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# Effects of Low-Protein Diet on Renal Oxidative Stress, Biochemistry and Histology in Weaned Rats

(Kesan Diet Rendah Protein terhadap Tekanan Oksidatif Buah Pinggang, Biokimia dan Histologi pada Tikus Cerai Susu)

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ABSTRACT

A low-protein diet (LPD) leads to low plasma protein and insufficient building blocks for normal kidney development, especially in children. This study aimed to determine the effects of short-term LPD on renal oxidative stress, biochemical profile and histologic changes in weaned rats. Three-week-old male and female Sprague Dawley rats were divided into the LPD group and the normal protein diet (NPD) group for 3 weeks. Renal oxidative stress, biochemical profile and histology were examined. Both male and female rats had significantly ($p<0.05$) lower body weight, smaller kidneys and higher advanced oxidation protein product concentrations after a 3-week LPD. Only LPD-fed females had lower malondialdehyde concentrations and superoxide dismutase activity but higher reduced glutathione concentrations compared to NPD-fed females. Histologic examination showed abnormal histologic features in the proximal and distal tubules, fibrosis in the cuboidal cells, reduced lumen diameter, smaller glomerular tuft area and glomerular tuft volume in LPD-fed male and female groups. In conclusion, short-term protein malnutrition leads to renal injury in male and female weaned rats. The different responses of male and female rats to protein malnutrition suggest sexual dimorphism and hormonal factors in kidney development, with females showing a higher susceptibility to oxidative damage.

Keyword: Kidney; low-protein diet; oxidative stress

ABSTRAK

Diet rendah protein (LPD) menyebabkan protein plasma yang rendah dan blok binaan yang tidak mencukupi untuk perkembangan ginjal yang normal, terutamanya pada kanak-kanak. Kajian ini bertujuan untuk menentukan kesan jangka pendek LPD terhadap tekanan oksidatif ginjal, profil biokimia dan perubahan histologi ginjal dalam tikus cerai susu. Tikus Sprague Dawley jantan dan betina berusia tiga minggu dibahagikan kepada kumpulan LPD dan kumpulan diet protein biasa (NPD) selama 3 minggu. Tekanan oksidatif ginjal, profil biokimia dan histologi ginjal telah diperiksa. Tikus jantan dan betina mempunyai berat badan yang lebih rendah ($p<0.05$), ginjal yang lebih kecil dan kepekatan pengoksidaan produk protein lanjutan yang lebih tinggi selepas 3 minggu LPD. Hanya tikus betina yang diberi LPD mempunyai kepekatan malondialdehid dan aktiviti superoksida dismutase yang lebih rendah dan kepekatan glutation terturun yang lebih tinggi berbanding tikus betina yang diberi NPD. Pemeriksaan histologi mendedahkan ciri histologi yang tidak normal dalam tubul proksimal dan distal, fibrosis dalam sel kuboid, diameter lumen yang berkurangan dan kawasan tuf glomerulus dan isi padu tuf glomerulus yang lebih kecil pada kumpulan jantan dan betina yang diberi LPD. Kesimpulannya, malpemakanan protein jangka pendek menyebabkan kecederaan ginjal pada tikus jantan dan betina yang telah bercerai susu. Tindak balas yang berbeza antara tikus jantan dan tikus betina terhadap kekurangan zat protein menunjukkan dimorfisme seks dan faktor hormon dalam perkembangan ginjal, dengan betina menunjukkan kerentanan yang lebih tinggi kepada kerosakan oksidatif.

Kata kunci: Ginjal; pemakanan rendah protein; tekanan oksidatif

## INTRODUCTION

Weaning is the transition phase of children from breast milk to infant formula to solid foods (National Health Service 2022). Depending on individual growth and cultural practices, children are usually introduced to complementary foods between six months and one year. Children's physical, cognitive and emotional growth during the weaning period is crucial for their development, as they have the fastest growth rates in human life after infancy. During the weaning period, children generally gain height and weight rapidly (Kang, Masilamani & Boegehold 2016). The adequacy of daily food intake easily influences the growth and development of children during the weaning period. Adequate intake of macro- and micronutrients in children, including protein and calcium for bone growth, tissue and muscle development, iron for cognitive development, and essential vitamins such as vitamin C for immune function and overall health, is essential for their continued growth and development (Williamson & Beatty 2015). However, an unbalanced diet can lead to malnutrition, which affects millions of people worldwide, especially children.

Malnutrition, an abnormal physiological state of energy or nutrient deficiency, imbalance or excess, comprises three main categories: Undernutrition (stunting, wasting and underweight), micronutrient-related malnutrition, and overweight and obesity (World Health Organization 2024). According to the World Health Organization (2024), 149 million children under the age of 5 were stunted worldwide in 2022, while an estimated 45 million children were wasted. Remarkably, almost half of the deaths in children under the age of 5 are associated with malnutrition. In Malaysia, the National Health Morbidity Survey 2022 reported that the national prevalence of undernutrition in children under the age of five was 15.3% for underweight, 10.1% for wasting and 21.2% for stunting (Institute for Public Health 2019).

