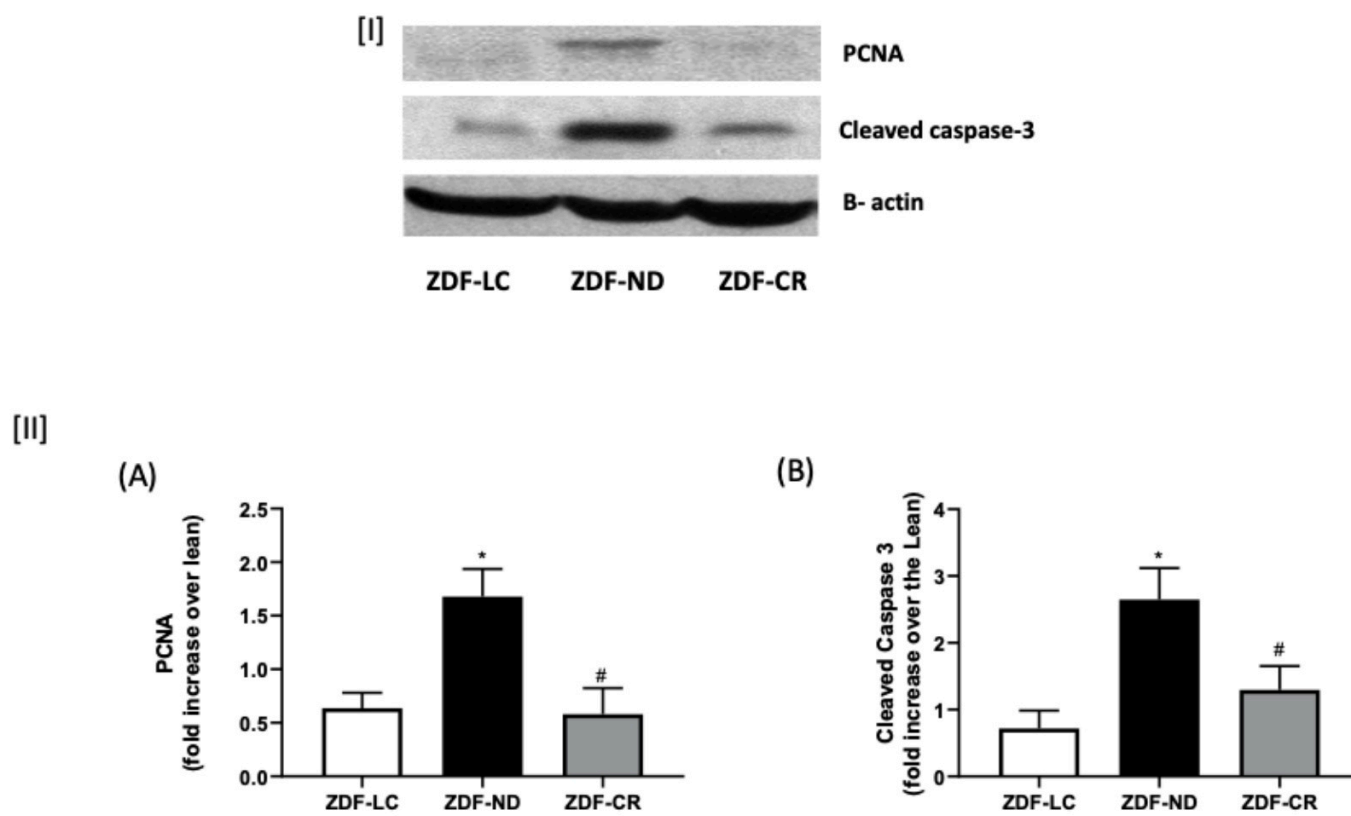


Figure 7

Panel [I] Representative western blot analysis of PCNA, and caspase-3 protein expression of kidney tissues, showing protein bands of each group. Panel [II] Representative graphs of densitometric analysis of protein expression of PCNA (A)F, and caspase-3 (B) in ZDF-LC, ZDF-ND and ZDF-CR groups. Data are represented as mean $\pm$ SEM, * Significant in comparison to ZDF-LC group, # significant in comparison to ZDF-ND group, $p \leq 0.05$. $n = 7$ for ZDF-L, $n = 8$ for ZDF-ND and $n = 5$ for ZDF-CR. ZDF-LC; lean normal group, ZDF-ND, normal diet, and ZDF-CR calorie restriction diet.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterialS1.pdf](#)
- [SupllemataryS2S7.pdf](#)