Undernutrition significantly increases children's susceptibility to diseases as it affects multiple organs (Wells 2019). It is also associated with delayed physical growth, behavioural problems and impaired immunity, which can lead to higher morbidity and mortality in adulthood (Wells 2019). Inadequate dietary intake, particularly inadequate protein and energy intake, is a major contributor to protein-energy malnutrition in children, which is associated with growth retardation such as underweight and stunting (Beckerman-Hsu et al. 2020; Mısırlıoğlu et al. 2023). Ensuring adequate protein intake is critical for improving children's nutritional status, preventing stunting, supporting growth and weight gain, and increasing bone density (Adi, Pratiwi & Amanda 2021; Uauy et al. 2015), thus, underlining the importance of protein in the diet in infancy and childhood to combat malnutrition. Therefore, addressing undernutrition through nutritional interventions is crucial to promote children's overall health and well-being. A comprehensive understanding of how a low-protein diet (LPD) affects body function is required to provide valuable insights for developing appropriate nutritional interventions to ensure optimal growth and development in children.

Studies have shown that low-protein diet (LPD) has profound effects on organ function and general health. For example, LPD leads to fatty liver, reduced energy digestibility and lower lean mass (Pezeshki et al. 2016). Restricted protein intake in early life can lead to many health disorders, such as hepatic steatosis, insulin resistance and glucose intolerance (Dalvi et al. 2018). Consistent with this, LPD also exerts a notable influence on renal function. In individuals with protein-energy malnutrition, LPD adversely affects renal function, including decreased glomerular filtration rate, decreased renal plasma flow, and impaired urine concentrating ability (D'Agati, Kaskel & Falk 2011). In addition, protein-energy malnutrition in children also leads to changes in renal morphology by affecting the size, depth, width and volume of the renal parenchyma (Ece et al. 2007). Long-term LPD also impairs the renal tubules and causes kidney injury (Snelson et al. 2021). LPD could affect the level of oxidative stress in the body. Theys et al. (2009) reported that early LPD exacerbated the imbalance of antioxidant enzymes. Similarly, LPD was observed to increase oxidative stress in pregnant rats (Vega et al. 2016). However, limited information on the adverse effects of LPD on renal functions related to biochemical profiles is available. Therefore, this study was conducted to determine the effects of short-term LPD on renal oxidative stress, biochemical profiles and histological changes in male and female weaned rats. The weaned rat model can provide invaluable insight into the intricacies of LPD-induced effects on the kidneys.

## MATERIALS AND METHODS

### DIET PREPARATION

In this study, the experimental diets LPD (6% protein) and normal protein diet (NPD; 18% protein) were prepared according to the formulation of Teklad Rodent Protein Diet TD.90016 and TD.96180 (Table 1), respectively (Envigo, Madison, Wisconsin, United States). All ingredients were thoroughly mixed in a stainless-steel blender to prepare the rat pellets. The pellets were then oven-dried at 37 °C for 24 h, packaged in a sealed bag and stored at -80 °C before feeding to the rats.

### EXPERIMENTAL ANIMAL PROTOCOL

This study was conducted in accordance with the Malaysian Code of Practice for the Care and Use of Animals for Scientific Purposes (MYCODE), and the experimental protocols were approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) (reference number: FSK/2022/SEE MENG/23-NOV./1288-NOV.-2022-AUG.-2025). Twenty-four male

TABLE 1. Composition of the experimental diets

| Ingredient (g) | Normal protein diet (NPD; 18% protein) | Low-protein diet (LPD; 6% protein) |
|---|---|---|
| Casein | 207.00 | 69.00 |
| DL-Methionine | 2.70 | 0.90 |
| Sucrose | 451.30 | 571.80 |
| Corn starch | 200.00 | 200.00 |
| Corn oil | 52.60 | 53.90 |
| Cellulose | 41.06 | 57.82 |
| Vitamin mix, Teklad (40060) | 10.00 | 10.00 |
| Ethoxyquin, antioxidant | 0.01 | 0.01 |
| Mineral mix, Ca-P Deficient (79055) | 13.37 | 13.37 |
| Calcium phosphate, dibasic | 17.36 | 21.60 |
| Calcium carbonate | 4.60 | 1.60 |

and female weaned Sprague-Dawley rats (50-80 g) aged three weeks were provided by the Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia (Kuala Lumpur, Malaysia). All rats were maintained under standard animal experimental conditions, a 12-h light-dark cycle and controlled room temperature (23 °C to 26 °C) in the animal house. After one week of acclimatisation to the chow diet, rats (n = 12/sex) were randomly divided into the LPD group (n = 6/group/sex) and the NPD group (n = 6/group/sex) for 21 days. All diet groups had free access to water and food throughout the experiment. The body weight of each rat was measured, and food and water intake were monitored weekly. Food intake (g/day) was calculated from the weight difference of food in the cages, and water intake (mL/day) from the volume difference in the water bottles.

### BLOOD AND RENAL TISSUE COLLECTION

Rats were anaesthetised with a mixture of ketamine (1 mL/100g body weight) and xylazine (0.01 mL/100g body weight) by intraperitoneal injection. Blood was collected through the orbital sinus, followed by cardiac puncture. The collected blood was centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was collected and stored at -80 °C for further analysis. The kidneys were surgically isolated and rinsed with phosphate-buffered saline. The weights were measured using an analytical balance. The kidneys were homogenised in an ice-cold buffer solution to prepare the kidney homogenate.

### DETERMINATION OF OXIDATIVE STRESS AND ANTIOXIDANT STATUS

The concentration of malondialdehyde (MDA) was determined using the colourimetric method described by Ledwoż et al. (1986). The determination of the concentration of advanced oxidation protein products (AOPP) was based on the principle of spectrophotometric determination of the oxidation of I- to I-3 (iodide ion) under acidic conditions according to the spectrophotometric method described by Witko-Sarsat et al. (1996). The determination of superoxide dismutase (SOD) enzyme activity was carried out using the method from Beyer and Fridovich (1987), which was conducted indirectly based on the reduction of nitroblue tetrazolium. The measurement of reduced glutathione (GSH) levels in the sample was based on the method of Ellman (1959), which involves the reaction of the acid 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) with GSH molecules.

### MEASUREMENT OF SERUM UREA AND CREATININE

Serum urea (mmol/L) and creatinine (μmol/L) concentrations were measured spectrophotometrically using a BA 400 automated biochemistry analyser (Biosystems, Spain) according to the manufacturer's standard protocols.

### HISTOMORPHOLOGICAL AND HISTOMORPHOMETRIC MEASUREMENT

For histologic examination, the kidneys were fixed in 10% formalin. The fixed kidney tissue was routinely processed using a Microm STP 120 Spin Tissue Processor (Thermo Scientific, MA, United States). The processed tissues were paraffin-embedded and cut into 3-5 μm thick slices using a Leica RM2135 rotary microtome (Leica, Nussloch, Germany). Staining was performed with haematoxylin and eosin (H&E) and Masson's trichrome. All prepared slides were viewed under a light microscope at ×400 magnification. For each sample, 10 sets of digital images were acquired using the X-PAD 97 Rax Vision camera (Rax Vision, FL, United States). The images were analysed

using ImageJ software (NIH, NY, United States) to detect glomerular structural changes. The glomerular tuft area (GA) and glomerular tuft volume (GV) of the renal tissue were measured and calculated. The GA measurement was converted to GV using the spherical approximation formula: $GV=1.2545(GA)^{1.5}$.

### STATISTICAL ANALYSIS

Data were expressed as mean ± standard error of the mean (SEM) and statistically analysed using GraphPad Prism version 8.2.1 (San Diego, CA, USA). Differences between groups were analysed using the independent t-test. For the longitudinal variables, a two-way analysis of variance (two-way ANOVA) was used to determine the main effects of diet and time, and the interactions between them. Treatment is reported as significantly different when $p<0.05$.

## RESULTS

### BODY WEIGHTS, FOOD AND WATER INTAKE

Figure 1 shows the changes in body weight, food intake and water intake of the rats in both diet groups in both sexes during the malnutrition period. In males and females, the body weight of the LPD group was significantly lower (Males: $F(1,10)=5.36$, $p=0.043$; Females: $F(1, 10)=14.34$, $p=0.004$) compared to the NPD group. However, food intake did not differ significantly (Males: $F(1,10)=2.6176$, $p=0.1368$; Females: $F(1, 10)=0.3565$, $p=0.5637$) between the LPD and NPD groups. In females, the LPD group had a significantly lower ($F(1, 10)=6.322$, $p=0.031$) water intake compared to the female NPD group. However, no significant difference ($p=0.055$) was found in male groups.

### KIDNEY WEIGHT

Figure S1 shows the average kidney weights of rats in NPD and LPD groups for both sexes. In males, the kidney weights of the LPD group (0.54 ± 0.04 g) were significantly lower ($p=0.0028$) compared to the NPD group (0.86 ± 0.06 g). In females, the kidney weights were not significantly different ($p=0.44$) between the LPD and NPD groups.

### OXIDATIVE STRESS AND ANTIOXIDANT STATUS

The effect of LPD on MDA, AOPP and GSH concentrations and SOD activity is shown in Figure 2. In male rats, the MDA level in the LPD group was not significantly different ($p=0.95$) compared to the NPD group. In female rats, however, the MDA level in the LPD group (55.38 ± 6.52 nmol/g protein) was significantly lower ($p=0.0194$) than in the NPD group (69.88 ± 4.83 nmol/g protein). After a 3-week LPD, significantly higher AOPP concentrations were observed in the LPD groups (Males: 142.20 ± 8.05 nmol/g protein, $p=0.0251$; Females: 204.70 ± 6.55 nmol/g protein, $p=0.0006$) compared to the NPD groups (Males: 125.90 ± 7.70 nmol/g protein; Females: 155.6 ± 5.80 nmol/g protein). In males, there were no significant differences ($p=0.64$) in GSH concentrations between the two groups. However, in females, GSH concentrations were significantly higher ($p=0.0134$) in the LPD group (0.22 ± 0.03 nmol/g protein) compared to the NPD group (0.14 ± 0.01 nmol/g protein). On the other hand, there was no significant difference ($p=0.24$) in SOD activities between the male LPD and NPD groups. However, in females, a significantly lower SOD activity ($p=0.0408$) was observed in the LPD group (40.45 ± 2.55 U/min/mg protein) compared to the NPD group (53.51 ± 2.68 U/min/mg protein).

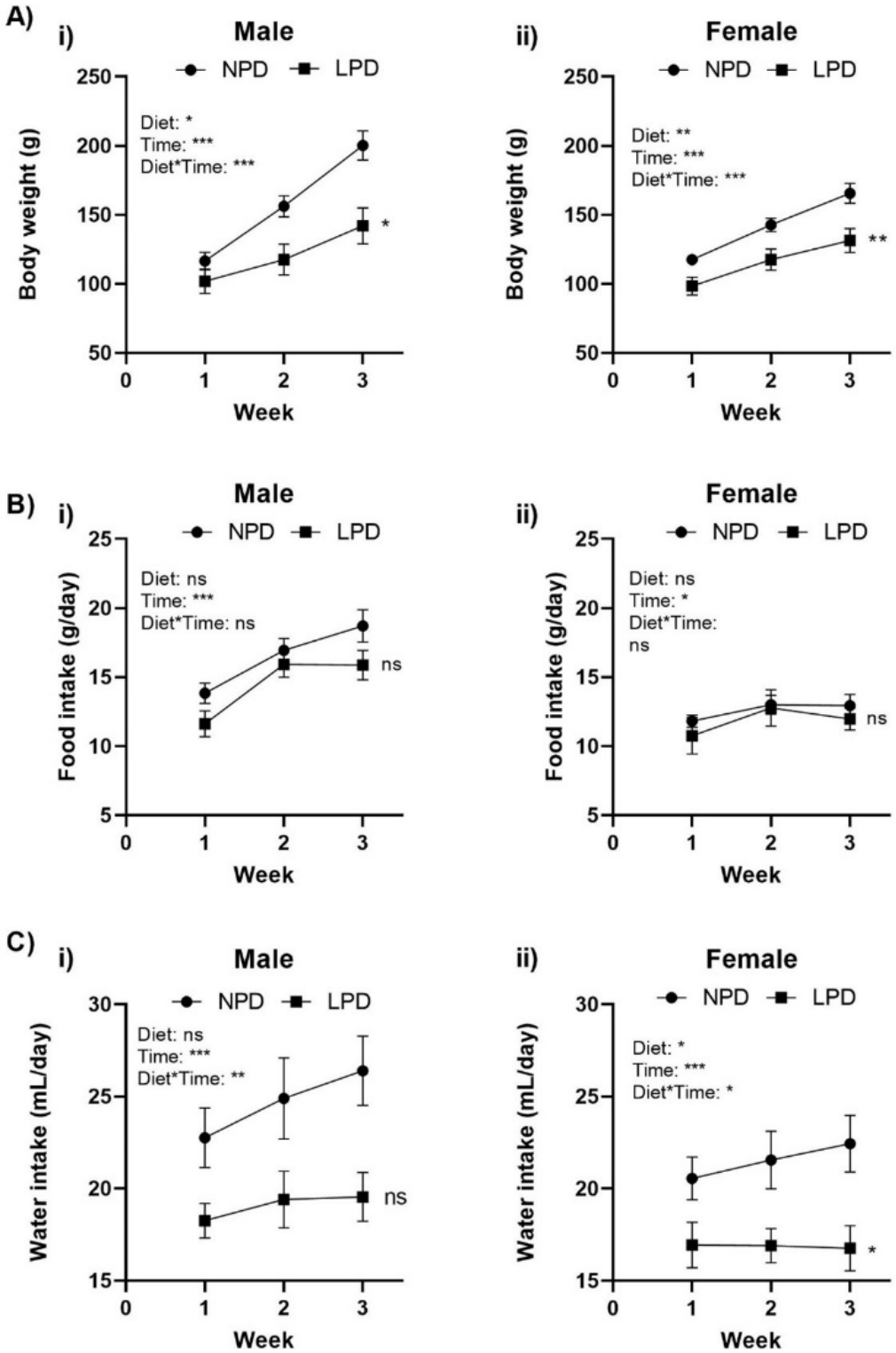
### SERUM UREA AND CREATININE CONCENTRATIONS

In males, the serum urea concentrations were significantly higher ($p<0.001$) in the LPD group (7.20 ± 0.61 mmol/L) compared to the NPD group (3.27 ± 0.52 mmol/L). In contrast, no significant difference ($p=0.1437$) was noted in females between the LPD and NPD groups (Figure S2). Serum creatinine concentrations were also not significantly different in both diet groups in either male ($p=0.38$) or female ($p=0.60$) rats.

### RENAL HISTOMORPHOLOGY

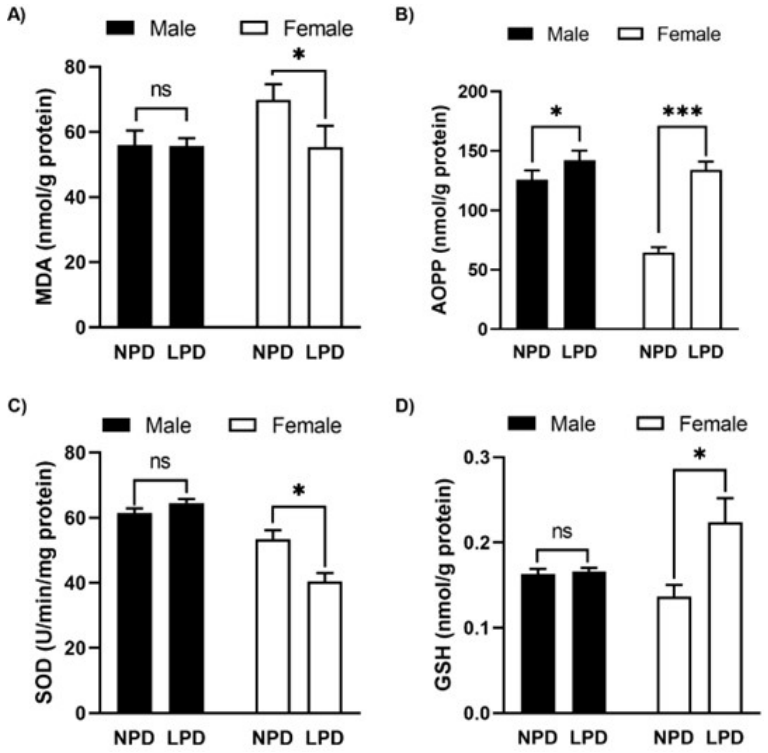
Histologic changes in the renal corpuscles and renal cortex were examined with H&E (Figure 3) and Masson's trichrome stain (Figure 4) at a magnification of ×400. In both sexes, the kidneys of the NPD groups showed normal glomerular morphology (Figure 3(A) and 3(C)) in a purple hue (Figure 4(A) and 4(C)). In contrast, a 3-week LPD resulted in a reduction in Bowman's capsule space (Figure 3(B) and 3(D)) and histologic fibrosis in the glomeruli, represented by a blue hue (Figure 4(B) and 4(D)), which was observed in both the male and female LPD groups. In addition, normal morphological structures of the proximal and distal tubules were observed in all NPD groups, characterised by tall cuboidal cells, small diameter lumens and a purple hue in the proximal tubules and lower cuboidal cells with larger diameter lumens in the distal tubules. However, the LPD groups showed abnormal histologic features in both the proximal and distal tubules, including fibrosis in the cuboidal cells and clear tubules with blue staining and reduced lumen diameter.

Figure 5 shows the microscopic structure of the renal medulla with Masson's trichrome staining and a magnification of ×100. In males and females, a normal histologic structure with purple hue was observed in the NPD groups (Figure 5(A) and 5(C)). In contrast, the LPD groups showed a blue hue, indicating the presence of renal fibrosis in the histology (Figure 5(B) and 5(D)). In the medullary region, the structure of the renal tubules appeared normal in the male LPD group, whereas it was enlarged and reduced in the female LPD group.



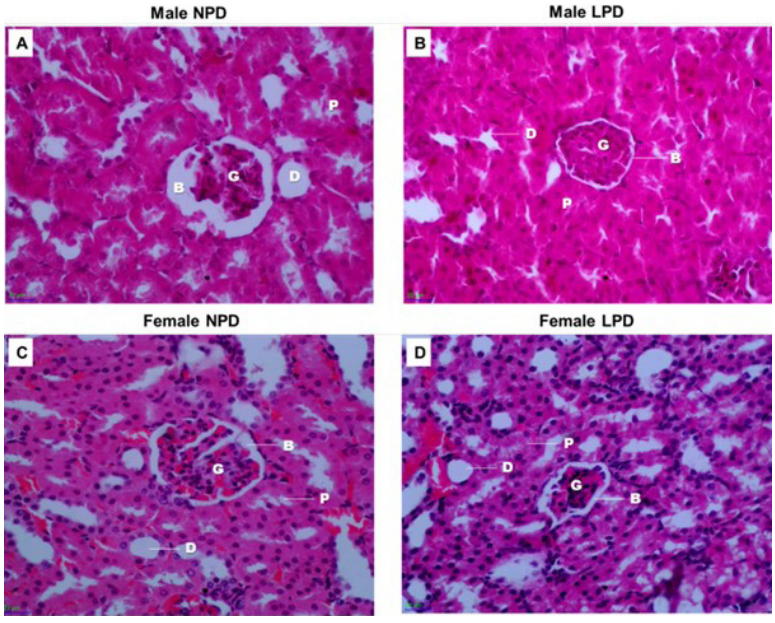

Values are expressed as mean ± SEM (n=6/group/sex). *p<0.05; **p<0.01; ***p<0.001. NPD: Normal protein diet, LPD: Low-protein diet, ns: not significant

FIGURE 1. Changes in body weight (A), food intake (B) and water intake (C) during the 3-week malnutrition feeding in male (i) and female (ii) weaned rats



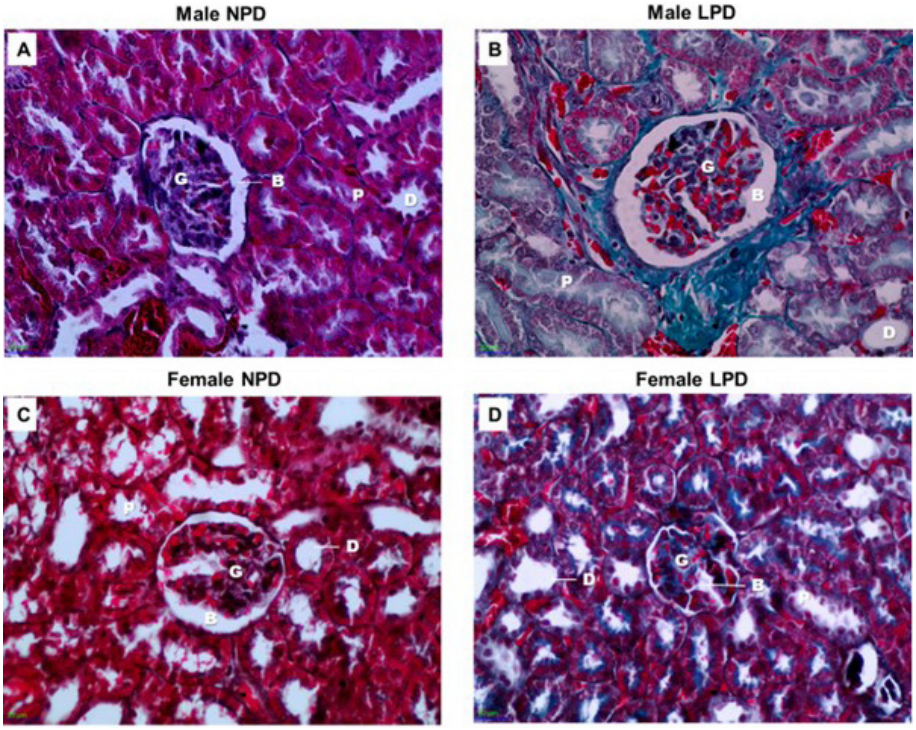

Values are expressed as mean ± SEM (n=6/group/sex). *p<0.05; ***p<0.001. NPD: Normal protein diet, LPD: Low-protein diet, MDA: Malondialdehyde, AOPP: Advanced Oxidation Protein Products, SOD: Superoxide dismutase, GSH: Reduced glutathione, ns: not significant

FIGURE 2. Effects of 3 weeks low-protein diet on the concentrations of MDA (A), AOPP (B), SOD activity (C) and GSH (D) in male and female weaned rats.



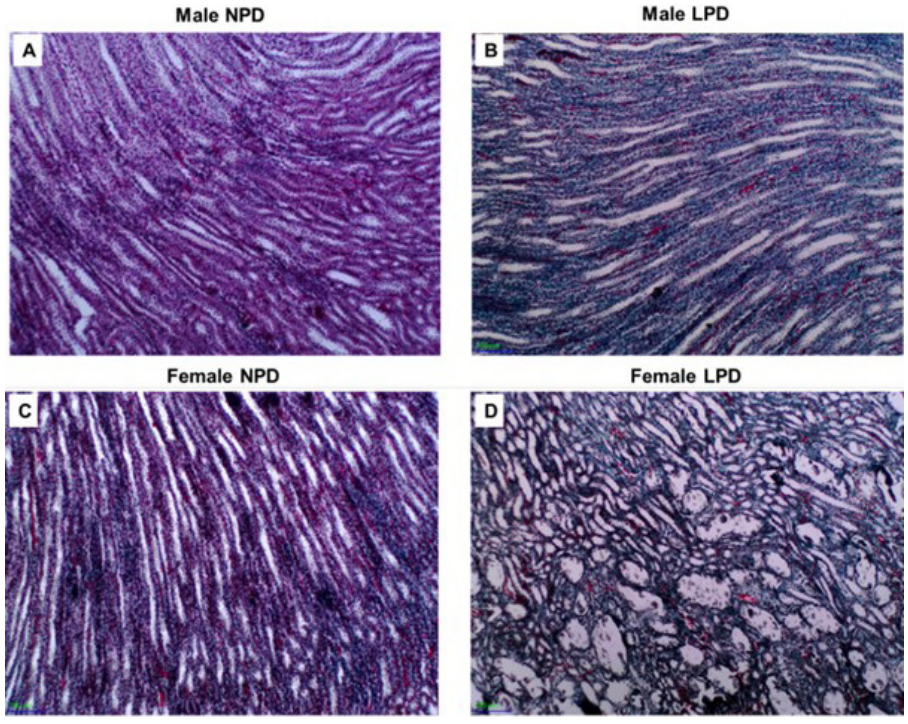

B: Bowman's capsule, D: Distal convoluted tubule, G: Glomerulus, LPD: Low-protein diet, NPD: Normal protein diet, P: Proximal convoluted tubule

FIGURE 3. Photomicrographs of the renal corpuscle and renal cortex with H&E staining and a magnification of ×400 for male NPD (A), male LPD (B), female NPD (C), and female LPD (D)




B: Bowman's capsule, D: Distal convoluted tubule, G: Glomerulus, LPD: Low-protein diet, NPD: Normal protein diet, P: Proximal convoluted tubule

FIGURE 4. Photomicrographs of the renal corpuscle and renal cortex with Masson's trichrome staining and a magnification of ×400 for male NPD (A), male LPD (B), female NPD (C), and female LPD (D)




NPD: Normal protein diet, LPD: Low-protein diet

FIGURE 5. Photomicrographs of the renal medulla with Masson's trichrome staining and a magnification of ×100 for male NPD (A), male LPD (B), female NPD (C), and female LPD (D)

### RENAL HISTOMORPHOMETRY

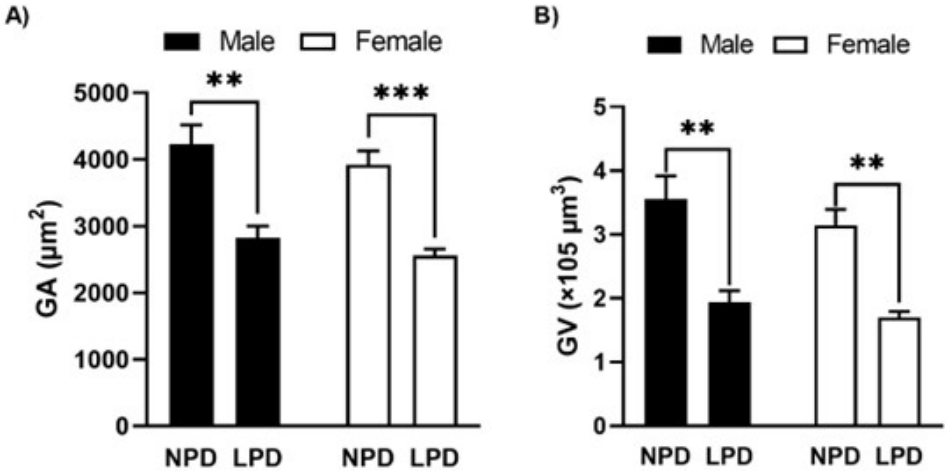
The effect of LPD on glomerular tuft area (GA) and glomerular tuft volume (GV) after 3 weeks of LPD is shown in Figure 6. In this study, both the male (p=0.0031) and female (p=0.0006) LPD groups exhibited significantly smaller GA (Males: 2821.61 ± 181.36 μm²; Females: 2561.38 ± 94.05 μm²) compared to their respective NPD groups (Males: 4226.44 ± 291.11 μm²; Females: 3923.96 ± 205.98 μm²). Similarly, the LPD groups showed significantly lower GV in males ($1.94 \pm 0.18 \times 10^5$ μm³, p=0.0032) and females ($1.70 \pm 0.09 \times 10^5$ μm³, p=0.0015) compared to the NPD groups (Males: $3.56 \pm 0.36 \times 10^5$ μm³; Females: $3.14 \pm 0.25 \times 10^5$ μm³).

## DISCUSSION

The present study investigated the effects of a 3-week LPD on renal oxidative stress, biochemical profile and histological changes in weanling rats. This study found that both male and female weaned rats had lower body weight and smaller kidneys after a 3-week of LPD. Both LPD-fed male and female rats had higher AOPP concentrations, but only the LPD-fed females had lower concentrations of MDA and SOD activity. Only females fed with a LPD had a higher GSH concentration. Serum urea concentrations were higher in males after a 3-week of LPD. Histologic examination showed abnormal histologic features in the proximal and distal tubules, fibrosis in the cuboidal cells and reduced lumen diameter in both males and females. Both males and females showed smaller GA and GV after a 3-week of LPD.

In this study, the findings that both male and female weaned rats showed lower body weight after 3 weeks of LPD are consistent with a previous study. The study showed that the adolescent rats given LPD had lower food intake and weight gain (Malta et al. 2014). In addition, a lower kidney weight was observed in the LPD group, indicating kidney atrophy and possible kidney damage due to protein malnutrition (Ying et al. 2022). While both males and females showed lower body and kidney weights, statistical significance was only observed in males for kidney weight. The observed differences between males and females suggest a possible sex-specific response of kidney weight to LPD. This observation is possibly due to sexual dimorphism in muscle protein turnover and hormonal fluctuations affecting kidney weight (Stamellou et al. 2024). Males tend to have a larger muscle mass than females and, therefore, need to consume more protein to meet their metabolic needs (Smith & Mittendorfer 2016). In addition, hormonal variations between the sexes can influence kidney development. Sex differences in kidney ammonia metabolism and kidney structure may be influenced by testosterone or androgen receptor (AR)-dependent signalling pathways in males (Harris et al. 2021).

Oxidative stress is involved in protein malnutrition due to an imbalance between the production of free radicals and their removal by antioxidants. Malnutrition in early life is strongly associated with increased DNA and oxidative lipid damage, which can be linked to a decrease in the status of antioxidant enzyme defence (Michael et al. 2022). In this study, oxidative stress markers showed different results, with the male LPD group showing no significant difference in MDA concentrations compared to the NPD group, while the female LPD group showed significantly lower MDA levels. The results of Long et al. (2022) indicate that the MDA concentration in kidney homogenates did not increase in the LPD group. This




Values are expressed as mean ± SEM (n=6/group/sex). **p<0.01; ***p<0.001. NPD: Normal protein diet, LPD: Low-protein diet, ns: not significant

FIGURE 6. Effect of 3 weeks low-protein diet on glomerular tuft area (GA; A) and glomerular tuft volume (GV; B) in male and female weaned rats

finding suggests that the antioxidant defence system within the nephron remains functional and plays a critical role in attenuating oxidative stress and reducing MDA production. Conversely, both the male and female LPD groups had significantly higher AOPP concentrations than the NPD group, indicating protein oxidation that may promote oxidative stress and inflammation in the kidney (Ou et al. 2017). Studies suggest that AOPP may contribute to the onset and progression of chronic kidney disease (Cao et al. 2013), possibly leading to various forms of kidney damage through activation of the tubular renin-angiotensin system, podocyte apoptosis, oxidative stress and inflammation in renal epithelial cells (Cao, Hou & Nie 2014; Sahoo et al. 2023).

Antioxidant status, as indicated by GSH concentrations and SOD activity, showed no significant difference between the LPD and NPD groups in males, suggesting that kidney cells can generally withstand mild oxidative stress. In addition to antioxidant defence mechanisms, oxidative stress may be regulated by cellular repair processes and other protective systems, such as cell signalling, changes in cell polarisation, and leukocyte uptake (Wang et al. 2023). However, in females, GSH concentrations were significantly higher in the LPD group than in the NPD group, while SOD activity was significantly lower. This observation suggests that the antioxidant defence system may be attempting to minimise reactive oxygen species (ROS) levels while allowing sufficient ROS for its beneficial functions (Valaei et al. 2021). Interestingly, this result suggests that the LPD-fed females in this study were more susceptible to oxidative damage than the males, indicating possible sex-specific differences in the response to oxidative stress caused by protein malnutrition.

Although the clinical signs of protein malnutrition are readily apparent on physical examination, changes in kidney function may not be apparent on initial assessment. Therefore, it is important to perform biochemical profile tests such as serum urea and creatinine determination to assess kidney function comprehensively. Urea, a primary metabolite derived from the turnover of dietary proteins and tissue proteins, is subject to a complex interplay influenced by factors such as age, gender, protein intake, endogenous catabolism, hydration status, hepatic synthesis, heart failure and kidney excretion (Liu et al. 2021). In the present study, the biochemical profiles in the male LPD group showed elevated serum urea concentrations compared to the NPD group, indicating increased protein catabolism and possible renal dysfunction. However, serum creatinine levels remained within the normal range in the LPD groups, suggesting that histologic studies are needed to clarify the effects of LPD on kidney structure and gain important insights into kidney health in protein malnutrition.

Histologic examination confirmed the presence of renal injury and fibrosis in both the male and female LPD groups, further supporting the effects of LPD on renal injury caused by protein malnutrition. Masson's trichrome staining showed marked fibrosis in the renal medulla and cortex, indicating progressive renal disease. Tubular epithelial cells, particularly susceptible to intrinsic oxidative stress, exhibit significant damage during critical prenatal developmental periods in male rats, leading to altered renal morphology and increased glomerular collagen deposition shortly after birth (Pedroza et al. 2019). In addition, a previous study showed significant histologic renal lesions in infants with protein malnutrition, suggesting a direct correlation (D'Marco et al. 2022). The present study confirms these findings and shows a significant reduction in the area and volume of glomerular tufts after LPD induction in both sexes, as demonstrated by H&E staining. The observed changes in glomerular morphology and the presence of fibrosis emphasise the effects of LPD on renal injury in male and female weaned rats.

This study aimed to explore the mechanistic aspects of the effects of short-term LPD on renal parameters in weaned rats. While the biochemical and histologic studies provide important information on structural and cellular changes, they do not necessarily correlate with functional outcomes. To fully understand the effects of a low-protein diet on renal health, future studies should also include functional tests such as glomerular filtration rate (GFR), creatinine clearance and other markers of renal function. In addition, the duration of the dietary intervention was relatively short. Chronic exposure to the dietary intervention could have different effects that may not be apparent in a short-term study. Longitudinal studies are needed to assess the long-term consequences and potential risks associated with longer-term LPD.

## CONCLUSION

This study demonstrates that feeding male and female weanling rats with an LPD for three weeks induces several changes, including lower body weight and smaller kidneys, higher renal oxidative stress, and renal injury with decreased glomerular size, fibrosis present, and decreased glomerular tuft area and volume. The differential response of males and females to LPD suggests sexual dimorphism and hormonal factors in kidney development, with females showing higher susceptibility to oxidative damage. Further research is needed to investigate the underlying mechanisms of the effects of protein malnutrition on the kidneys.

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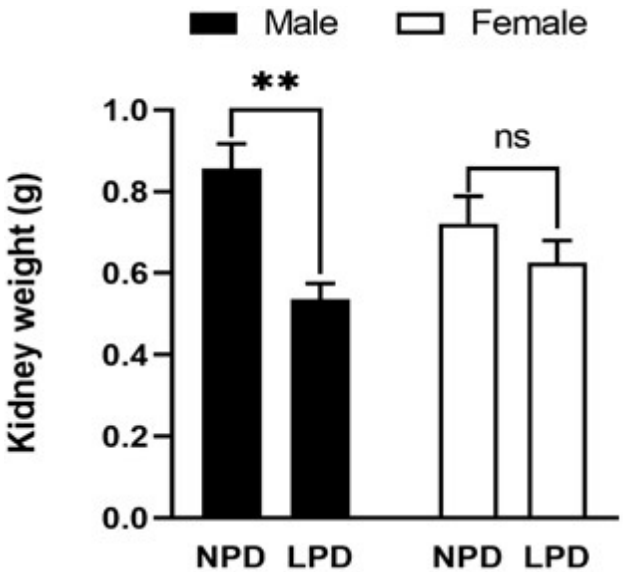
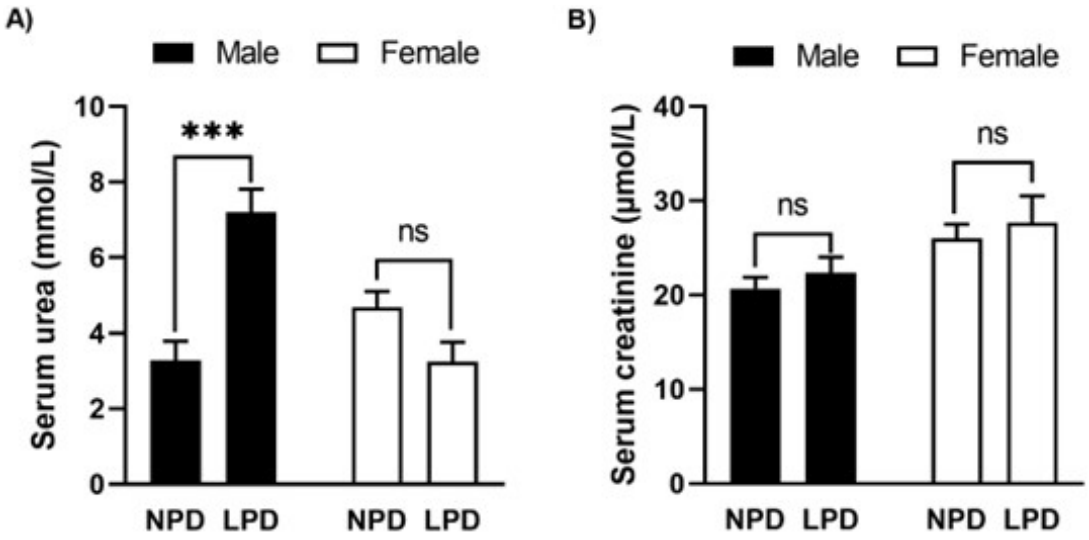



Values are expressed as mean ± SEM (n=6/group/sex). **p<0.01. NPD: Normal protein diet, LPD: Low-protein diet, ns: not significant

FIGURE S1. Average kidney weights in male and female weaned rats after 3 weeks of low-protein diet




Values are expressed as mean ± SEM (n=6/group/sex). ***p<0.001. NPD: Normal protein diet, LPD: Low-protein diet, ns: not significant

FIGURE S2. Effect of 3 weeks low-protein diet on serum urea (A) and creatinine (B) concentrations in male and female weaned rats